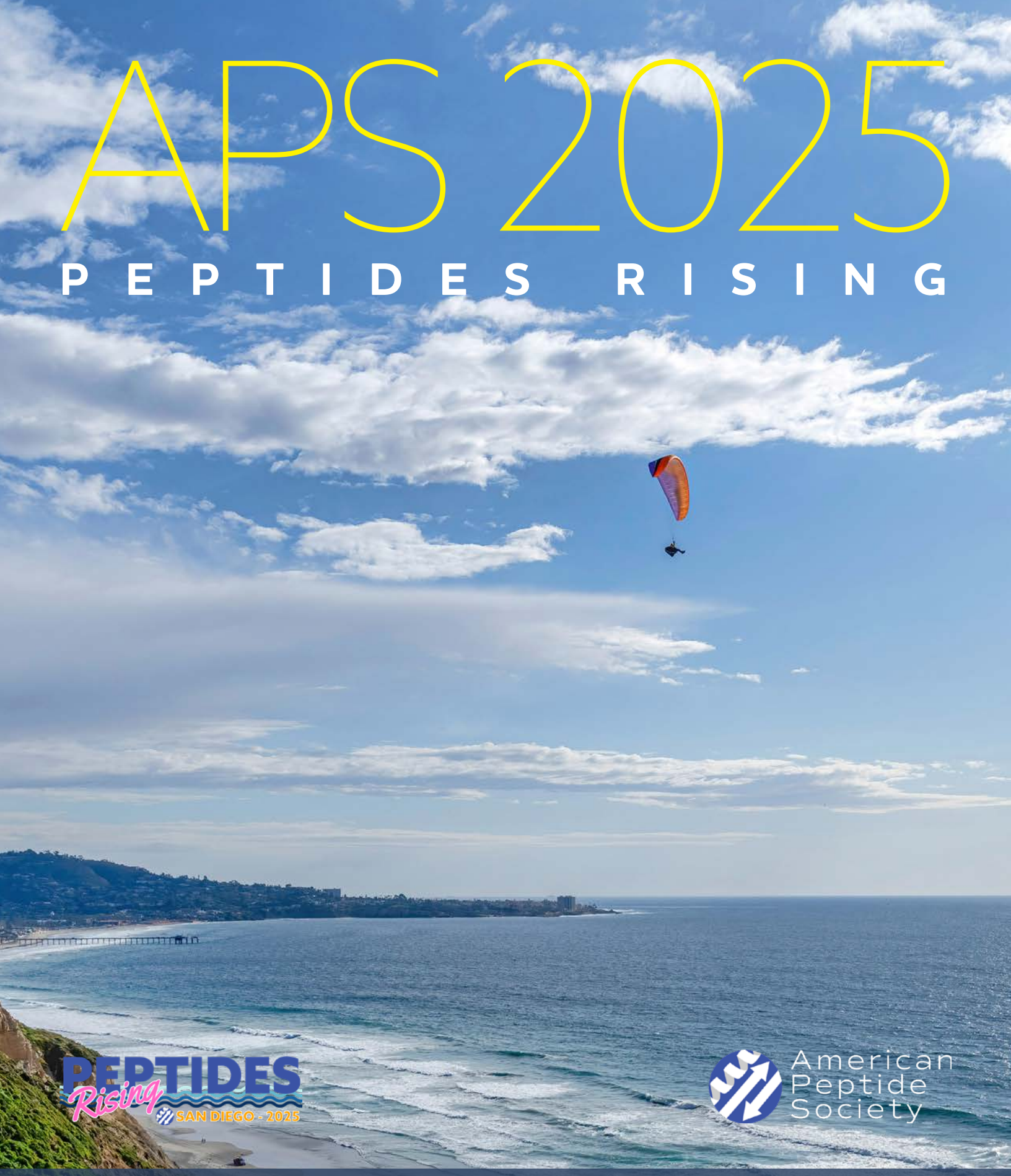
APS 2025

PEPTIDES RISING



PEPTIDES Rising SAN DIEGO - 2025

American Peptide Society

29th American Peptide Society & 15th International Peptide Society Symposium
San Diego, California | 15th - 19th June

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WELCOME FROM THE CO-CHAIRS

Welcome to the 29th American Peptide Symposium, in conjunction with the 15th International Peptide Symposium, hosted in sunny San Diego, California! Our theme, Peptides Rising, highlights the ascending importance of peptide research in our community, which will have substantial, long-lasting impacts on health and disease.

The 2025 program will feature innovative talks from two world-renowned keynote speakers. Our opening keynote presentation will be held on June 15. Dame Margaret Brimble, Distinguished Professor and Director of Medicinal Chemistry at the University of Auckland, will speak on "Applications of Cysteine Lipidation "CLipPA" Technology. Our closing keynote lecture, held on June 19, will be presented by Dr. Scott Miller, Sterling Professor of Chemistry at Yale University. He will present "Peptide-Based Catalysis Version 2.0."

Multiple award lectures will also feature cutting-edge science in the field of peptide chemistry. The du Vigneaud Lectures will include Dek Woolfson, University of Bristol, and Ashraf Brik, Technion-Israel Institute of Technology. The R. Bruce Merrifield Award Lecture will be presented by Phil Dawson, The Scripps Research Institute. The Rao Makineni Award Lecture will be presented by Krishna Kumar, Tufts University. The Murray Goodman Award Lecture will be presented by William Lubell, University of Montreal. In addition, the Early Career Award Lecture will be presented by Betsy Parkinson, Purdue University.

The conference will encompass a wide breadth of talks encompassing areas such as sustainability, peptide materials, therapeutic discoveries, computational design and artificial intelligence and epigenetics. In addition, we will have a special session encompassing the drug discovery space surrounding incretins. This year, we also have a very exciting addition to our agenda: a Young Investigator Symposium scheduled on June 15. This will provide additional opportunities for young investigators to present, share ideas and network.

We express our sincere gratitude for the tremendous support of our sponsors and exhibitors. Without this support, the conference would not be possible. We appreciate their dedicated commitment to peptide science, and we highly encourage you to visit their exhibits to learn about their new products and services.

Thank you for participating in this year's symposium as we strive together to promote Peptides Rising!

Jean Chmielewski
Distinguished Professor
Purdue University

Wendy Hartsock
Director of Strategic
Partnerships Aralez Bio

Eileen Kennedy
Professor of Pharmacy
University of Georgia

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Wednesday Live Session

4.00 pm at the booth

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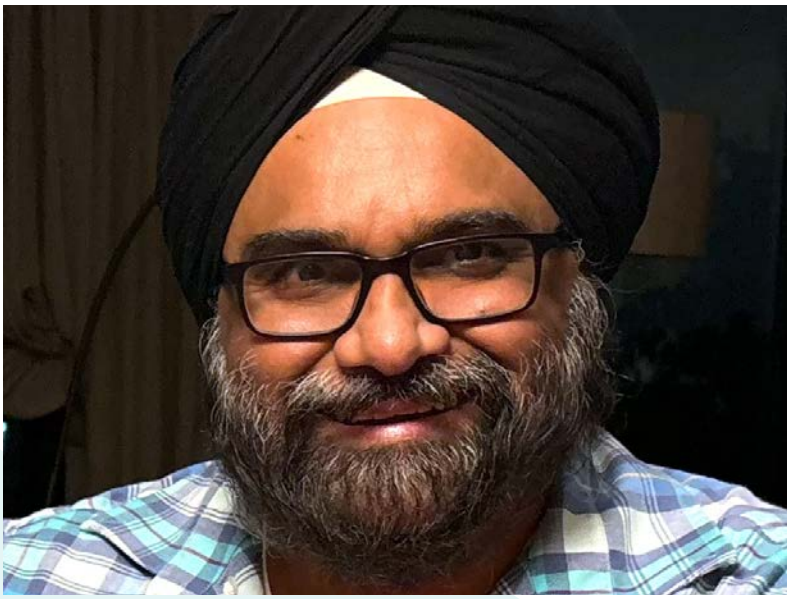
WELCOME FROM THE APS PRESIDENT

Dear Peptide Enthusiasts,



It is my great pleasure to welcome you to the 29th American Peptide Symposium and 15th International Peptide Symposium. We come together at a time when the pursuit of science faces extraordinary challenges. Yet, our distinguished and diverse community of scientists remain united by a shared commitment to advancing knowledge and innovation in our field. In this spirit, this conference is not just a platform for presenting data; it is a reaffirmation of our collective values to cherish scientific discoveries and celebrate the perseverance of our researchers and the curiosity of our students.

Our symposium chairs Jean Chmielewski, Wendy Hartsock and Eileen Kennedy have captured the resurgence of peptides as therapeutics and materials in the theme of the meeting, "Peptides Rising". Once viewed as limited by stability and delivery challenges, peptide-based drugs are now experiencing a renaissance, thanks to advances in synthetic chemistry, formulation technologies, and molecular design. Peptides are offering novel solutions for diseases that were once considered untreatable, with growing impact in oncology, metabolic disorders, and infectious diseases. This revival not only reflects the ingenuity of our field but also the translational power of fundamental research when paired with interdisciplinary collaboration.

The Chairs have organized a stellar program that will begin with a Young Investigator Symposium on Sunday and feature talks by leaders in the field throughout the week. We are also delighted to host a vibrant exhibitor hall, where leading companies and emerging startups will showcase cutting-edge technologies, reagents, and tools that help to accelerate discovery. From next-generation synthesizers and chromatography instruments to new classes of reagents for synthesis, biological assays and novel biomaterials, these contributions from industry partners are an essential part of the scientific ecosystem. I encourage you to explore the exhibits, engage with our partners, and discover new resources.

Our Society is blessed by several individuals who tirelessly serve the community and make this Symposium possible. I would like to extend my deepest gratitude to the Fundraising Committee for their exceptional efforts in making this conference possible during very uncertain times - kudos to Steve Miller, Chloe Mitchell and Wendy Hartsock! You will personally witness the dedication of the Student Affairs Committee (SAC) headed by Emel Adaligil, Mike Bertucci and James Checco at the poster sessions and all the young investigator activities. Lars Sahl is our website designer and conference photographer – he is also the person who highlights new publications on the APS website. Please say Aloha! to Lars when you see him in his Hawaiian shirt.

The Chairs have worked tirelessly over many months to plan, coordinate, and deliver this outstanding event. Their leadership, vision, and attention to detail are reflected in every aspect of the program - from the thoughtfully curated sessions to the smooth logistics. Please join me in thanking the Chairs! Finally, a special shout-out to our Peptide Society Manager, Lauren Cline. There's not enough space on this page to enumerate all the ways that Lauren keeps this Society functioning. Thank you, Lauren!

Lastly, thank you for attending and for being part of this remarkable community. We look forward to the discoveries, connections, and conversations that will emerge in the days ahead.

Keep on keeping on,
Paramjit Arora

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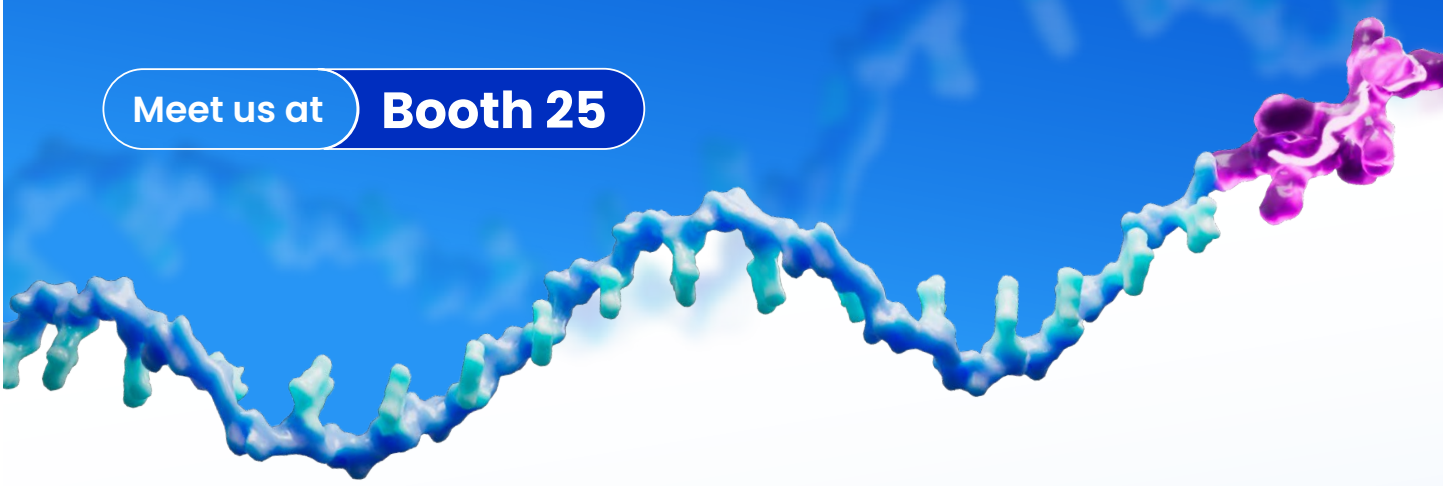

Peptide Chemistry Platform

Unnatural Amino Acids	Peptides	Peptide Conjugates
• α,β,γ-substituted AAs	• Long Linear Peptides	• Peptide-PMO (PPMO)
• N-alkylated AAs	• Cyclic Peptides	• Peptide-Oligonucleotide (POC)
• Glyco AAs	• Modified Peptides	• Peptide-Drug (PDC)
	• PEGylated Peptides	• Radionuclide Drug (RDC)
	• Peptidomimetics	

American Peptide Society People

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American Peptide Society

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2023-2025 Nominating Committee
Marcey Waters, Chair, Kathlynn Brown, Arundhati, Juan Del Valle, Champak Chatterjee, Stephen Miller, and Jon Lai

APS SYMPOSIA CHRONOLOGY

1st	**1968**	**Saul Landa** - Yale University **Boris Weinstein** - University of Washington at Seattle	Yale University New Haven, CT
2nd	**1970**	**F. Merlin Bumpus** - Cleveland Clinic	Cleveland Clinic Cleveland, OH
3rd	**1972**	**Johannes Meinhofer** - Harvard Medical School	Children's Cancer Research Foundation Boston, MA
4th	**1975**	**Roderich Walter** - University of Illinois Medical Center, Chicago	The Rockefeller University and Barbizon Plaza Hotel New York, NY
5th	**1977**	**Murray Goodman** - University of California at San Diego	UCLA at San Diego San Diego, CA
6th	**1979**	**Erhard Gross** - National Institute of Health	Georgetown University Washington, DC
7th	**1981**	**Daniel H. Rich** - University of Wisconsin at Madison	University of Wisconsin Madison, WI
8th	**1983**	**Victor J. Hruby** - University of Arizona	University of Arizona Tucson, AZ
9th	**1985**	**Kenneth D. Kopple** - Illinois Institute of Technology **Charles M. Deber** - University of Toronto	University of Toronto Toronto, Ontario Canada
10th	**1987**	**Garland R. Marshall** - Washington University School of Medicine	Washington University St. Louis, MO
11th	**1989**	**Jean E. Rivier** - The Salk Institute of Biological Studies, La Jolla	UCLA at San Diego San Diego, CA
12th	**1991**	**John A. Smith** - Massachusetts General Hospital	Massachusetts Institute of Technology, Cambridge, MA
13th	**1993**	**Robert S. Hodges** - University of Alberta at Edmonton	Edmonton Convention Ctr. Alberta, Canada
14th	**1995**	**Pravin T.P. Kaumaya** - The Ohio State University	The Ohio State University Columbus, OH
15th	**1997**	**James P. Tam** - Vanderbilt University	Nashville Convention Center Nashville, TN
16th	**1999**	**George Barany** - University of Minnesota **Gregg B. Fields** - Florida Atlantic University	Minneapolis Convention Ctr. Minneapolis, MN

APS SYMPOSIA CHRONOLOGY

17th	**2001**	**Richard A. Houghten** - Torrey Pines Institute Mol. Stud. **Michael Lebl** - Illumina Inc., CA	Town and Country Resort San Diego, CA
18th	**2003**	**Michael Chorev** - Beth Israel Medical & Harvard Med Sch **Tomi K. Sawyer** - ARIAD Pharmaceuticals, Inc.	Marriott Copley Place Boston, MA
19th	**2005**	**Jeffrey W. Kelly** - The Scripps Research Institute **Tom W. Muir** - Rockefeller University, NY	Town and Country Resort San Diego, CA
20th	**2007**	**William D. Lubell** - University of Montreal **Emanuel H.F. Escher** - Univeristy of Sherbrooke	Palais des Congres Montreal, Canada
21st	**2009**	**Richard diMarchi** - Indiana University **Hank Mosberg** - University of MIchigan	Indiana University Bloomington, IN
22nd	**2011**	**Philip Dawson** - The Scripps Research Institute **Joel Schneider** - National Cancer Institute	Sheraton San Diego San Diego, CA
23rd	**2013**	**David Lawrence** - University of North Carolina at Chapel Hill **Marcey Waters** - University of North Carolina at Chapel Hill	Hilton Waikoloa Village, Waikoloa, Hawai'i
24th	**2015**	**Ved Srivastava** - GlaxoSmithKline **Andrei Yudin** - University of Toronto	Hyatt Regency Grand Cypress, Orlando, FL
25th	**2017**	**Jonathan Lai** - Albert Einstein College of Medicine **John Vederas** - University of Alberta	Whistler Conference Center Whistler, BC, Canada
26th	**2019**	**Paramjit Arora** - New York University **Anna Mapp** - University of Michigan	Portola Hotel and Monte-rey Conference Center
27th	**2022**	**Mark D. Distefano** - University of Minnesota **Les Miranda** - Amgen, Inc.	Whistler Conference Cen-ter Whistler, BC, Canada
28th	**2023**	**David Chenoweth** - University of Pennsylvania **Robert Garbaccio** - Merck	Westin Kierland Resort Scottsdale, AZ
29th	**2025**	**Jean Chmielewski**, Purdue University **Wendy Hartsock**, Aralez Bio **Eileen Kennedy**, University of Georgia	Sheraton San Diego San Diego, CA

SCHEDULE OF EVENTS - OVERVIEW

Sunday, June 15, 2025

12:00pm - 01:00pm	Young Investigator's Lunch - by invitation only	Eventide Gardens
01:00pm - 03:45pm	Young Investigator Symposium	Silver Pearl 1, 2, & 3
03:45pm - 04:45pm	Workshop on Career Development	Silver Pearl 2
04:00pm – 05:00pm	International Liaison Meeting	Tidepool 4
05:30pm - 07:00pm	Opening Reception	Eventide & Gardens
07:00pm - 07:15pm	President's Welcome & General Assembly Remarks	Pacific Jewel Ballroom
07:15pm - 08:00pm	Opening Plenary Keynote Lecture	Pacific Jewel Ballroom

Monday, June 16, 2025

07:30am - 08:25am	Breakfast with Exhibitors	Eventide
08:25am - 08:30am	Opening Remarks	Pacific Jewel Ballroom
08:30am - 10:00am	Session 1: Strategies for Membrane Permeability or Oral Bioavailability	Pacific Jewel Ballroom
10:00am - 10:25am	Coffee with Exhibitors & Posters	Eventide
10:30am - 12:00pm	Session 2: Bioactive Peptides	Pacific Jewel Ballroom
12:00pm - 02:00pm	Lunch with Exhibitors and Poster Session 1	Eventide
02:05pm - 03:40pm	Session 3: Peptide Synthesis and Innovation	Pacific Jewel Ballroom
02:05pm - 02:10pm	Sponsor Flash Talk	Pacific Jewel Ballroom
03:15pm - 03:40pm	Early Career Lectureship Award	Pacific Jewel Ballroom
03:40pm - 04:05pm	Afternoon Break	Pacific Jewel Ballroom
04:10pm - 06:10pm	Session 4: Peptide Materials	Pacific Jewel Ballroom
04:10pm - 04:40pm	du Vigneaud Lecture	Pacific Jewel Ballroom
06:30pm - 07:30pm	Student Mixer	Eventide Gardens
08:00pm - 10:00pm	Pharma Panel - Beyond Potency: PK, Formulation, IP, and More	Pacific Jewel Ballroom

Tuesday, June 17, 2025

07:30am - 08:40pm	Breakfast with Exhibitors	Eventide
08:40am - 08:45am	Opening Remarks	Pacific Jewel Ballroom
08:45am - 10:00am	Session 5: Sustainability in Peptide Science	Pacific Jewel Ballroom
10:00am - 10:25am	Coffee with Exhibitors & Posters	Eventide
10:30am - 12:35pm	Session 6: Peptide Therapeutics from Discovery to the Clinic	Pacific Jewel Ballroom
12:45pm - 02:15pm	Schram Young Scientists' Lunch	Silver Pearl 3
08:00pm - 10:00pm	Pharma Panel - Designing and Developing Orally Available Peptides	Pacific Jewel Ballroom

Wednesday, June 18, 2025

07:30am - 08:25am	Breakfast with Exhibitors	Eventide
08:25am - 08:30am	Opening Remarks	Pacific Jewel Ballroom
08:30am - 10:00am	Session 7: Protein Modification, Structural Insights, and Disease State	Pacific Jewel Ballroom
10:00am - 10:25am	Coffee with Exhibitors & Posters	Eventide
10:30am - 12:00pm	Session 8: Peptides in Oncology	Pacific Jewel Ballroom
11:50pm - 11:55pm	Poster Flash Talk	Pacific Jewel Ballroom
11:55pm - 12:00pm	Poster Flash Talk	Pacific Jewel Ballroom
12:00pm - 02:05pm	Lunch with Exhibitors and Poster Session 2	Eventide
12:30pm - 02:05pm	Poster Judging and Vendor Interactions	Eventide
02:10pm - 03:45pm	Session 9: Computational Empowerment in Peptide Science	Pacific Jewel Ballroom
03:45pm - 04:15pm	Afternoon Break with Exhibitors	Eventide
04:20pm - 06:15pm	Session 10: Exploration of Selectivity and Methods for Targeting Disease	Pacific Jewel Ballroom

SCHEDULE OF EVENTS - OVERVIEW

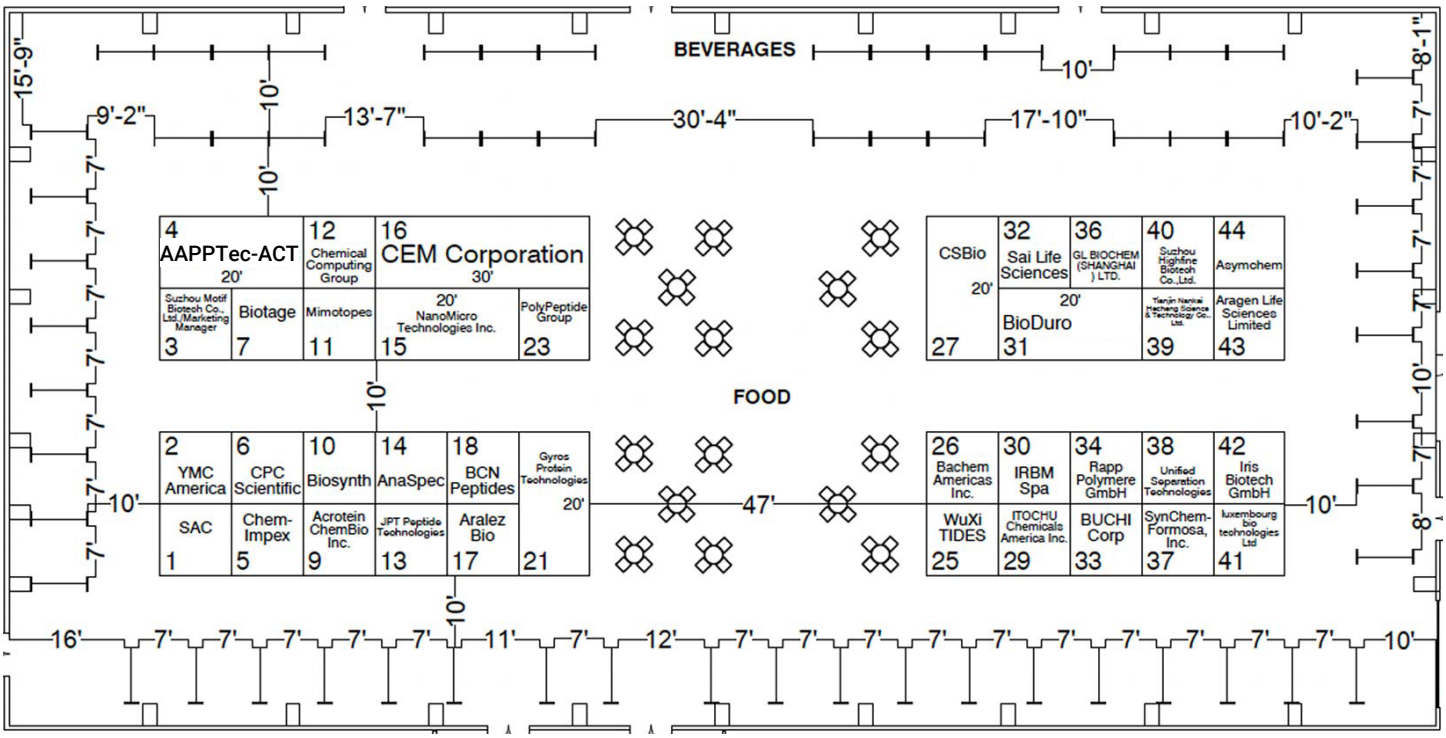
Wednesday, June 18, 2025, cont'd...

05:30pm - 06:15pm	**Merrifield Award Lecture**	Pacific Jewel Ballroom
08:00pm - 10:00pm	**Pharma Panel: Sustainability in Peptide Manufacturing**	Pacific Jewel Ballroom

Thursday, June 19, 2025

08:15am - 08:20am	**Opening Remarks**	Pacific Jewel Ballroom
08:20am - 08:25am	**Sponsor Flash Talk**	Pacific Jewel Ballroom
08:25am - 10:20am	**Session 11: Incretins 2025 - Transforming the Transformation**	Pacific Jewel Ballroom
09:50am - 10:20am	**Makineni Lecture**	Pacific Jewel Ballroom
10:20am - 10:40am	**Coffee Break**	Pacific Jewel Foyer
10:40am - 12:30pm	**Session 12: Disease-Focused Peptide Discovery**	Pacific Jewel Ballroom
12:00pm - 12:30pm	**Goodman Lecture**	Pacific Jewel Ballroom
12:30pm - 01:40pm	**Lunch on Your Own**	
12:30pm - 01:40pm	**du Vigneaud Luncheon - by invitation only**	Seaglass
12:30pm - 01:40pm	**Young Investigator Appreciation Lunch**	Eventide Gardens
01:45pm - 03:30pm	**Session 13: Implications and Applications of PTMs**	Pacific Jewel Ballroom
03:00pm - 03:30pm	**du Vigneaud Lecture**	Pacific Jewel Ballroom
03:30pm - 04:00pm	**Afternoon Break**	Pacific Jewel Foyer
04:00pm - 05:05pm	**Session 14: New Frontiers in Peptide Science**	Pacific Jewel Ballroom
05:05pm - 05:50pm	**Closing Plenary Keynote Lecture**	Pacific Jewel Ballroom
05:50pm - 06:00pm	**Closing Remarks**	Pacific Jewel Ballroom
07:00pm - 10:00pm	**Closing Banquet**	Eventide

EVENTIDE BALLROOM FLOOR PLAN AND EXHIBITOR BOOTH ASSIGNMENTS



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SCHEDULE OF EVENTS - DETAIL

Sunday, June 15, 2025

12:00pm - 01:00pm	Young Investigator's Lunch - by invitation only	Eventide Gardens
01:00pm - 03:45pm	Young Investigator Symposium	Silver Pearl 1, 2, and 3
03:45pm - 04:45pm	Workshop on Career Development	Silver pearl 2
04:00pm - 05:00pm	International Liaison Meeting	Tidepool 4
05:30pm - 07:00pm	Opening Reception	Eventide and Gardens
07:00pm - 07:15pm	President's Welcome & General Assembly Remarks	Pacific Jewel Ballroom
07:15pm - 08:00pm	Opening Plenary Keynote Lecture	Pacific Jewel Ballroom

L01 Margaret Brimble, Distinguished Professor, University of Auckland. |
Applications of Cysteine Lipidation "CLipPA" Technology : Drugs Vaccines and Biomaterials

Pacific Jewel Ballroom

Monday, June 16, 2025

07:30am - 08:25am	Breakfast with Exhibitors	Eventide
08:25am - 08:30am	Opening Remarks	Pacific Jewel Ballroom
08:30am - 10:00am	**Session 1:** Strategies for Membrane Permeability or Oral Bioavailability **Session Chairs:** Jayanta Chatterjee, Indian Institute of Science Ikuhiko Nakase, Osaka Metropolitan University	Pacific Jewell Ballroom

08:30am - 08:55am

L02 John Gleeson, Senior Scientist, Merck. |
Development, Validation, and Implementation of Intestinal Cell Models to Guide Oral Peptide Formulation Design

Pacific Jewel Ballroom

08:55am - 09:10am

L03 Severin Schneebeli, Associate Professor, Purdue University |
The Molecular Basis of Oral Peptide Delivery with Permeation Enhancers

Pacific Jewel Ballroom

09:10am - 09:20am

L04-YI1 Laura M. Poller, Graduate Student, ETH Zürich |
Collagen Cross-Linking: Structure and Sequence Selectivity of Lysyl Oxidase-Like 2

Pacific Jewel Ballroom

09:20am - 09:35am

L05 Marco Pires, Professor and Director of Graduate Studies, University of Virginia |
Systematic Determination of the Impact of Structural Edits on Accumulation into Mycobacteria

Pacific Jewel Ballroom

09:35am -10:00am

L06 Joshua Kritzer, Professor, Tufts University |
Chemical Biology Approaches for Measuring Intracellular Drug Delivery

Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Monday, June 16, 2025, cont'd...

10:00am - 10:25am Coffee with Exhibitors & Posters — Pacific Jewel Ballroom

10:30am - 11:55am **Session 2:** Bioactive Peptides — Pacific Jewell Ballroom

Session Chairs:
Mike Bertucci, Lafayette College
Alan Cameron, University of Auckland

10:30am - 10:55am  **L07 Michelle Arkin**, Senior Scientist, Merck. | *Development, Validation, and Implementation of Intestinal Cell Models to Guide Oral Peptide Formulation Design* — Pacific Jewel Ballroom

10:55am - 11:10am  **L08 Jaehoon Ju**, Associate Professor, Purdue University | *The Molecular Basis of Oral Peptide Delivery with Permeation Enhancers* — Pacific Jewel Ballroom

11:10am - 11:20am  **L09-YI2 Caroline Almeida**, Graduate Student, Federal University of Rio de Janeiro and UC Merced | *Transcriptional Regulation of Aspergillus nidulans Biofilms from Environmental and Clinical Isolates Exposed to Pisum sativum Defensin 2* — Pacific Jewel Ballroom

11:20am - 11:45am  **L10 David Lawrence**, Distinguished Professor, University of North Carolina | *Peptides as Therapeutic Partners: From Drug Delivery to Molecular Sentinels* — Pacific Jewel Ballroom

11:45am - 12:00pm  **L11 Jun Ohata**, Assistant Professor, North Carolina State University | *Potential Roles of Solid-State Reactivity of Dipeptides in Prebiotic Polypeptide Synthesis* — Pacific Jewel Ballroom

12:00pm - 02:00pm Lunch with Exhibitors and Poster Session 1 - **poster session starts 12:30pm** — Eventide

02:05pm - 03:40pm **Session 3:** Peptide Synthesis & Innovation — Pacific Jewell Ballroom

Session Chairs:
Stephanie Barros, Johnson & Johnson
Chloe Mitchell, Gyros Protein Technologies

Session Sponsor:
GYROS PROTEIN Technologies

02:05pm - 02:10pm **Sponsor Flash Talk** — Pacific Jewell Ballroom

Driving Peptide Synthesis Forward: A Platform for Innovation and Scientific Excellence

Chloe Mitchell
Regional Sales Manager, Canada; Field Application Scientist, Americas
Gyros Protein Technologies

SCHEDULE OF EVENTS - DETAIL

Monday, June 16, 2025, cont'd...

02:10pm - 02:25pm 
L12 Gong Chen, Professor, Nankai University |
Simple C1 Chemistry for Peptide Modification
Pacific Jewel Ballroom

02:25pm - 02:50pm 
L13 Lara Malins, Professor, Australian National University |
Advances in Late-Stage Peptide Modification Chemistry: Inspiration from Nature
Pacific Jewel Ballroom

02:50pm - 03:00pm 
L14-YI3 Zhenquan Sun, Postdoctoral Scholar, University of Chicago |
Chemical Synthesis of Difficult Peptide and Protein by N,O/S-Benzylidene (thio)Acetals (NBA) Strategy
Pacific Jewel Ballroom

03:00pm - 03:15pm 
L15 Timothy Reichart, Elliott Assistant Professor of Chemistry, Hampden-Sydney College |
Total Chemical Synthesis of a 97-Amino Acid Target-Binding Monobody via Cysteine-Free Conformationally-Assisted Ligation
Pacific Jewel Ballroom

03:15pm - 03:40pm **Early Career Lectureship Award**
Pacific Jewell Ballroom


L16-AW Elizabeth, 'Betsy,' Parkinson, Assistant Professor, Purdue University |
Utilizing Bioinformatics, Biocatalysis, and Synthetic Chemistry to Access Natural Product-Inspired Cyclic Peptides
Pacific Jewel Ballroom

03:40pm - 04:10pm Afternoon Break
Pacific Jewell Ballroom

04:10pm - 06:10pm **Session 4:** Peptide Materials
Pacific Jewell Ballroom

Session Chairs:
James Checco, University of Nebraska-Lincoln
Matthew Kubasik, Fairfield University

04:10pm - 04:40pm **du Vigneaud Lecture**
Pacific Jewell Ballroom


L17-AW Dek Woolfson, Professor, Bristol University |
From Peptides to Proteins to Functions by Design
Pacific Jewel Ballroom

04:40pm - 04:55pm 
L18 Beate Koksch, Professor, Freie Universitat Berlin |
Fluoropeptides as Biodegradable Biopolymers
Pacific Jewel Ballroom

04:55pm - 05:05pm 
L19-YI4 Ashutosh Agrahari, Graduate Student, Purdue University |
Growth Mechanism of Coiled-Coil Peptide Nanocrystals and their Application for Intracellular Delivery of Proteins
Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Monday, June 16, 2025, cont'd...

05:05pm - 05:30pm  **L20 Vincent P. Conticello** Professor, Emory University | *Peptide-Based Nanomaterials: Progress from Structural Analysis to Design* — Pacific Jewel Ballroom

05:30pm - 05:45pm  **L21 Tania Lopez-Silva,** Postdoctoral Fellow, National Cancer Institute | *Peptide Hydrogels Control Neutrophil Extracellular Trap, NET, Formation in vivo with Locoregional Precision* — Pacific Jewel Ballroom

05:45pm - 06:10pm  **L22 Akif Tezcan**, Professor of Chemistry and Biochemistry, University of California, San Diego | *Design of Out-of-Equilibrium Biomolecular Assemblies* — Pacific Jewel Ballroom

06:30pm - 07:30pm Student Mixer — Eventide Gardens

08:00pm - 10:00pm **Pharma Panel:** Beyond Potency: PK, Formulation, IP, and More — Pacific Jewel Ballroom
Moderated by Nicolas Boyer, Principal Scientist, Merck

Panelists:
Elisabetta Bianchi, Director, Discovery Chemistry, Curium
John DiMaio, Patent Attorney, Senior Associate, Hodgson Russ LLP
Ruchia Duggal, Principal Scientist, Merck
Sivaneswary Genapathy, Associate Director, Bicycle Therapeutics
Christopher John, Scientific Director, Johnson and Johnson Innovative Medicines

Tuesday, June 17, 2025

07:30am - 08:40am Breakfast with Exhibitors — Eventide

08:40am - 08:45am Opening Remarks — Pacific Jewel Ballroom

08:45am - 10:00am **Session 5:** Sustainability in Peptide Science — Pacific Jewell Ballroom
Session Chairs:
Fernando Albericio, University of Kwazulu-Natal
Victor Outlaw, University of Missouri

08:45am - 09:10am  **L24 Anna Maria Papini,** Professor, University of Florence *Advancing Sustainable Peptide Synthesis: Green Strategies for Scalable and Efficient Automatised Solid-Phase Manufacturing* — Pacific Jewel Ballroom

09:10am - 09:20am  **L25-YI5 Sikabwe Noki** Postdoctoral Research Fellow, University of KwaZulu-Natal | *"Base Labile" Safety Catch Linker. Synthesis and Applications in SPPS in a Green Context* — Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Tuesday, June 17, 2025, cont'd...

09:20am - 09:35am  **L26 Francesco Merlino,** Assistant Professor, University of Naples Federico II | *Ultrasound-Powered Sustainable Chemical Synthesis of Bioactive Peptides* — Pacific Jewel Ballroom

09:35am - 10:00am  **L27 Michael Kopach,** Associate Vice President, Eli Lilly | *Retatrutide: A Novel Triagonist for Metabolic Disorders and CMC Advancements* — Pacific Jewel Ballroom

10:00am - 10:25am Coffee with Exhibitors and Posters — Eventide

10:30am - 12:35pm **Session 6:** Peptide Therapeutics from Discovery to the Clinic — Pacific Jewel Ballroom

Session Chairs:
Yoshimasa Kawaguchi, Kyoto University
Emilia Oueis, Khalifa University

10:30am - 10:55am  **L28 Carrie Haskell-Luevano,** Professor, University of Minnesota | *Paradigms for the Discovery of Unknown Naturally Occurring GPCR Ligands in Chemical Neuroscience: Melanocortins and Opioids* — Pacific Jewel Ballroom

10:55am - 11:05am  **L29-YI6 Tiancheng Chen,** Graduate Student, University of Georgia | *C-tail Mimics of LRRK2 Downregulate Kinase Activity in Parkinson's Disease* — Pacific Jewel Ballroom

11:05am - 11:20am  **L30 Aphrodite Kapurniotu,** Professor, Technical University of Munich | *Designed Macrocyclic Peptides as Nanomolar Inhibitors of Self- and Cross-Sseeded Amyloid Self-Assembly of Alpha-Synuclein* — Pacific Jewel Ballroom

11:20am - 11:35am  **L31 Federica Tucci,** Principal Research Scientist, IRBM| *R2R01, a Potent Long-Acting RXFP1 Peptide Agonist as Clinical Candidate in Phase 2 Studies for Cardiovascular and Renal Diseases* — Pacific Jewel Ballroom

11:35am - 11:50am  **L32 Robin Polt,** Professor, The University of Arizona | *O-Linked Glycopeptides Derived from Endogenous Neurotransmitters as a Source of Brain-Penetrant CNS Drugs for the Treatment of Stroke, mTBI and Neurodegeneration* — Pacific Jewel Ballroom

11:50am - 12:35pm  **L33 Pascale Guiton,** Assistant Professor, Santa Clara University | *Inclusive Mentoring* — Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Tuesday, June 17, 2025, cont'd...

12:45pm - 02:15pm Schram Young Scientists' Lunch — Silver Pearl 3

08:00pm - 10:00pm **Pharma Panel:** — Pacific Jewel Ballroom
Designing and Developing Orally Available Peptides
Moderated by Kaustav Biswas, Sr. Principal Scientist/Sr. Director, Merck

Panelists:
Ashok Bhandari, Executive Vice President, Chief Drug Discovery and Preclinical Development Officer, Protagonist Therapeutics
Gaurav Bhardwaj, Assistant Professor, University of Washington, Co-Founder Vilya
Callie Bryan, Scientific Director and Peptide Lead, Global Discovery Chemistry, Johnson and Johnson Innovative Medicines
Ryuji Hayashi, Group Head, Discovery Chemistry, Chugai Pharmaceutical Co., Ltd.
Josh Schwochert, Co-founder and CSO, Unnatural Products

Wednesday, June 18, 2025

07:30am - 08:25am Breakfast with Exhibitors — Eventide

08:25am - 08:30am Opening Remarks — Pacific Jewel Ballroom

08:30am - 10:00am **Session 7:** Protein Modification, Structural Insights, and Disease State — Pacific Jewell Ballroom

Session Chairs:
Stephen Miller, Genentech
Leah Witus, Macalester College

08:30am - 08:55am  **L34 William DeGrado,** Professor, Univ. of California, San Francisco | — Pacific Jewel Ballroom
De novo Protein Design of Functional Proteins

08:55am - 09:10am  **L35 Jutta Eichler,** Professor of Medicinal Chemistry, University of Erlangen-Nurnberg| — Pacific Jewel Ballroom
Bind & Bite: Covalently Stabilized Heterodimeric Coiled-Coil Peptides for the Site-Selective Chemical Modification of Proteins

09:10am - 09:20am  **L36-YI7 Thu Nguyen,** Ph.D. Candidate in Chemistry, New York University | — Pacific Jewel Ballroom
A Proteomimetic Strategy for Modulation of Intrinsically Disordered Protein MYC

09:20am - 09:30am  **L37-YI8 Kira Podolsky,** Postdoctoral Fellow, Massachusetts Institute of Technology | — Pacific Jewel Ballroom
A Peptide can Replace an Essential Enzyme in Yeast

SCHEDULE OF EVENTS - DETAIL

Wednesday, June 18, 2025, cont'd...

09:30am - 09:45am

L38 - Richard Bayliss, Professor of Molecular Medicine, University of Leeds|
Hydrocarbon-Stapled Peptidomimetic of TACC3 Disrupts CHC Interaction and Delays Mitotic Progression

Pacific Jewel Ballroom

09:45am - 10:00am

L39 - Norman Metanis, Professor, The Hebrew University of Jerusalem |
Chemical Protein Synthesis as a Tool for Therapeutic Applications: The development of Mirror-Image RaPID Technology & New Insulin Analogues

Pacific Jewel Ballroom

10:00am - 10:25am Coffee with Exhibitors and Posters

Eventide

10:30am - 12:00pm **Session 8:** Peptides in Oncology

Session Chairs:
Danielle Guarracino, The College of New Jersey
Jody Mason, University of Bath

Pacific Jewell Ballroom

10:30am - 10:55am

L40 - Tom Muir, Van Zandt Williams Jr. Class of 1965 Professor of Chemistry, Princeton University |
Discovery and Characterization of Oncohistones

Pacific Jewel Ballroom

10:55am - 11:05am

L41 Andy Wilson, Professor, University of Birmingham |
Understanding and Manipulating Protein-Protein Interactions of Aurora-A Kinase Employing Intrinsically Disordered Regions

Pacific Jewel Ballroom

11:10am - 11:25am

L42 - Jacky C.G. Ngo, Associate Professor, The Chinese University of Hong Kong |
Design of a Covalent Protein-Protein Interaction Inhibitor of SRPKs to Suppress Angiogenesis and Invasion of Cancer Cells

Pacific Jewel Ballroom

11:25am - 11:50am

L43 - Sivaneswari Genapathy, Associate Director, Bicycle Therapeutics|
Bicycle® Molecules as an Innovative and Unique Therapeutic Class

Pacific Jewel Ballroom

11:50am - 11:55am **Poster Flash Talk**
A High-diversity mRNA-platform for the Discovery of Multicyclic Peptides

Peter Timmerman
Head of Peptide Science, BioSynth

Pacific Jewel Ballroom

11:55am - 12:00pm **Poster Flash Talk**
Comprehensive Peptide Analysis with High-Resolution NMR Spectroscopy: NMR as a Powerful Tool

Nadine Peez
Business Innovation Manager, Spectral Service AG

Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Wednesday, June 18, 2025, cont'd...

12:00pm - 02:05pm	Lunch with Exhibitors and Poster Session 2 - **poster session starts 12:30pm**	Eventide
12:30pm - 02:05pm	Poster Judging and Vendor Interactions	Eventide
02:10pm - 03:45pm	**Session 9:** Computational Empowerment in Peptide Science **Session Chairs:** Parisa Hosseinzadeh, University of Oregon Ved Srivastava, Perpetual Medicines	Pacific Jewel Ballroom
02:10pm - 02:35pm	 **L44 - Laurie Parker,** Professor, University of Minnesota | *Using Experimental Approaches and AI-Based Structural Modeling to Understand Kinase-Substrate Interactions*	Pacific Jewel Ballroom
02:35pm - 02:50pm	 **L45 Ingrid Dijkgraaf,** Professor, Maastricht University | *Computational Epitope Mapping and Chemical Engineering of Tick Proteins for Vaccine Development*	Pacific Jewel Ballroom
02:50pm - 03:15pm	 **L46 - Yu-Shan Lin**, Professor - Department Chair, Tufts University | *Design of a Covalent Protein-Protein Interaction Inhibitor of SRPKs to Suppress Angiogenesis and Invasion of Cancer Cells*	Pacific Jewel Ballroom
03:15pm - 03:30pm	 **L47 - Ewa Lis,** Chief Executive Officer, Koliber Biosciences | *AI-Driven Peptide Discovery: Unlocking the Potential of Peptide Arrays for Therapeutic Development*	Pacific Jewel Ballroom
03:30pm - 03:45pm	 **L48 - Gaurav Bhardwaj,** Assistant Professor, University of Washington | *Generative Deep Learning for Accurate de novo Design of Macrocyclic Peptides*	Pacific Jewel Ballroom
03:45pm - 04:15pm	Afternoon Break with Exhibitors	Pacific Jewel Ballroom
04:20pm - 06:15pm	**Session 10:** Exploration of Selectivity and Methods for Targeting Disease **Session Chairs:** Elizabeth Stone, Fairfield University Christina Schroeder, Genentech	Pacific Jewel Ballroom
04:20pm - 04:45pm	 **L49 - Lila Gierasch,** Distinguished Professor, University of Massachusetts | *How Hsp70 Molecular Chaperones Bind Substrates with Selective Promiscuity*	Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Wednesday, June 18, 2025, cont'd...

04:45pm - 05:00pm

L50 - Mark Lipton, Associate Professor, Purdue University |
Eradicating HIV-1 Latency Through the Development of Dual Inhibitors of HIV-1 Protease and Histone Deacetylase 3

Pacific Jewel Ballroom

05:00pm - 05:15pm

L51 - Ana Salome Vega, Assistant Professor, Gulbenkian Institute for Molecular Medicine |
Unveiling the Mode of Aaction of SARS-CoV-2 Putative Fusion Peptides and their Exploitation as Antiviral Targets

Pacific Jewel Ballroom

05:15pm - 05:30pm

L52 - Gosuke Hayashi, Associate Professor, Nagoya University |
Mirror-Image Monobody Targeting MCP-1 Generated via mRNA Display and Peptide Ligation

Pacific Jewel Ballroom

05:30pm - 06:15pm **Merrifield Lecture**

Award Introduction: Stephen Kent - University of Chicago

Pacific Jewel Ballroom

L53 - AW Philip E. Dawson, Professor, Scripps Research Center |
Building Proteins from Scratch

Pacific Jewel Ballroom

08:00pm - 10:00pm **Pharma Panel:**
Sustainability in Peptide Manufacturing
Moderated by Michael Kopach, Associate VP, Eli Lilly

Pacific Jewel Ballroom

Panelists:
Fernando Albericio, Professor, University of KwaZulu-Natal
Matthew Bio, President and CEO, Snapdragon
Stefan Eissler, Vice President Peptide Manufacturing Upstream, Bachem
Susan Zultanski, Principal Scientist, Merck

SCHEDULE OF EVENTS - DETAIL

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Thursday, June 19, 2025

08:15am - 08:20am Opening Remarks — Pacific Jewel Ballroom

08:20am - 10:20am **Session 11:** Incretins 2025 - Transforming the Transformation — Pacific Jewel Ballroom

Session Chairs:
Emel Adaligil, Eli Lilly
Yvonne Angell, WuXi

Session Sponsor:

08:20am - 08:25am **Sponsor Flash Talk**
Synthesis and Purification Strategies for Complex and Challenging Peptides
Yvonne Angell
Regional Sales Manager, Canada; Field Application Scientist, Americas
Gyros Protein Technologies

08:25am - 08:50am  **L54 - Richard DiMarchi**, Distinguished Professor, Indiana University | *The Chemical Evolution of Peptides To Transform Human Health* — Pacific Jewel Ballroom

08:50am - 09:15am  **L55 - Timo Műller,** Professor, Ludwig Maximilians University München | *Novel Insights into Regulation of Energy and Glucose Metabolism by GIP and GIPR:GLP-1R Co-Agonists* — Pacific Jewel Ballroom

09:15am - 09:30am  **L56 - Poanna Tran ,** Research Scientist, Gubra ApS | *Machine Learning Guided Peptide Drug Discovery Speeds up Lead Identification as Demonstrated with Novel GLP-1R Agonists* — Pacific Jewel Ballroom

09:30am - 09:50am  **L57 - Chtristoffer Clemmensen,** Associate Professor, University of Copenhagen | *Peptide-Based Therapeutics for Treatment of Cardiometabolic Diseases* — Pacific Jewel Ballroom

09:50am - 10:20am **Makineni Lecture** — Pacific Jewel Ballroom
Award Introduction: Emel Adaligil, Eli Lilly

 **L58 - AW Krishna Kumar**, Robinson Professor of Chemistry, Tufts University | *Two Bites at the Apple: Design and Repair of Peptide Therapeutics* — Pacific Jewel Ballroom

10:20am - 10:40am Coffee Break — Pacific Jewel Ballroom

Thursday, June 19, 2025, cont'd...

10:40am - 12:30pm **Session 12:** Disease-Focused Peptide Discovery — Pacific Jewel Ballroom

Session Chairs:
Christian Becker, University of Vienna
Alan Cameron, University of Auckland

10:40am - 11:05am  **L59 - James Nowick**, Distinguished Professor, University of California, Irvine | *Potent Peptide Antibiotics Against Pernicious Pathogens* — Pacific Jewel Ballroom

11:05am - 11:20am  **L60 - Juan Del Valle,** W.K. Warren Family Professor, University of Notre Dame | *δ-Arch Macrocycles as Functional Tau Proteomimetics* — Pacific Jewel Ballroom

11:20am - 11:45am  **L61 - Maja Köhn,** Professor, University of Bonn | *Targeting Phosphatases with Peptides and Phosphomimetics* — Pacific Jewel Ballroom

11:45am - 12:00pm  **L62 - Huong Kratochvil,** Assistant Professor, University of North Carolina | *Going with the Flow: Proton-Selective Transport through de novo Designed Peptide Bundles* — Pacific Jewel Ballroom

12:00pm - 12:30pm **Goodman Lecture** — Pacific Jewel Ballroom
Award Introduction: Eileen Kennedy

 **L58 - AW William Lubell**, Professor, Université de Montréal | *Semicarbazides as Amino Amide Surrogates in Peptide Mimicry for Treating Unmet Medical Conditions* — Pacific Jewel Ballroom

12:30pm - 01:40pm Lunch on your own

12:30pm - 01:40pm Young Investigators Appreciation Luncheon — Eventide Gardens

12:30pm - 01:30pm du Vigneaud Luncheon - by invitation only — Seaglass

Sponsored by BACHEM

01:45pm - 03:30pm **Session 13:** Implications and Applications of PTMs — Pacific Jewel Ballroom

Session Chairs:
Yuki Goto, Kyoto University
Katherine Albanese, Waker Forest University

01:45pm - 02:10pm  **L64 Marcey Waters**, Glen H. Elder, Jr. Distinguished Professor, University of North Carolina | *Addressing the Challenge of Selective Inhibition of Histone Trimethyllysine Reader Proteins through Electrostatic Pocket Mapping* — Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Thursday, June 19, 2025, cont'd...

02:10pm - 02:25pm  **L65 - Mark Distefano,** Distinguished Professor, University of California, Irvine | *Potent Peptide Antibiotics Against Pernicious Pathogens* — Pacific Jewel Ballroom

02:25pm - 02:35pm  **L66 - Muhammad Jbara,** Assistant Professor, Tel Aviv University | *Cracking the Code of PTMs on Transcription Factor-DNA Interactions Using Synthetically Modified Proteins* — Pacific Jewel Ballroom

02:35pm - 02:45pm  **L67 - Chi Ting,** Assistant Professor, Brandeis University | *Chemical Synthesis of Ribosomally Synthesized and Post-translationally Modified Peptides, RiPPs* — Pacific Jewel Ballroom

02:45pm - 03:00pm  **L68 - Y. George Zheng,** Panoz Professor of Pharmacy, University of Georgia | *Identification of Lysine Acetoacetylation as a Novel Protein Post-Translational Modification* — Pacific Jewel Ballroom

03:00pm - 03:30pm **du Vigneaud Lecture** — Pacific Jewel Ballroom
Award Introduction: Philip Dawson

 **L69 - AW Ashraf Brik,** Technion-Israel Institute of Technology | *Ubiquitin Signaling: Chemistry, Biology and Drug Discovery* — Pacific Jewel Ballroom

04:00pm - 06:00pm **Session 14:** New Frontiers in Peptide Science — Pacific Jewel Ballroom
Session Chairs:
Jane Aldrich, University of Florida
Andrew Jamieson, University of Glasgow

04:00pm - 04:25pm  **L70 - Hiroaki Suga,** Professor, University of Tokyo | *De Novo Discovery of Pseudo-Natural Products* — Pacific Jewel Ballroom

04:25pm - 04:50pm  **L71 - Christian Heinis,** Associate Professor, EPFL | *Membrane-Permeable Cyclic Peptides Against Intracellular Targets and for Oral Delivery* — Pacific Jewel Ballroom

04:50pm - 05:05pm  **L72 - Louise Walport,** Group Leader Imperial College London and The Francis Crick Institute | *A Cyclic Peptide Toolkit to Modulate Protein Citrullination* — Pacific Jewel Ballroom

SCHEDULE OF EVENTS - DETAIL

Thursday, June 19, 2025, cont'd...

05:05pm - 05:50pm **Closing Plenary Keynote Lecture** — Pacific Jewel Ballroom

Closing Remark Introduction: Jean Chmielewski

L01 - Scott Miller, Sterling Professor of Chemistry, Yale University | — Pacific Jewel Ballroom

Peptide-Based Catalysis Version 2.0

05:50pm - 06:00pm **Closing Remarks** — Pacific Jewel Ballroom

07:00pm - 10:00pm **Closing Banquet** — Eventide

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American Peptide Society Awards

Chair of the Awards Committee, 2023-2025
Professor Sam Gellman,
University of Wisconsin - Madison

The 2024-2025 Awards are as follows:

- 2024 APS Early Career Lectureship
- 2025 R. Bruce Merrifield Award
- 2025 Vincent du Vigneaud Awards, 2 awards
- 2025 Murray Goodman Scientific Excellence & Mentorship Award
- 2025 Rao Makineni Lectureship
- 2025 APS Early Career Lectureship

Selection Process:
A Chair for the Awards Committee is elected by the Council from among Active Members. The Awards Committee Chair serves for at least two years and thereafter remains a Member of the Awards Committee for a period of at least two years. For each award of the Society, the Awards Committee Chair:

1| Publishes a call for submission of nominations for the Award

2| Chooses a current Award Nominating Committee of three or more Active Members of the Society, who shall actively solicit the submission of nominations of worthy individuals for the Award

3| Chooses an Active or Honorary Member to Chair the specific Award Selection Committee.

The Chair of each Award Selection Committee chooses at least six confidential members of this committee from the members of the Society. The members of the committee remain anonymous from each other, from the Awards Committee Chair, from all members of the Executive Committee, Council, and membership at large.

After the selection process is complete, the Chair of each Award Selection Committee submits to the Chair of the Awards Committee a confidential written report of the specific details of the procedures used to select the recipient(s) of the Award. The names of the selection committee members are not given in this report. The Chair of the Award Committee shares the results with the APS President who sends a letter to the award recipient.

R. BRUCE MERRIFIELD AWARD

Robert Bruce Merrifield, July 15, 1921 — May 14, 2006, was an American biochemist who won the Nobel Prize in Chemistry in 1984 for the invention of solid phase peptide synthesis. His wife Elizabeth, Libby, a biologist by training, joined the Merrifield laboratory at Rockefeller University where she worked for over 23 years. The Merrifield Award recognizes the lifetime achievement of a peptide scientist, whose work exemplifies the highest level of scientific creativity.

AWARD WINNERS

Philip E. Dawson	Scripps Research Institue	2025
Sam Gellman	University of Wisconsin - Madison	2023
Padmanabhan Balaram	Indian Institute of Science, Bangalore, India	2021
Lila Gierasch	University of Massachusetts at Amherst	2019
Charles Deber	University of Toronto, Hospital for Sick Children	2017
Robert Hodges	University of Colorado at Denver	2017
Horst Kessler	TU München Institute for Advanced Study	2015
James P. Tam	Nanyang Technological University, Singapore	2013
Richard DiMarchi	Indiana University	2011
Stephen B.H. Kent	University of Chicago	2009
Isabella Karle	Naval Research Laboratory	2007
Richard A. Houghten	Torrey Pines Institute for Molecular Studies	2005
William F. DeGrado	University of Pennsylvania	2005
Garland R. Marshall	Washington University Medical School, St. Louis	2001
Daniel H. Rich	University of Wisconsin, Madison	1999
Shumpei Sakakibara	Peptide Institute, Inc.	1997
John M. Stewart	University of Colorado at Denver	1995
Victor J. Hruby	University of Arizona	1993
Daniel F. Veber	Merck, Sharp & Dohme	1991
Murray Goodman	University of California at San Diego	1989
Cho Hao Li	University of California at San Francisco	1987
Robert Schwyzer	Swiss Federal Institute of Technology	1985
Ralph F. Hirschmann	Merck, Sharp & Dohme	1983
Klaus Hofmann	University of Pittsburgh - School of Medicine	1981
Bruce Merrifield	The Rockefeller University	1979
Miklos Bodanszky	Case Western Reserve University	1977

2025 R. BRUCE MERRIFIELD AWARD

Philip E. Dawson

Scripps Research Institute - La Jolla, California

Professor Philip E. Dawson is a renowned chemist specializing in synthetic protein chemistry and peptide science. He currently serves as a Professor in the Department of Chemistry at Scripps Research in La Jolla, California, and chairs the Graduate School Advisory Committee of the Skaggs Graduate School of Chemical and Biological Sciences.

Dr. Dawson earned his A.B. in Chemistry from Washington University in St. Louis in 1992. He completed his Ph.D. in Macromolecular and Cellular Structure and Chemistry at Scripps Research in 1996. Following postdoctoral research at the California Institute of Technology, he returned to Scripps Research as an Assistant Professor in 1997, advancing to full Professor by 2016. He served as Dean of Graduate and Postdoctoral Studies from 2017 to 2024 and currently chairs the Graduate School Advisory Committee.

Professor Dawson's research centers on developing chemoselective methods for protein synthesis and bioconjugation. His lab has pioneered techniques such as native chemical ligation, enabling the assembly of complex polypeptides and the incorporation of non-natural amino acids into proteins. These methodologies have broad applications in understanding protein function and developing novel therapeutics.

Dr. Dawson has authored over 190 peer-reviewed publications, contributing significantly to the fields of peptide chemistry and chemical biology. His work has facilitated advancements in protein engineering, drug development, and the study of protein-protein interactions.

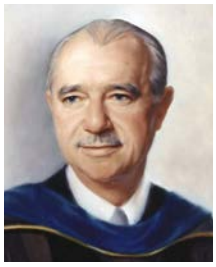
Professor Dawson's contributions have been recognized with numerous awards, including Arthur C. Cope Scholar Award, American Chemical Society, 2024, Cathay Award, Chinese Peptide Society, 2024, Akabori Memorial Award, Japanese Peptide Society, 2020, Leonidas Zervas Award, European Peptide Society, 2014, Max Bergmann Gold Medal, 2011, Vincent du Vigneaud Award, American Peptide Society, 2010, and a Alfred P. Sloan Research Fellowship, 1999–2001.

Beyond his research, Dr. Dawson has served as President of the American Peptide Society, 2013–2018, and has been involved in organizing major scientific conferences, including the American Peptide Symposium and the Gordon Research Conference on the Chemistry and Biology of Peptides. He also contributes to the scientific community through editorial roles and participation in advisory boards.

Through his innovative research and leadership, Professor Philip E. Dawson continues to advance the field of peptide science, making significant contributions to our understanding of protein chemistry and its applications in medicine.

VINCENT DU VIGNEAUD AWARD

Vincent du Vigneaud, May 18, 1901 – December 11, 1978, was an American biochemist. He was recipient of the 1955 Nobel Prize in Chemistry "for his work on biochemically important sulphur compounds, especially for the first synthesis of a polypeptide hormone," a reference to his work on the peptide hormone oxytocin. The Vincent du Vigneaud Awards recognize outstanding achievement in peptide research at mid-career. The du Vigneaud Awards are sponsored by Bachem, and are awarded to two deserving recipients at the biennial American Peptide Symposia.



AWARD WINNERS

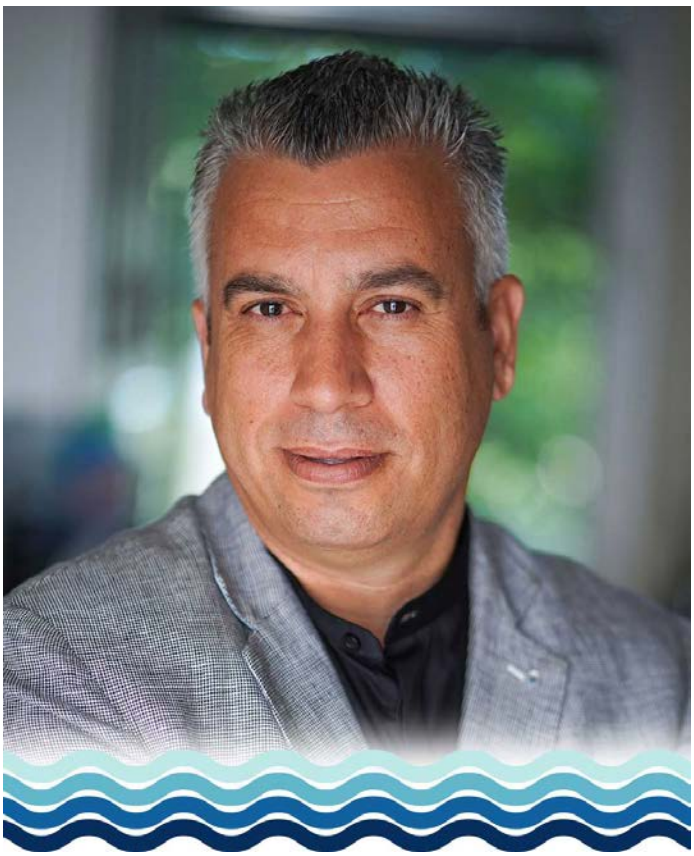
2025	Ashraf Brik	Technion-Israel Institute of Technology
2025	Dek Woolfson	University of Bristol
2023	Helma Wennemers	ETH Zurich
2023	Marcey Waters	University of North Carolina at Chapel Hill
2021	Alanna Schepartz	University of California, Berkeley
2021	Joel Schneider	Center for Cancer Research, National Cancer Institute
2019	Annette Beck-Sickinger	Leipzig University
2019	Hiroaki Suga	The University of Tokyo
2017	Ronald Raines	University of Wisconsin at Madison
2017	Wilfred van der Donk	University of Illinois at Urbana-Champaign
2015	Jean Chmielewski	Purdue University
2015	David Craik	University of Queensland
2013	Michael Chorev	Harvard Medical Scool
2013	Kit Sang Lam	University of California, Davis School of Medicine
2011	Fernando Albericio	University of Barcelona
2011	Morten Meldal	Carlsberg Laboratories, Copenhagen
2010	Philip Dawson	Scripps Research
2010	Reza Ghadiri	Scripps Research
2008	Jeffery W. Kelly	Scripps Research
2008	Thomas W. Muir	Rockefeller University
2006	Samuel H. Gellman	University of Wisconsin
2006	Barbara Imperiali	Massachusetts Institute of Technology
2004	Stephen B. H. Kent	University of Chicago
2004	Dieter Seebach	Swiss Federal Institute of Technology at Zurich
2002	Robert Hodges	University of Colorado, School of Medicine
2002	Horst Kessler	Technical University of München
2000	Charles M. Deber	University of Toronto
2000	Richard A. Houghten	Torrey Pines Institute for Molecular Studies
1998	Peter W. Schiller	Clinical Research Institute of Montreal
1998	James A. Wells	Genentech, Inc.
1996	Arthur M. Felix	Hoffmann-La Roche, Inc.
1996	Richard G. Hiskey	University of North Carolina at Chapel Hill
1994	George Barany	University of Minnesota at Minneapolis
1994	Garland R. Marshall	Washington University Medical School at St. Louis
1992	Isabella Lugoski Karle	Naval Research Laboratory
1992	Wylie W. Vale	The Salk Institute for Biological Studies
1990	Daniel H. Rich	University of Wisconsin at Madison
1990	Jean E. Rivier	The Salk Institute for Biological Studies
1988	William F. DeGrado	DuPont Central Research
1988	Tomi K. Sawyer	The Upjohn Company
1986	Roger M. Freidinger	Merck, Sharp & Dohme
1986	Michael Rosenblatt	Massachusetts General Hospital
1986	James P. Tam	The Rockefeller University
1984	Betty Sue Eipper	The Johns Hopkins University
1984	Lila M. Gierash	University of Delaware
1984	Richard E. Mains	The Johns Hopkins University

VINCENT DU VIGNEAUD AWARD

Ashraf Brik

Technion-Israel Institute of Technology

Professor Ashraf Brik is a distinguished chemist and chemical biologist, currently serving as a full professor at the Schulich Faculty of Chemistry at the Technion–Israel Institute of Technology. Renowned for his pioneering work in protein synthesis and post-translational modifications, he will be honored with the 2025 Vincent du Vigneaud Award at the American Peptide Society's 2025 Symposium, recognizing his significant contributions to peptide research at the mid-career level.



Born in Abu Snan, Israel, Professor Brik earned his bachelor's degree in chemistry from Ben-Gurion University of the Negev. He pursued his master's and doctoral studies at the Technion, with his Ph.D. research conducted jointly with The Scripps Research Institute under the mentorship of Professors Chi-Huey Wong and Ehud Keinan. His doctoral thesis focused on the design and synthesis of novel catalytic proteins based on polypeptide scaffolds. Following postdoctoral research, he joined the Department of Chemistry at Ben-Gurion University in 2007, where he was promoted to full professor in 2012. In 2014, he returned to the Technion, where he currently holds the Jordan and Irene Tark Chair in Chemistry.

Professor Brik's research centers on developing innovative chemical and semisynthetic methods for the site-specific synthesis of post-translationally modified proteins, such as ubiquitinated and phosphorylated proteins. His work has provided critical insights into the molecular mechanisms of ubiquitin signaling, which plays a pivotal role in various biological processes and diseases. By creating homogeneous protein conjugates, his lab facilitates structural, biochemical, and functional analyses that were previously challenging due to the heterogeneity of naturally modified proteins.

Among his significant achievements, Professor Brik's group was the first to chemically synthesize K48-linked tetraubiquitin chains, enabling detailed studies of ubiquitin-mediated processes. His research has led to the development of novel modulators targeting deubiquitinases, DUBs, enzymes implicated in cancer and neurodegenerative diseases. With over 200 publications and more than 11,000 citations, his work has been featured in prestigious journals, including Science, Nature Chemistry, and the Journal of the American Chemical Society.

Professor Brik is actively involved in the scientific community, serving on editorial boards of journals such as Cell Chemical Biology and ChemBioChem. He has received numerous accolades, including the Rappaport Prize for Excellence in Biomedical Research in 2024. His research is supported by various international funding agencies, including the European Research Council, ERC, the Israel Science Foundation, ISF, and the US-Israel Binational Science Foundation, BSF. Through his innovative research and leadership, Professor Brik continues to advance the field of chemical biology, contributing to the development of novel therapeutic strategies.

VINCENT DU VIGNEAUD AWARD

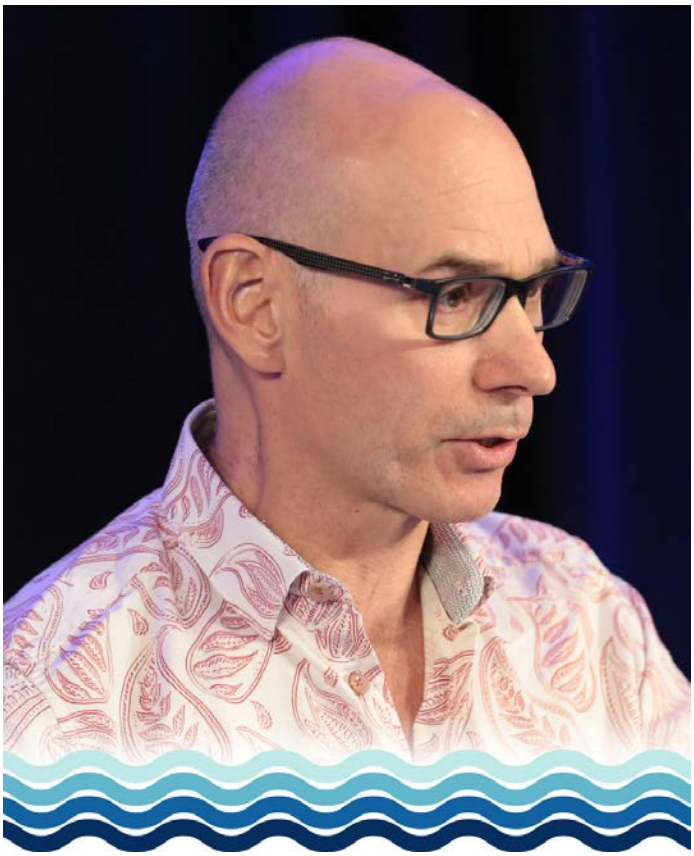
Dek Woolfson

University of Bristol, United Kingdom

Award Sponsored By:

BACHEM

Professor Dek N. Woolfson holds a joint appointment in the Schools of Chemistry and Biochemistry at the University of Bristol. He is also the Director of the Bristol BioDesign Institute and a founding director of the Max Planck-Bristol Centre for Minimal Biology. His research focuses on the rational design of novel protein structures and assemblies, bridging the disciplines of chemistry, biology, and synthetic biology.



Professor Woolfson earned his B.A. in Chemistry from the University of Oxford in 1987, conducting undergraduate research with Professor Christopher M. Dobson. He completed his Ph.D. in Chemistry and Biochemistry at the University of Cambridge in 1991 under the supervision of Professor D.H. Williams and Dr. P.A. Evans. Following postdoctoral research at University College London and the University of California, Berkeley, he held academic positions at the University of Sussex before joining the University of Bristol in 2005.

Professor Woolfson's laboratory specializes in de novo protein design, aiming to create entirely new protein structures not found in nature. His team employs computational and experimental approaches to design coiled-coil proteins, peptide assemblies, and synthetic biomaterials. These designed proteins have applications in understanding fundamental aspects of protein folding, as well as in developing novel biomaterials and therapeutic agents.

Professor Woolfson has made significant contributions to the field of protein design, including the development of computational tools for modeling coiled-coil structures and the creation of self-assembling peptide-based materials. His work has been recognized with several awards, such as the Medimmune Protein and Peptide Science Award, 2011, the Royal Society Wolfson Research Merit Award, 2014, the Interdisciplinary Prize of the Royal Society of Chemistry, 2016, and the Humboldt Research Award, 2020.

Beyond his research, Professor Woolfson is actively involved in mentoring and teaching, supervising numerous graduate students and postdoctoral researchers. He is also the founder of Rosa Biotech, a spin-out company focused on developing biosensing technologies based on synthetic protein scaffolds.

Through his innovative research and leadership in synthetic biology, Professor Dek N. Woolfson continues to advance the frontiers of protein design and its applications in science and medicine.

THE MURRAY GOODMAN SCIENTIFIC EXCELLENCE AND MENTORSHIP AWARD

The **Murray Goodman** Scientific Excellence & Mentorship Award was established in 2007 by an endowment from Zelda Goodman. The Goodman Award recognizes an individual who has demonstrated career-long research excellence in the field of peptide science. In addition, the selected individual should have been responsible for significant mentorship and training of students, post-doctoral fellows, and/or other co-workers. This award is presented at the biennial American Peptide Symposia.

AWARD WINNERS

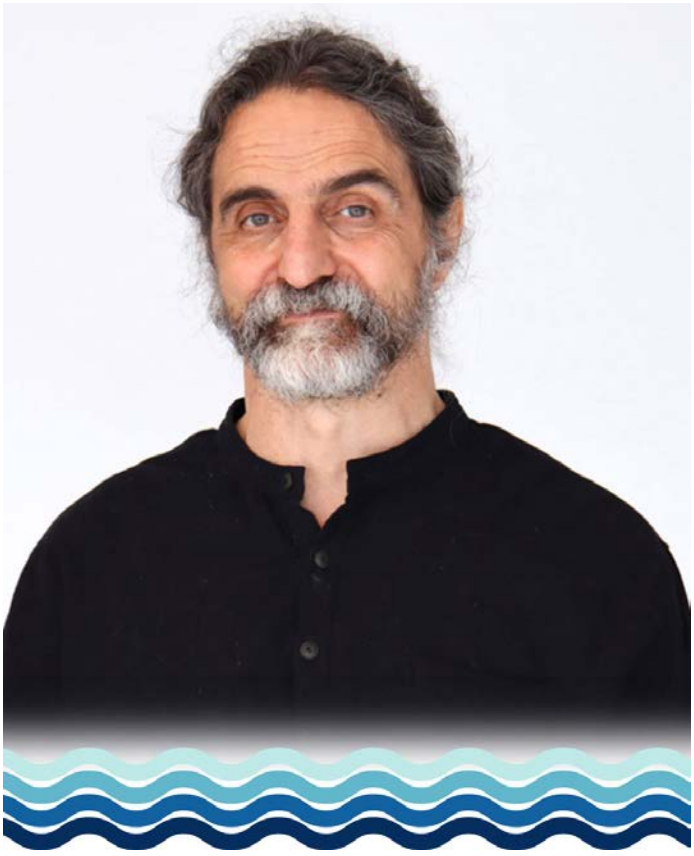
2025	William Lubell	Université du Montréal
2023	James P. Tam	Nanyang Technological University
2021	Jean Chmielewski	Purdue University
2019	Fernando Albericio	University of KwaZulu-Natal
2017	Paul Alewood	The University of Queensland
2015	George Barany	University of Minnesota at Minneapolis
2013	Robert S. Hodges	University of Colorado at Denver
2011	Victor J. Hruby	University of Arizona
2009	Charles M. Deber	University of Toronto, Hospital for Sick Children

THE MURRAY GOODMAN SCIENTIFIC EXCELLENCE AND MENTORSHIP AWARD

William Lubell

Université du Montréal

Professor William D. Lubell is a Full Professor in the Department of Chemistry at the Université de Montréal. He is renowned for his contributions to medicinal chemistry and peptide science, particularly through the development of innovative peptides and peptidomimetics that target and modulate biologically relevant receptors for drug discovery. In recognition of his career-long research excellence and mentorship, Professor Lubell has been selected to receive the 2025 Murray Goodman Scientific Excellence and Mentorship Award from the American Peptide Society.



Dr. Lubell earned his A.B. in Chemistry from Columbia University in 1984 and his Ph.D. in Chemistry from the University of California, Berkeley, in 1989, under the mentorship of Professor H. Rapoport. His doctoral research focused on the synthesis of alpha-amino carbonyl compounds, including the cyclosporine MeBmt amino acid. He conducted postdoctoral research with Professor R. Noyori at Nagoya University in Japan, further honing his expertise in organic synthesis.

Professor Lubell's research encompasses the solution-phase and solid-phase synthesis of heterocycles, amino acids, peptides, and peptide mimics. His work aims to develop new methodologies for effectively synthesizing these novel structures for drug discovery and to explore their use in understanding protein folding, molecular recognition, and bioorganic catalysis. His laboratory applies the strength of organic synthesis to explore the chemical biology of peptides through conformational restriction, innovating methods for amino acid, polyamide, and heterocycle synthesis.

Dr. Lubell has authored over 250 scientific publications and has been instrumental in developing intellectual property used to launch start-up companies. His collaborative efforts with biochemists, pharmacologists, and physicians have been critical in drug discovery teams developing interventions to treat Alzheimer's disease, premature birth, and age-related macular degeneration. He is also the originator of "Molecules of Life," an initiative aimed at enhancing public understanding of science through experiential education techniques.

Through his innovative research, mentorship, and commitment to science education, Professor William D. Lubell continues to make significant contributions to the fields of chemistry and biomedical science.

THE RAO MAKINENI LECTURESHIP

The **Rao Makineni** Lectureship was established in 2003 by an endowment by PolyPeptide Laboratories and Murray and Zelda Goodman. The Lectureship honors Rao Makineni, a long-time supporter of peptide science, peptide scientists, and the American Peptide Society. The Makineni Lectureship recognizes an individual who has made a recent contribution of unusual merit to research in the field of peptide science. The award is intended to recognize original and singular discoveries rather than cumulative or lifetime contributions. The award is presented at the biennial American Peptide Symposia.

AWARD WINNERS

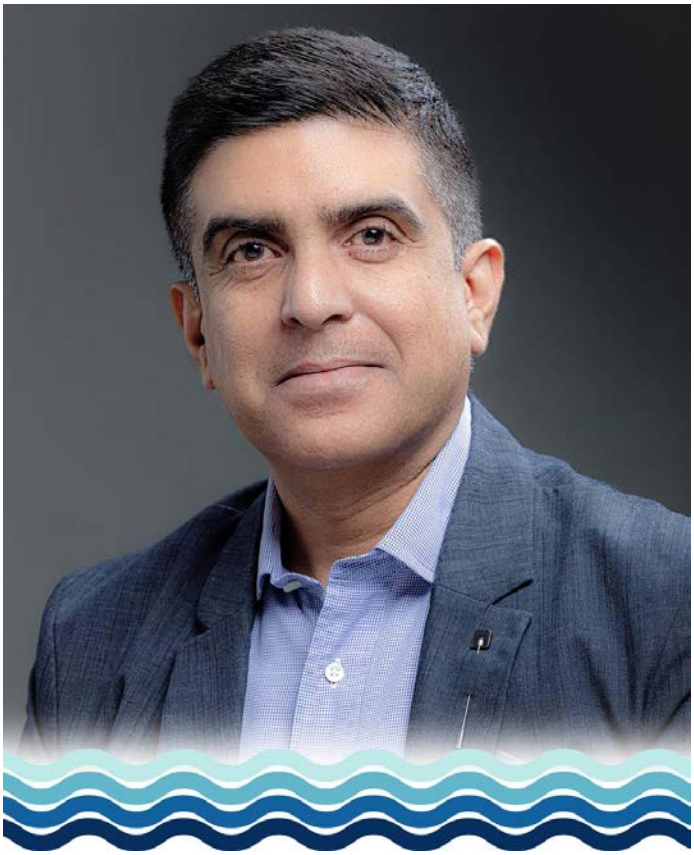
2025	Krishna Kumar	Tufts University
2023	César de la Fuente	University of Pennsylvania
2021	Bradley L. Pentelute	Massachusetts Institute of Technology
2019	Xuechen Li	The University of Hong Kong
2017	Thomas Kodadek	Scripps Research Institute
2015	Paramjit Arora	New York University
2013	Samuel H. Gellman	University of Wisconsin at Madison
2011	Jeffery W. Kelly	Scripps Research
2009	William DeGrado	University of Pennsylvania
2007	Ronald T. Raines	University of Wisconsin at Madison
2005	Robin E. Offord	Centre Medical Universitaire, Switzerland
2003	James P. Tam	Vanderbilt University

THE RAO MAKINENI LECTURESHIP

Krishna Kumar

Tufts University

Professor Krishna Kumar holds the Robinson Professorship in Chemistry at Tufts University, where he also serves as an Adjunct Professor of Biomedical Engineering and is a member of the Cancer Center at Tufts Medical Center. His interdisciplinary research bridges chemistry, biology, and medicine, focusing on the design of novel molecules to understand and manipulate biological processes.



Dr. Kumar earned his B.Sc. in Chemistry with honors from St. Stephen's College, Delhi, and his Ph.D. in Chemistry from Brown University in 1996, under the mentorship of Professor Matthew B. Zimmt. He conducted postdoctoral research at The Scripps Research Institute with Professor M. Reza Ghadiri before joining the faculty at Tufts University in 1998. He served as Chair of the Chemistry Department from 2006 to 2009 and again from 2012 to 2018.

Professor Kumar's research group operates at the interface of chemistry, biology, and medicine. Their work includes designing molecules to probe fundamental biological mechanisms and developing therapeutics. Key areas of interest encompass the origin of life, therapeutic molecule design for conditions such as Type 2 diabetes, cancer, non-alcoholic steatohepatitis, NASH, Alzheimer's and Parkinson's diseases, smoking cessation, and the development of novel antibiotics. The group also investigates the penetration of peptide molecules through biological barriers like the gut and the blood-brain barrier.

Dr. Kumar has pioneered the incorporation of fluorinated amino acids into proteins, enhancing their stability and therapeutic potential. His work has led to the development of fluorinated drug delivery systems and imaging agents, as well as methods for cell surface engineering and imaging of cancer cells with metastatic potential.

Beyond his research, Dr. Kumar is actively involved in mentoring students and contributing to the academic community through teaching and leadership roles. He has served as a Visiting Scientist at the Center for Cancer Research at MIT and has been recognized for his excellence in teaching and mentorship at Tufts University. He also serves as the secretary of the American Peptide Society.

Through his innovative research and dedication to education, Professor Krishna Kumar continues to make significant contributions to the fields of chemistry and biomedical science.

THE APS EARLY CAREER LECTURESHIP AWARD

Established in 2019 and sponsored by the **American Peptide Society**, the Early Career Lectureship recognizes outstanding early career investigators who have demonstrated innovative research in peptide science. Two recipients will be chosen biennially and each will deliver a talk at the Symposium in a session appropriate to their work. The APS will support the registration, lodging at the conference hotel and up to $1000 in travel expenses of the awardees.

AWARD WINNERS

2025	Nina Hartrampf	University of Zürich
2024	Elizabeth Parkinson	Purdue University
2023	Lara Malins	Australian National University
2022	Danny Chou	Stanford University
2021	Caroline Proulx	North Carolina State University
2020	Yftah Tal-Gan	University of Nevada, Reno
2019	Monika Raj	Auburn University
2019	Jevgenij Raskatov	University of California at Santa Cruz

THE APS EARLY CAREER LECTURESHIP AWARD FOR 2024

Elizabeth Parkinson

Purdue University

Assistant Professor Elizabeth I., "Betsy," Parkinson holds a joint appointment in the Department of Chemistry and the Department of Medicinal Chemistry and Molecular Pharmacology at Purdue University. Her research focuses on the discovery and development of novel bioactive natural products from cryptic bacterial biosynthetic gene clusters, aiming to address pressing challenges such as antibiotic resistance and cancer.

Dr. Parkinson earned her B.S. in Chemistry from Rhodes College in 2010, where she conducted undergraduate research at St. Jude Children's Research Hospital under Dr. Philip Potter, focusing on reducing the toxicity of the chemotherapeutic irinotecan. She completed her Ph.D. in Chemistry at the University of Illinois at Urbana-Champaign in 2015, working with Professor Paul Hergenrother on synthesizing derivatives of anticancer natural products and studying their mechanisms of action. Following her doctoral studies, she pursued postdoctoral research with Professor William Metcalf in Microbiology at UIUC, investigating the biosynthesis of phosphonate-containing natural products and developing metabologenomics approaches for natural product discovery.

Professor Parkinson's laboratory is dedicated to uncovering novel natural products from soil-dwelling bacteria, particularly Streptomyces species. Her team employs chemical synthesis, bioinformatics, and high-throughput screening to activate and study cryptic biosynthetic gene clusters. By exploring these uncharacterized pathways, they aim to discover new antibiotics and anticancer agents, as well as understand the unique chemistries performed by natural product biosynthetic enzymes.

Dr. Parkinson has significantly advanced the field of natural product chemistry through her work on the synthesis and characterization of bioactive compounds. Her research has led to the development of more soluble derivatives of anticancer and antibiotic natural products, enhancing their therapeutic potential. Additionally, her efforts in metabologenomics have provided new methodologies for the discovery of natural products from previously silent gene clusters.

Beyond her research, Professor Parkinson is actively involved in teaching and mentoring at Purdue University, where she has also contributed to initiatives supporting mental health awareness among graduate students. She is a member of the American Chemical Society, the Society for Industrial Microbiology and Biotechnology, and the American Society of Pharmacognosy. Her commitment to education and outreach reflects her dedication to fostering the next generation of scientists.

Through her innovative research and dedication to education, Assistant Professor Elizabeth I. Parkinson continues to make significant contributions to the fields of natural product chemistry and chemical biology.

THE APS EARLY CAREER LECTURESHIP AWARD FOR 2025

Nina Hartrampf

University of Zürich

Nina Hartrampf is an Assistant Professor in the Department of Chemistry at the University of Zürich, UZH, in Switzerland. She studied chemistry and biochemistry at Ludwig-Maximilians-Universität Munich, and obtained her Ph.D. in the field of natural product synthesis and chemical biology in the group of Dirk Trauner.

In 2018, she moved to the group of Brad Pentelute at the Massachusetts Institute of Technology, as a postdoctoral researcher, where she worked on the flow-based synthesis of peptides and proteins using an automated synthesis platform.

Nina relocated to Switzerland in 2020, to launch her independent career as an assistant tenure-track professor. Her lab specializes in the chemical synthesis of post-translationally modified peptides and proteins, including peptidic natural products such as lasso peptides and the oncogenic transcription factor MYC. Additionally, the lab develops innovative tools for flow-based peptide synthesis to enable, for example, the synthesis of "difficult sequences."

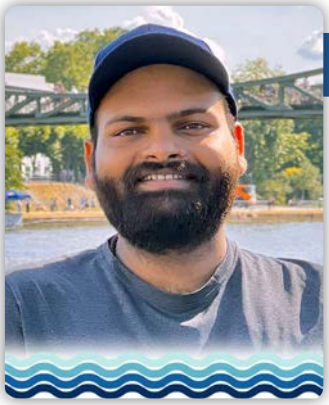
Her independent work has been recognized with several awards including the DECHEMA "Nachwuchswissenschaftler-Preis für Naturstoffforschung," 2025, the "ADUC Young Talent Award," 2025, from the GDCh, the "2024 Chemical Biology Lectureship," the RSC "CEM Emerging Investigator in Protein and Peptide Science Award," 2024, and the "Bachem Award for Peptide Science," 2021, from the Austrian Peptide Society.

The Hartrampf Group

DR. ELIZABETH SCHRAM YOUNG INVESTIGATOR ORAL PRESENTATION COMPETITION

At each symposium, the ESCOM Foundation sponsors the Dr. Elizabeth Schram Young Investigator, YI, Oral Presentation Competition. The selected YIs are graduate students or postdoctoral scholars whose research has been recognized as cutting edge or work of high interest to the greater peptide community. Consequently, they have been invited to give an oral presentation on their research at the 2025 American Peptide Symposium. Their presentations will be judged by academic and industry professionals and winners of the competition will be declared at the end of the Symposium.

2025 PARTICIPANTS



L19-YI4

Ashutosh Agrahari
Purdue University

L09-YI2

Caroline Almeida
Federal University of Rio de Janeiro and UC Merced

L29-YI6

Tiancheng Chen
University of Georgia

L36-YI7

Thu Nguyen
New York tUniversity

L25-YI5

Sikabwe Noki
University of KwaZulu-Natal

L37-YI8

Kira Podolsky
MIT

L04-YI1

Laura M. Poller
ETH Zürich

L14-YI3

Zhenquan Sun
University of Chicago

DR. ELIZABETH SCHRAM YOUNG INVESTIGATOR POSTER COMPETITION

At the biennial American Peptide Symposium, young investigators may elect to enter the Dr. Elizabeth Schram Young Investigator Poster Competition sponsored by the ESCOM Foundation. Young Investigators are defined as undergraduates, graduates and post docs. Cash prizes are awarded to the winners. These are this year's competitors.

2025 PARTICIPANTS - ALPHABETICAL

Mohaddeseh Abbasi	Iowa State University
Sota Adachi	Tokyo University of Agriculture and Technology
Sebin Abraham	Texas A&M University
A'Lester Allen	University of Illinois Chicago
Haley Anchukaitis	Tufts University
Isaac Angera	University of Notre Dame
Avraz Anwar	University of Notre Dame
Mónica Aróstica	Pontificia Universidad Católica de Valparaíso
Maxwell Austin	Stanford University
Noora Azadvari	University of Oregon
Meghna Bajaj	Cornell University
Anju Basnet	University of Nevada
Nomindari Bayaraa	Tufts University
Lena Beiersdörfer	ETH Zurich
Dror Ben Abba Amiel	The Hebrew University of Jerusalem
Diptomit Biswas	Pennsylvania State University
Silvia Bracci	University of Florence
Ferdinand Braun	Friedrich-Alexander-University Erlangen
Elizabeth Bredice	University of North Carolina Wilmington
Ida Boccino	University of Naples Federico II
Jackson Brunicardi	University of California, Irvine
Jenna Cain	Fordham University
Natalia Cano-Sampaio	University of Notre Dame
Yihui Cao	The University of Hong Kong
Karizza Catenza	University of Alberta
Saibal Chanda	Texas A&M University
Subir Chatterjee	Indian Institute of Science
Eleni Chatzilakou	Imperial College London
Brittney Chau	Boston College
Chia-Yuan Chen	University of Texas at Dallas
Pei Hsuan Chen	University of Kansas
Ashley Clemente	Fordham University
Gabriela Coy	Purdue University
Leander Crocker	Leibniz-Forschungsinstitut für Molekulare Pharmakologie
Achyut Dahal	National Cancer Institute
Abha Dangi	University of Notre Dame
Luca Danieli	The University of Tokyo
Juan Dantis	University of California, Irvine
Rachita Dash	University of Virginia

2025 PARTICIPANTS - ALPHABETICAL, cont'd...

Naysilla Dayanara	University of British Columbia
Stepan Denisov	University of Vienna
Magdalena DiGiorno	Fordham University
Taylor Dill	University of Georgia
Tristan Dinsmore	Tufts University
Alisha Doda	University of Nebraska-Lincoln
Gopal K. Dubey	Texas A&M University
Emma Elsdon	Fordham University
Kathryn Fincham	Monash University
Jonathan Franke	Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)
Mayuresh Gadgil	Vanderbilt University
Pragati Ganatra	University of Illinois Chicago
Stephanie Garcia	Brigham Young University
Adriana Gauna	Universidad de Chile
Anamika Ghosh	JIS Institute of Advanced Studies and Research Kolkata
Patrick Gonschorek	Max-Planck-Institute for Terrestrial Microbiology
Nicola Grasso	University of Naples Federico II
Louis-David Guay	Université Laval
Ingyu Han	Yonsei University
Mahdi Hasan	Technion-Israel Institute of Technology
Samantha Hatfield	Brigham Young University
Rahel Heeb	ETH
Jordi Hintzen	University of Pennsylvania
Andrew Hong	University of Rochester
Chris Howard	North Carolina State University
Ruiqi Huang	UC Davis
Ellie Hyde	University of East Anglia
Rida Ibrahim	Iowa State University
Seungyoon Jang	POSTECH
Tyler Jones	University of Utah
Albert Kakkis	Scripps Research Institute
Grace Kaul	IIT Kanpur
Shahrukh Khan	New York University
Ariel Kuhn	University of Wisconsin - Madison
Syuan-Ting Kuo	Texas A&M
Alexander J. Lander	University of Basel
Yen Jea Lee	Lawrence Berkeley National Laboratory
Minhee Lee	Columbia University
Simon Leukel	FAU Erlangen-Nuremberg
Wenchao Li	Stanford University
Yuhan Lin	University of Pittsburgh
Zichen Liu	University of Virginia
Heng Liu	University of South Florida
Matthew Lloyd	University of Utah
Sydney Mager	University of Massachusetts Amherst

2025 PARTICIPANTS - ALPHABETICAL, cont'd...

Diana A. Martinez-Baquero	Hunter College of CUNY
Isabel Mathiesen	Francis Crick Institute
Ciaran McGrory	University of Galway
Karlee McKinney	NC State University
Katherine Menendez	City University of New York
Esther Meron	Gubra
Marcus C. Mifflin	University of Utah
Yutaro Miura	Institute of Science Tokyo
Ivan Montes de Oca Estrada	University of A Coruña
Danielle Morgan	Princeton University
Dhanya Mahalakshmi Murali	National University of Singapore
Yuya Nakajima	Nagoya University
Genki Nakamura	Nagoya university
Siavash Shahbazi Nia	Purdue University
Karl Ocius	University of Virginia
Whitney S. Y. Ong	Merck & Co., Inc.
Amarachi Osuji	Tufts University
Daniel Ou	University of Toronto
Henry Pan	The University of California, San Francisco
Jake Pedigo	University of Utah
Chuanhao Peng	University of Alberta
Kerry-Anne Perkins	Philadelphia College of Osteopathic Medicine
Jordyn Pieper	College of Charleston
Andrew Powers	University Of Oregon
Viktor Prypoten	Monash Institute of Pharmaceutical Sciences
Nina Raasch	FAU Erlangen-Nürnberg
Nishant Raj	Indian Institute of Science, Bangalore
Shivani Sachdev	NIH
Chitra Pandiparambil Sadanandhan	Université de Montréal
Subhrodeep Saha	Indian Institute of Science
Jesus Sandres	University of Utah
Megan Sargent	Tufts University
Carly Schissel	UC Berkeley
Adeline Schmitt	ETH Zürich
Hassan Seyrani	University of Missouri
Lucrezia Sforzi	University of Florence
Dhriti Santosh Shenoy	University of Milan
Oscar Shepperson	University of Glasgow
Shingo Shinjo-Nagahara	Tokyo University of Agriculture and Technology
Kouki Shinohara	Institute of Science Tokyo
Diedra Shorty-Duffie	Eli Lilly
Izabela Siekierska	University of Warsaw
Maxwell Sigal	The University of Tokyo
Rupali Singh	New York University
Troy Smith	The University of Arizona

2025 PARTICIPANTS - ALPHABETICAL, cont'd...

Yuanming Song	University of California, Irvine
Marina Spenciner	Tufts University
Syrah Starnes	University of Notre Dame
Chase Stebbing	University of Nebraska - Lincoln
Madeline Swanson	University of North Carolina Wilmington
Oliver Swart	New York University
Raj Talukdar	Tufts University
Robert Tancer	Rutgers University
Weiyi Tang	Scripps Research
Lauren Tran	University of Wisconsin- Madison
Linh Tran	University of Minnesota, Twin Cities
An T. Trinh	University of Minnesota
Liam Tucker	University of East Anglia
Claiborne Tydings	Vanderbilt University
Sven Ullrich	University of Tokyo
Tayla Van Oers	University of Alberta
Saipriyaa Purushotham Vasan	The University of Queensland
Dhanya Karipal Padinjare Veedu	The Australian National University
Stephanie A. Vogt	University of Basel
Noelle Waltenburg	University of Nebraska-Lincoln
Lei Wang	Purdue University
Anjalee Wijewardane	University of Alberta
Jake Wilkinson	University of North Carolina at Chapel Hill
Yu Yu Win	Univerisity of South Florida
Kristiana Witte	North Carolina State University
Jacob Wolfe	University of Pittsburgh
Mulate Zerihun Workeneh	Bar-Ilan University
Madison Wright	University of Notre Dame
Hongkang Wu	The Florey, The University of Melbourne
Songyi Xue	University of Illinois Urbana-Champaign
Kenichi Yamamoto	Institute of Science Tokyo
Kejia Yan	University of Alberta
Amy Yang	New York University
Danial Yazdan	University of Alberta
Koki Yoshida	Konan University
Minghao Zhang	The Scripps Research Institute
Belinda Zhang	University of Sydney
Jing Zhao	University of Copenhagen
Xue Zhao	University of South Florida
Jason Zhu	University of California, Irvine

2025 TRAVEL GRANT RECIPIENTS

Recipients - Alphabetical

Mohaddeseh Abbasi
Iowa State University

Isaac Angera
University of Notre Dame

Anju Basnet
University of Nevada Reno

Diptomit Biswas
Pennsylvania State University

Elizabeth Bredice
University of North Carolina

Brittney Chau
Boston College

Pei Hsuan Chen
University of Kansas

Tiancheng Chen
University of Georgia

Gabriela Coy
Purdue University

Noah Daniecki
University of Delaware

Sneha Dasgupta
Purdue University

Rachita Dash
University of Virginia

Naysilla Dayanara
University of British Columbia

Magdalena DiGiorno
Fordham University

Pragati Ganatra
University of Illinois Chicago

Louis-David Guay U
Université Laval

Mahdi Hasan
Technion Israel Institute of Technology

Rahel Heeb
ETH Zurich

Andrew Hong
University of Rochester

Ruiqi Huang
University of California Davis

Minhee Lee
Columbia University

Wenchao Li
Stanford University

Yuhan Lin
University of Pittsburgh

Isabel Mathiesen
Francis Crick Institute

Karlee McKinney
North Carolina State University

Sikabwe Noki
University of KwaZulu-Natal

Kira Podolsky
MIT

Chitra Pandiparambil Sadanandhan
Université de Montréal

Samuel Scherer
University of Utah

Hassan Seyrani
University of Missouri

Maxwell Sigal
The University of Tokyo

Troy Smith
University of Arizona

Chase Stebbing
University of Nebraska - Lincoln

Linh Tran
University of Minnesota

Claiborne Tydings
Vanderbilt University

Lei Wang
Purdue University

Mulate Zerihun Workeneh
Bar-Ilan University

Kejia Yan
University of Alberta

Amy Yang
New York University

Xue Zhao
University of South Florida

Inaugural Session

We're excited to introduce the inaugural Young Investigator, YI, Symposium, debuting at the 2025 American Peptide Symposium! This unique half-day event is dedicated to showcasing innovative research by graduate students and postdoctoral scholars in peptide science. It provides emerging scientists a platform to share insights, connect with peers, and engage with established experts, advancing both research and professional growth. Join us for an inspiring session that's set to become a staple for the next generation of leaders in peptide science.

Symposium Events

Dynamic 10-minute student presentations will take place in two consecutive 75-minute blocks. To conclude the event, the YI Symposium will host a Career Workshop, where students will have the chance to connect one-on-one with experienced professionals.

Everyone is Welcome

The YI Symposium kicks off on Sunday, June 15, 2025, at 1:00 PM. Both students and professionals are invited to attend the presentations to provide the students with exposure to potential future mentors and employers.

SAC Young Investigators Symposium Co-Chairs & Working Group

Emel Adaligil
Eli Lilly

Michael Bertucci
Lafayette College

James Checco
University of Nebraska

Whitney Ong - Merck
Gopal Dubey - Texas A&M
A'Lester Wiggins-Allen - University of Illinois Chicago
Karl Ocius - University of Virginia

Hassan Seyrani - University of Missouri
Karlee McKinney - NC State
Brittney Chau - Boston College
Nishant Raj - Indian Institute of Science

Symposium Schedule - Sunday, June 15th

TIME	EVENT
01:00pm - 02:15pm	Presentations in Concurrent Rooms - Session 1
02:15pm - 02:30pm	Coffee Break
02:30pm - 03:45pm	Presentations in Concurrent Rooms - Session 2
03:45pm - 04:45pm	Career Workshop

Symposium Speakers - Alphabetical

FIRST NAME	LAST NAME	TALK TITLE
Haley	Anchukaitis	Activation of Class B GPCRs via Cell-Penetrable Membrane-Anchored Peptides
Nima	Adhami	An Organometallic Strategy for Peptide Macrocyclization
Tayla	Van Oers	Designing FRET Substrates and Peptidomimetics for the Development of Broad-Spectrum Inhibitors of Coronaviral Main Proteases
Ruiqi	Huang	A Two-component Two-step (TCTS) Transformable Nanoplatform against Cancers Based on Bioorthogonal Click Chemistry
Dror	Benabba	Expeditious synthesis of peptides with multiple glycosylations
Belinda	Zhang	Discovery of Selective Cyclic D-Sulfopeptide Ligands of the Chemokine CCL22 via Mirror-Image mRNA Display with Genetic Reprogramming
Linh	Tran	Calcium Mobilization Assay as a High Throughput Screening Assay for Mixture-based Positional Peptide Libraries on Opioid Receptors
Patrick	Gonschorek	Split inteins for generating combinatorial nonribosomal peptide libraries
Avraz	Anwar	Total Synthesis of Marformycins A and D
Jacob	Wolfe	NMR-Guided Design of Heterogeneous-Backbone Mini-Metalloprotein Catalysts
Syuan-Ting	Kuo	A Dual Assembly Pathway of Liraglutide Uncovered by Advanced Integrative Native Mass Spectrometry
Anju	Basnet	Developing Competence Stimulating Peptide (CSP)-Based Quorum Sensing Modulators in Streptococcus sanguinis
Leander	Crocker	Mapping peptide interactomes using an organic energy transfer photocatalyst
Isabel	Mathiesen	RaPID Discovery of Covalent Cyclic Peptide Binders
Kejia	Yan	Late-stage reshaping of phage-displayed libraries to macrocyclic landscapes for ligand discovery
Stepan	Denisov	Photoprobes for target identification of Trefoil Factor Family Peptide 2 (TFF2) and its therapeutic use in inflammatory bowel disease
Maxwell Jacob	Sigal	De novo discovery of α-helical peptides containing α,α-disubstituted α-amino acids
Stephanie	Vogt	Breaking the boundaries of a clinically approved cyclic peptide: Development of rodent-active and long-duration compstatin analogs
Anita	Chen	A Bi-facial Tris-benzamide Scaffold Mimicking Two Helical Surfaces
Naysilla	Dayanara	Chemoselective, regioselective, and positionally selective fluorogenic stapling of unprotected peptides for cellular uptake and direct cell imaging
Kathryn	Fincham	Enhancing the Cytosolic Delivery of Bioactive Peptides
Subir	Chatterjee	Design of Miniproteins: From Ultrastable α-Helical Hairpins to α-Helical Macrocycles
Esteban	Suarez Picado	Electrochemical Deprotection of Amino Groups: Advancing Sustainable Peptide Synthesis
Esther	Meron	Combining AlphaFold and mRNA display technologies - de novo discovery and maturation of functional CD206 peptide binders
Jordi	Hintzen	A Versatile Fluorescent System for Studying Diverse Post-Translational Modifications Using Simple Peptide Substrates
Lucrezia	Sforzi	Environmentally sustainable synthesis of cosmetic peptides
Minhee	Lee	Substrate-derived peptides for selective covalent inhibition of protein tyrosine kinases
Madison	Wright	Impact of backbone-oxidized proline surrogates on the stability of complex polyproline II folds
Jake	Wilkinson	Evaluation of Benzoyllysine Isostere Interactions and Removal by Deacetylase Enzyme Hst2
Pei-Hsuan	Chen	DhaSulf: A Latent Handle for Introducing Dehydroamino Acids into Peptides & Applications for Anti-Aggregation Therapies.
Marcelo	Munoz	Photoactive Peptide Injectable Materials for Restoring Thinning Corneas

Symposium Speakers - Alphabetical, cont'd...

Benjamin	Tombling	Engineering LRRC15-binding Disulfide-Constrained Peptides for Enhanced Tumor Imaging
Zichen	Liu	Systematic Assessment of Backbone N-methylation on Peptide Permeation Across the Mycomembrane in Live Mycobacteria
Hongkang	Wu	Developing Simplified Insulin-like Peptide 5 (INSL5) Analogues as Colon Motility Regulators
Juan	Dantis	Development of Immunoproteasome Substrate Labeling Assays (iSLAy)
Heng	Liu	α/Sulfonyl-ɣ-AApeptide Foldamers Mitigate Alzheimer's Disease Pathology by Stabilizing Transient Helical Domains in Aβ
Sven	Ullrich	Non-symmetric cysteine stapling in native peptides and proteins
Ferdinand	Braun	Peptide inhibitors of the human cytomegalovirus core nuclear egress complex
Alisha	Doda	Progress towards evaluating the functional selectivity of endogenous D-amino acid-containing neuropeptides
Marcy	Mitchell	Chemical Synthesis of Uropathogenic E. coli Adhesion Proteins as Mirror-Image Drug Targets of Urinary Tract Infections
Diptomit	Biswas	Retro-Inversion Imparts Antimycobacterial Specificity to Host Defense Peptides
Diana Alexandra	Martinez Baquero	Targeting Transient Receptor Potential Channels with Teretoxins for the Treatment of Liver Cancer

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LECTURE 01

Applications of Cysteine Lipidation "CLipPA" Technology : Drugs Vaccines and Biomaterials

Margaret Brimble
Distinguished Professor, University of Auckland

Opening Plenary Keynote Lecture

Our research group focuses on the synthesis of bioactive natural products and peptides as potential therapeutic agents. This lecture will highlight the development of peptidomimetics for the treatment of neurogenetic diseases and the synthesis of antimicrobial peptides to combat infectious disease. We also demonstrate the use of the thiol-ene reaction as an expedient method to chemoselectively lipidate a cysteine residue within a polypeptide.

Application of this patented CLipPA, **C**ys **Lip**idation on a **P**eptide or **A**mino acid, technology to the synthesis of novel lipopeptides as self-adjuvanting cancer vaccines, antimicrobial peptides, peptide hormones and peptidebased biomaterials will be illustrated. Our use of the thiol-ene reaction to expand the repertoire of methods to effect peptide stapling and bioconjugation will also be presented.

LECTURE 02

Development, Validation, and Implementation of Intestinal Cell Models to Guide Oral Peptide Formulation Design

John Gleeson
Senior Scientist, Merck

Oral delivery is the most patient-preferred route of drug delivery, especially for chronic disease treatment. Predicting the clinical performance of BCS Class III and IV drugs including oral peptides is difficult due to their low intestinal permeability and limitations of current in vitro and in silico models.

The Caco-2 Transwell model is the gold standard for permeability assessment, as per FDA and ICH guidance; however, it has poor predictability for low permeability compounds and permeation enhancer efficacy that are often required in oral peptide formulations. To improve the predictability of these models, we took a number of approaches: **1|** utilizing a microfluidic Chip platform instead of Transwell, **2|** adapting the cellular source from Caco-2 to biopsy-derived organoids, and **3|** improving the buffers and matrices overlaid on cells to mimic physiological conditions.

We validated the clinical predictability of a Caco-2 Chip model using 19 small molecule drugs, $r^2 = 0.71$, compared to conventional Transwells, $r^2 = 0.76$, and assessed permeation enhancer efficacy, sodium caprate and sucrose monolaurate. The Chip model showed more modest enhancement, 2-fold and 8-fold, versus Transwells, 10-fold and 150-fold, in line with preclinical and clinical observations.

Biopsy-derived organoids are an emerging tool to better recapitulate the intestine, as they are said to retain their regional specificity. However, in ileum and colon matched samples, we observed only one donor of three with expected regional size-based permeability (ileum $_{Papp}$ > colon $_{Papp}$) to macromolecules, and equivalent permeability (ileum $_{Papp}$ = colon $_{Papp}$) to BCS I and II drugs in both Transwells and Chips, platform

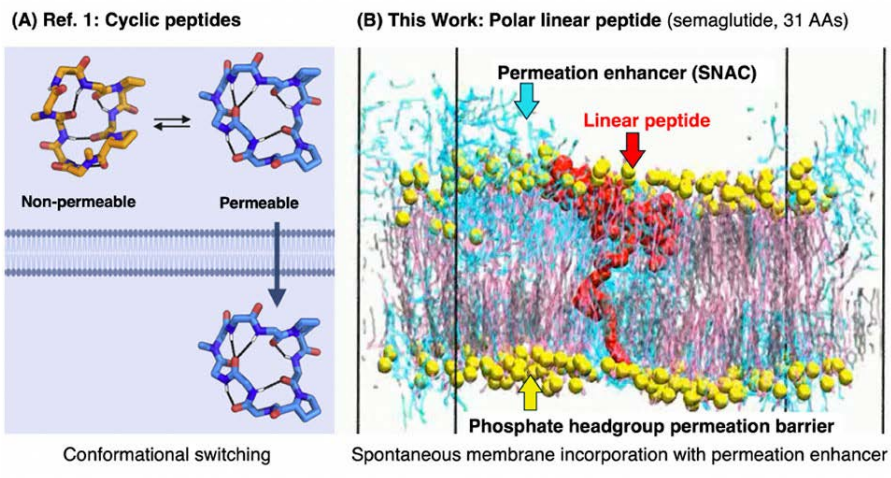
independent. Our approach has enabled us to triage formulations and peptides using cell-based platforms prior to preclinical species to improve outcomes and success toward the clinic.

LECTURE 03

The Molecular Basis of Oral Peptide Delivery with Permeation Enhancers

Severin Schneebeli
Associate Professor, Purdue University

GLP-1 agonists, such as semaglutide, the active ingredient in Ozempic, are commonly used peptide drugs for treating type 2 diabetes and obesity, with their pharmacokinetic properties—particularly absorption—being crucial in peptide drug research and development. It is projected that more than 9% of Americans will be taking GLP-1 agonists by 2030, and oral formulations of these peptide drugs play a vital role in enhancing patient compliance, convenience, and reducing healthcare costs.



However, while the membrane permeation mechanisms of many cyclic peptides are relatively well understood1, there remains limited knowledge about how a large, polar peptide like semaglutide in an oral formulation, for instance, with a permeation enhancer, can cross the intestinal barrier. This fundamental gap in understanding has hindered the rational design of improved oral peptide drugs and formulations.

To shed light on this essential mechanism and ultimately improve oral peptide drugs, this presentation will discuss recent advances from the Schneebeli lab, focusing on high-throughput peptide selection and mechanistic insights into how linear, polar peptide drugs like semaglutide can permeate membranes, aided by permeation enhancers, which are key components of oral peptide formulations like Rybelsus—the oral version of Ozempic. Our new permeation mechanism for peptides is supported by computational and experimental results, in particular accurate constant pH molecular dynamics simulations, which demonstrate for the first time how semaglutide can spontaneously embed itself into a membrane, thereby validating a long-hypothesized oral absorption mechanism.

[1] Baker, D. et al. *Cell* **2022**, 185, 3520–3532.
[2] Colston, K. J.; Faivre, K. T.; Schneebeli, S. T. *ChemRxiv* **2025**; DOI: 10.26434/chemrxiv-2025-n24f8

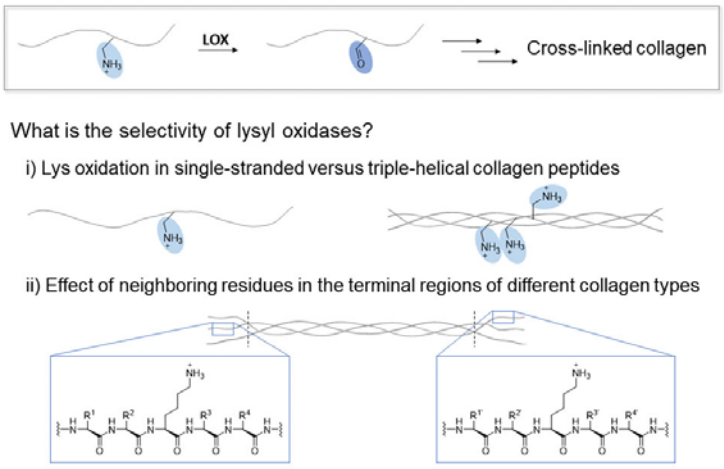
LECTURE 04

The Molecular Basis of Oral Peptide Delivery with Permeation Enhancers

Laura M. Poller
Graduate Student, ETH, Zűrich

Young Investigator Lecture 1

The remodelling and maturation of collagen, the dominant structural protein in mammals, is crucial for the integrity of organs and wound healing.[1,2] A key process is the cross-linking of collagen strands triggered by the post-translational oxidative deamination of lysine to allysine residues through lysyl oxidases, LOXs.



Excessive LOX activity is, however, implicated in fibrotic and malignant diseases which are associated with more than one third of deaths woldwide.[1–3] Understanding the role of LOXs in specific tissues could facilitate addressing these diseases. Surprisingly little is, however, known about the selectivity of LOXs.

In this study, we deciphered structure and sequence effects on aldehyde formation by LOXL2. This LOX isoform is of particular interest as it is over-expressed in many cancers.[4] Using fluorogenic enzyme activity assays, we investigated proline-rich peptides, comprising a lysine residue, that mimic either an intact or disrupted triple-helical structure, as found in connective tissue disorders.[5]

Our findings revealed the importance of the lysine-containing peptide sequence with a remarkable impact of charged residues on LOXL2 activity. We also show that LOXL2 is most active on single-stranded proline-rich peptides, as present in pathologically disturbed collagen triple helices.[6]

[1] **a|** Vallet, S. D.; Ricard-Blum, S. *Essays Biochem*. **2019**, 63, 349-364. **b|** Shoulders, M. D.; Raines, R. T. *Annu. Rev. Biochem*. **2009**, 78, 929-958.

[2] **a|** Aronoff, M. R.; Hiebert, P.; Hentzen, N. B.; Werner, S.; Wennemers, H. *Nat. Chem. Biol.* **2021**, 17, 865-871. **b|** Hiebert, P.; Antoniazzi, G.; Aronoff, M.; Werner, S.; Wennemers, H. *Matrix Biol.* **2024**, 128, 11-20.

[3] Henderson, N. C.; Rieder, F.; Wynn, T. A. *Nature* **2020**, 587, 555-566.

[4] **a|** Perryman, L; Erler, J. T. Future Oncol. 2014, 10, 1709-1717. **b|** Trackman, P. C. *Expert Opin. Ther. Targets* **2016**, 20, 935-945.

[5] Bateman, J. F.; Shoulders, M. D.; Lamandé, S. R. *Connective Tissue Res.* **2022**, 63, 210–227.

[6] Poller, L. M.; Wennemers, H. Manuscript in submission.

LECTURE 05

Systematic Determination of the Impact of Structural Edits on Accumulation into Mycobacteria

Marco Pires
Professor, and Director of Graduate Studies, University of Virginia

Most antibiotics currently used in the treatment of tuberculosis, such as isoniazid, rifampicin, ethambutol, and pyrazinamide, are small, hydrophobic molecules that were developed several decades ago. These molecular features of efficacious TB antibiotics have been purported to be a direct consequence of the difficult-to-cross mycomembrane barrier. The overuse of these therapeutics, compounded by suboptimal patient adherence due to the prolonged treatment regimens, has led to the emergence of multi-drug resistant, MDR, and total drug-resistant, XDR, strains of Mtb.

There are notable exceptions, for example rifampicin, to the size constraint purported to operate in TB antibiotics. Peptidic molecules that are well beyond Lipinski's Rule of Five, bRo5, have attracted interest in drug design because of their ability to bind targets with greater specificity and affinity. Broadly, peptide drugs have drawn considerable interest across disease areas including oncology and metabolic disorders. A noteworthy antibacterial example is the development of an entirely new class of macrocyclic peptide antibiotics, Zosurabalpin developed by Roche, that specifically target the lipopolysaccharide transporter in carbapenem-resistant *Acinetobacter baumannii*. Peptidic candidates are also emerging on the mycobacterial side. This growth in interest is highlighted by the recent discovery of evybactin and cyclomarin A, which was also the basis of the promising series of BacPROTACs.

Deciphering the principles that govern molecular accumulation in mycobacteria is central for achieving high antimycobacterial efficacy. A comprehensive understanding of how structure drives accumulation will enable the identification of high-potential drug candidates and the strategic redesign of existing chemical scaffolds to enhance their accumulation within mycobacterial cells. A prior challenge in the field was the general lack of tools to readily measure the arrival of molecules past the mycomembrane, which we addressed with the development of the Peptidoglycan Accessibility Click-Mediated Assessment, PAC-MAN, assay for live-cell analysis.

Herein, we systematically evaluated two structural alterations that could potentially enhance molecular accumulation past the mycomembrane: backbone N-methylation and macrocyclization. Through a comprehensive series of peptides, we demonstrated that specific structural features significantly influence accumulation levels in mycobacteria. This discovery lays the groundwork for a set of prescriptive modifications that can be employed in developing more effective antibiotics targeting Mycobacteria. By applying these strategies to a poorly permeable antibiotic, we observed that, in certain cases, these structural modifications can substantially enhance antibiotic activity against Mycobacteria.

LECTURE 06

Chemical Biology Approaches for Measuring Intracellular Drug Delivery

Joshua Kritzer
Professor, Tufts University

Large-molecule therapeutics including peptides, oligonucleotides, and proteins make up a large and growing portion of the drug development pipeline. One of the greatest barriers to developing these drugs is cell penetration. Most enter the cell through a complex pathway involving endocytosis followed by endosomal escape. This process is so poorly understood and difficult to study that it is challenging simply to measure how much compound has actually accessed the cytosol at any given point.

The Kritzer Lab has developed new tools for making these and related measurements. The Chloroalkane Penetration Assay, CAPA, is a versatile assay that measures cell penetration using cellularly expressed HaloTag protein and a small chloroalkane tag on the molecule-of-interest. CAPA has been used by the Kritzer group to measure cell penetration for many classes of peptide and oligonucleotide therapeutics, to measure penetration to different subcellular compartments, and to measure relative penetration in different cell types. CAPA has also been adopted by academic and industrial groups all over the world to investigate cell penetration.

The Kritzer group has also used molecular evolution to produce new HaloTag variants which work optimally with a fluorogenic benzothiadiazole dye. The resulting "BenzoTag" system allows for turn-on, no-wash cell labeling in seconds. BenzoTag is currently being applied to produce a "turn-on" version of CAPA for continued investigation of drug delivery and mechanisms of endosomal escape.

LECTURE 07

Chemical Biology Approaches for Measuring Intracellular Drug Delivery

Michelle Arkin
Professor, University of California at San Francisco

Molecular glues – compounds that induce or stabilize protein-protein interactions, PPI, – are fundamentally changing the way drug hunters think about targeting previously undruggable targets. Natural and synthetic molecular glues can induce non-native, neomorphic, interactions or further stabilize native complexes; they can also lead to pathway activation, inhibition, or even degradation of one of the target proteins. Most molecular glues have been discovered serendipitously. To capitalize on this new mechanism of action, the field requires new methods for prospective discovery of molecular glues for a particular PPI.

Our team has developed cell-active molecular glues for several proteins, including the kinase CRAF and transcription factors estrogen receptor, ER, and yes-associated protein, YAP, that bind to the phosphoprotein-chaperone 14-3-3. Our systematic approach uses disulfide tethering and other reversible covalent libraries to screen for cooperative binding with phosphopeptides derived from the intrinsically disordered regions of 14-3-3 client proteins. These peptide-protein complexes serve as reliable models for wildtype

complexes and for rare diseases where the PPI has been weakened through mutations. Through screening and structure-guided optimization, we are developing the rules-of-thumb for designing molecular glues.

LECTURE 08

Mitochondrial Targeting α-Helical Amphipathic Peptides As Drug Candidates for Sarcopenia

Jaehoon Yu
CAMP Therapeutics

Mitochondrial dysfunction is linked to degenerative diseases, resulting from cardiolipin, CL-induced disruption of cristae structure in the inner mitochondrial membrane, IMM; therefore, preventing CL remodeling offer effective strategies to maintain mitochondrial function.

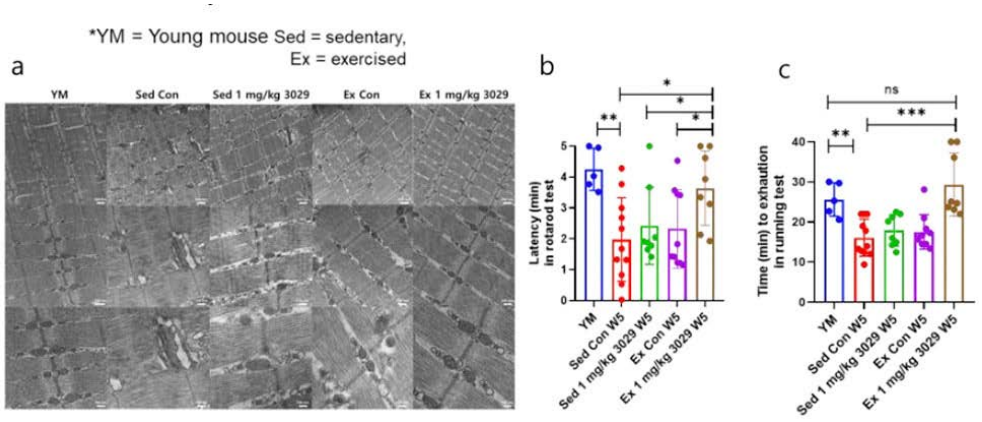


Figure **a|** Mitochondrial morphology of old and 3029-treated mice samples; **b|** Latancy of rotaroad test of old and 3029-treated mice; **c|** Treadmill running test of old and 3029-treated mice.

To identify reactive oxygen species, ROS-blocking agents against mitochondrial dysfunction, a library of cyclohexylamine-containing cell-penetrating α-helical amphipathic "bundle" peptides were screened. Among these, CMP3029 is selectively bound to abnormal mitochondria, preserving the cristae structure impaired by mitochondria-damaging agents. With a stronger affinity for CL compared with other lipid components, CMP3029 exhibited high selectivity. Consequently, it protected cristae, reduced ROS production, and enhanced ATP generation.

Using old mouse models of sarcopenia, the exercise-peptide dual-modality treatment group showed dramatic improvement of exercise capacity, overwhelming that of young mice control, especially in time exhaustion test using treadmill. The results highlight its potential as a therapeutic agent for sarcopenia. Overall, CMP3029 represents a promising agent for mitigating mitochondrial dysfunction and associated diseases.

LECTURE 09

Transcriptional Regulation of *Aspergillus nidulans* Biofilms from Environmental and Clinical Isolates Exposed to *Pisum sativum* Defensin 2

Caroline Almeida
Professor, Tufts University

Young Investigator Lecture 2

Fungal infections cause approximately 1.5 million deaths annually, with *Aspergillus spp*. being a major contributor. The ability of these fungi to form biofilms on catheters, combined with increasing resistance to available antifungal treatments, highlights the urgent need for new therapeutic strategies targeting biofilm formation. *Ps*d2 is a pea defensin with selective antifungal activity against planktonic cells of pathogenic fungi, interacting with lipid rafts enriched in glucosylceramide and ergosterol. Beyond its antifungal effect, *Ps*d2 inhibits Aspergillus nidulans GR5 biofilms, decreasing cell viability and extracellular matrix, and reducing hyphal length and diameter while inhibiting conidiophore formation.[1]

Thus, this study aimed to evaluate the gene expression profile of *A. nidulans* biofilms from GR5 and TNO2A3, environmental, and SP260548, clinical isolate, treated with *Ps*d2 using RNA-Seq. The strains exhibited distinct transcriptional responses to *Ps*d2 treatment, with 27 biological processes affected in GR5, 154 in TNO2A3, and 153 in SP260548. From these, only 9 processes were differentially regulated among the three strains, suggesting a strain-dependent effect. Notably, processes related to apical growth were downregulated in GR5 and TNO2A3 which could be associated with the inhibition of conidiophore development led by *Ps*d2 action, found in our previous studies.[1] Different processes related to transcription and secondary metabolism were upregulated in both TNO2A3 and SP260548 strains, while the metabolism of glucose, fatty acids, and lipids was downregulated. That suggests that *Ps*d2 can act at the transcriptional level of *A. nidulans* biofilms and also modify their metabolism.

[1] Corrêa-Almeida, Caroline, Luana P. Borba-Santos, Rodrigo Rollin-Pinheiro, Eliana Barreto-Bergter, Sonia Rozental, e Eleonora Kurtenbach. "Characterization of *Aspergillus Nidulans* Biofilm Formation and Structure and Their Inhibition by Pea Defensin *Psd2*". *Frontiers in Molecular Biosciences* 9, **2022**, 1–15. https://doi.org/10.3389/fmolb.2022.795255.

LECTURE 10

Peptides as Therapeutic Partners: From Drug Delivery to Molecular Sentinels

David Lawrence
Distinguished Professor, University of North Carolina at Chapel Hill

Peptides have played an important role in the acquisition of novel therapeutics, including as drugs themselves as well as targeting agents. We have employed peptides in the design of drug delivery vehicles as well as molecular sentinels.

Protein therapeutics are a powerful class of drugs known for their potency. However, the potential efficacy of these therapeutics is commonly offset by short circulatory half-lives and undesired action at otherwise healthy tissue. We've developed a protein delivery system that employs engineered red blood cells, RBCs, as carriers and light as the external trigger that promotes hemolysis and drug release. RBCs internally

loaded with therapeutic proteins are readily surface-modified with a dormant hemolytic peptide. The latter is activated via easily assigned wavelengths that extend into the optical window of tissue. We have demonstrated that photo-release transpires with spatiotemporal control and that the liberated proteins display the anticipated biological effects *in vitro* and *in vivo*, including clot lysis, with potential applications to stroke, pulmonary embolism, and other clotting disorders.

The protein kinase interaction network is essential for understanding disease development and therapeutic resistance. In this regard, several strategies have been described for assessing the active state of protein kinases in cells. However, these technologies do not actually measure catalytic activity nor are they applicable to single, or a relatively few, cells. We've developed and merged two technologies, protein kinase biosensors and thin-layer chromatography to address this issue. The peptide-based biosensors are cell-permeable, light-activatable, kinase-selective, wavelength-tunable, and readily imaged on TLC plates designed to accommodate lysate from single cells, picoliter TLC or pTLC. We will provide a brief discussion of potential applications.

LECTURE 11

Potential Roles of Solid-State Reactivity of Dipeptides in Prebiotic Polypeptide Synthesis

Jun Ohata
Assistant Professor, North Carolina State University

Prebiotic emergence of functional polypeptides, that would be essential for the formation of cellular systems, was likely to have been driven through a series of nonenzymatic organic chemistry reactions to combine building blocks, but the pathways for the abiotic synthesis of those biomacromolecules or even some of the building blocks, canonical amino acids, are not yet fully understood.

In other words, the longstanding question of "how the polypeptides were formed without the aid of enzymes before the first cell was formed?" remains unanswered to date. Many plausible chemical-evolution scenarios have been proposed in that context including wet-dry cycle and hydrothermal vent theories. This presentation demonstrates potential roles of dipeptides for origin of polypeptides under solid-state conditions.

LECTURE 12

Simple C1 Chemistry for Peptide Modification

Gong Chen
Professor, Nankai University

Selective biomolecule modification techniques are crucial to advancing modern biopharmaceuticals. As the field rapidly evolves, the demand for new crosslinking methods that offer greater precision, flexibility, and practicality has become paramount. Crosslinking biomolecules via their native endogenous functional groups, as opposed to post-introduced exogenous reaction handles, provides significant advantages in simplicity and accessibility.

However, practical methods of this kind have been largely limited to those based on thiol groups, which, despite their distinct reactivity and specificity, are naturally scarce. To expand this native modification toolkit, leveraging other common functional groups within biomolecules and bioactive compounds, particularly the ubiquitous amino groups, is vital. In this lecture, I will discuss our recent exploration of modifying native peptides and proteins through amino handles with simple organic reagents such as formaldehyde, glyoxylic acid, and CS_2 under mild conditions.

LECTURE 13

Advances in Late-Stage Peptide Modification Chemistry: Inspiration from Nature

Lara Malins
Professor, Australian National University

The development of new synthetic technologies for the precise modification of amino acids has been an enduring goal for peptide and protein chemists. Over the past decade, our growing understanding of the enormous array of structural and functional diversity incorporated into peptide natural products[1,2] has provided new inspiration for the development of versatile chemical tools to both mimic and expand upon Nature's repertoire of peptide modifications.

This talk will highlight our recent efforts toward the development of strategies for late-stage peptide modification for applications in the synthesis of valuable peptide natural products and therapeutic leads. Methods which specifically leverage the use of electrochemical[3] and photochemical[4] activation to accomplish the precise modification of peptide substrates will be discussed. Looking beyond the scope of the common proteinogenic amino acids, the modification of functional groups inspired by naturally occurring, albeit "non-canonical," structural motifs will also be examined as a broader tool for fine-tuning peptide structure and function.

[1] Montalbán-López M. et al. *Nat. Prod. Rep.* **2021**, 38, 130–239.
[2] Süssmuth R.D., Mainz A. *Angew. Chem. Int. Ed.* **2017**, 56, 3770–3821.
[3] Karipal Padinjare Veedu D., Connal L.A., Malins L.R. *Angew. Chem. Int. Ed.* **2023**, 62, e202215470.
[4] Hammond J.M., Gardiner M.G., Malins L.R. *Org. Lett.* **2023**, 25, 3157–3162.

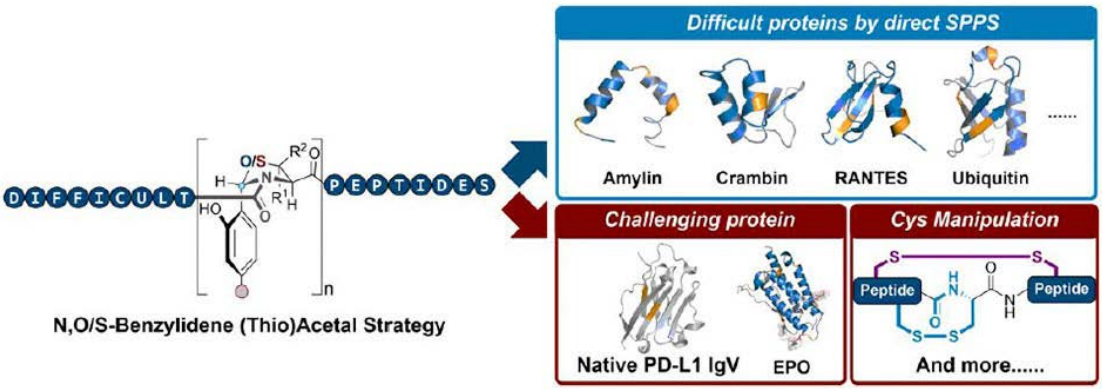
LECTURE 14

Chemical Synthesis of Difficult Peptide and Protein by *N,O/S*-Benzylidene (thio)Acetals, NBA, Strategy

Zhenquan Sun
Postdoctoral Scholar, University of Chicago

Young Investigator Lecture 3

The development of solid phase peptide synthesis, SPPS, and chemical ligation strategies enable the chemical synthesis of peptide or protein at atomic level precision.[1] However, difficult peptide/protein that are highly prone to aggregate, are still challenging to be synthesized due to their serious residue deletion in SPPS, poor reactivity in ligation and miserable separation in purification.



Herein we present a novel and general strategy based on the N,O/S-benzylidene (thio)acetal, NBA, to address above obstacles. During the peptide synthesis, the twisted NBA structure from the Ser/Thr ligation serves as an efficient aggregation disruptor. Also, both O- and S- NBA scaffolds can be readily introduced at different stages of synthesis, either by the SPPS coupling of easily prepared building blocks[2,5] or via late-stage chemical ligation[3]. Meanwhile, the unnatural Cys/Pen residue in the S-NBA can be converted into Ala/Val by desulfurization,[4] covering broader substrate scope of NBA insertion. The utility of NBA strategy was further demonstrated by successful syntheses of various challenging peptides and proteins, including amylin, crambin, RANTES, ubiquitin, histones, erythropoietin and human programmed death ligand 1 (PD-L1 IgV). To sum up, the NBA strategy provides a flexible and robust platform to synthesize difficult peptides and proteins in an efficient way.

[1] Sun, Z.; Liu, H.; Li, X. Chem, 2024, 10, 767–799.
[2] Wu, H.; Sun, Z.; Li, X. Angew. Chem. Int. Ed. 2023, e202310624.
[3] Wu, H.; Sun, Z.; Li, X. Angew. Chem. Int. Ed. 2024, e202403396.
[4] Sun, Z. et al. Chem, 2022, 8, 2543-2557.
[5] Sun, Z.; Wu, H.; Zhang, Y.; Li, X. in preparation.

LECTURE 15

Total Chemical Synthesis of a 97-Amino Acid Target-Binding Monobody via Cysteine-Free Conformationally-Assisted Ligation

Timothy Reichart
Elliott Assistant Professor of Chemistry, Hampden-Sydney College

Native chemical ligation enables stepwise assembly of proteins via unprotected ligation at cysteine.[1] Total chemical synthesis of proteins is limited by the need to strategically place cysteines for native chemical ligation with possible subsequent desulfurization. In special cases where different protein fragments self-assemble, the resulting high effective concentration allows direct aminolysis of the thioester, enabling ligation without a cysteine.[2]

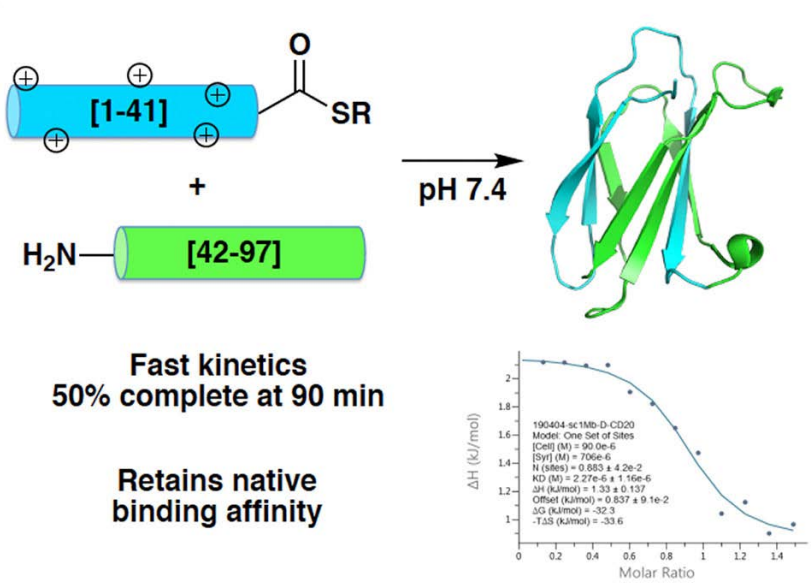


Figure 1. Conformationally-assisted ligation proof of concept using monobodies, synthetic binding proteins based on the tenth fibronectin III domain.

We have applied this principle to the synthesis of a 97-amino acid β-sheet monobody protein based on the tenth fibronectin type III domain. This required the combination of two separate strategies. First, the insoluble N-terminal portion was selectively modified by arginine substitution to enhance solubility.[3] Second, the disconnection was chosen to permit self-assembly, maximizing high local concentration, and permitting rapid, direct aminolysis. The resulting protein retained binding to its original target as measured by ITC.

[1] Dawson, P., Muir, T., Clark-Lewis, I., and Kent, S. *Science* **1994**, 266, 776–779.

[2] Beligere, G.S., and Dawson, P.E. *J. Am. Chem. Soc.* **1999**, 121, 6332–6333.

[3] Reference for arginine substitution not provided in abstract.

LECTURE 16

Utilizing Bioinformatics, Biocatalysis, and Synthetic Chemistry to Access Natural Product-Inspired Cyclic Peptides

Elizabeth, 'Betsy,' Parkinson
Assistant Professor, Purdue University

Early Career Lectureship Award

Cyclic peptide natural products, NPs, from the soil-dwelling *Actinomycetota* are a bountiful source of bioactive molecules, including medicines, agricultural products, and chemical tools to study biological processes. Additionally, the biosynthetic enzymes that produce them perform unique chemistries and are excellent starting points for biocatalysts.

Unfortunately, many biosynthetic gene clusters, BGCs, clusters of genes that encode for the enzymes that produce NPs) are cryptic. Accessing cyclic peptide NPs from these cryptic BGCs is quite challenging, thus slowing the discovery of novel bioactive cyclic peptides that can serve as leads for medicines and agricultural products.

Herein, we are using bioinformatics predictions followed by direct chemical synthesis to access NP-inspired cyclic peptides from cryptic BGCs. These peptides have been screened for a variety of activities, leading to novel antibiotic and antiamoebic leads. Additionally, we are discovering and utilizing the biosynthetic enzymes to access otherwise challenging-to-access cyclic peptides.

Overall, these approaches enable us to access cyclic peptides that are otherwise inaccessible, thus helping to expand the medicinal and agricultural pipelines.

LECTURE 17

From Peptides to Proteins to Functions by Design

Dek Woolfson
Professor, University of Bristol

du Vigneaud Lecture

It is now possible to generate many stable peptide assemblies and proteins from scratch using rational and computational design approaches. One challenge in this field of *de novo* protein design is to move past structures found in nature and target the 'dark matter of protein space'; that is, structures that should be possible in terms of chemistry and physics, but which biology seems to have overlooked. This talk will illustrate what is currently possible in this nascent area using *de novo* designed coiled-coil peptides and proteins.

I will describe our "toolkit" of de novo coiled-coil assemblies,[1] and how we are converting these peptides bundles and barrels into single-chain proteins through rationally seeded computational protein design.[2] Then I will turn to subcellular applications. I will describe new designs and systems including **i|** *de novo* cell-penetrating peptides,[3] and **ii|** high-affinity kinesin-binding peptides,[4] and how these can be combined to hijack and control active motor proteins in living cells.[5]

[1] Understanding a protein fold: The physics, chemistry, and biology of alpha-helical coiled coils DN Woolfson *J Biol Chem* **2023** 299, ARTN: 104579. DOI: 10.1016/j.jbc.2023.104579

[2] Rationally seeded computational protein design of α-helical barrels KI Albanese, R Petrenas, F Pirro, EA Naudin, U Borucu, WM Dawson, DA Scott, GJ Leggett, OD Weiner, TAA Oliver, DN Woolfson *Nat Chem Biol* **2024** 20, 991-9. DOI: 10.1038/s41589-024-01642-0

[3] *De novo* designed peptides for cellular delivery and subcellular localisation GG Rhys, JA Cross, WM Dawson, HF Thompson, S Shanmugaratnam, NJ Savery, MP Dodding, B Höcker, DN Woolfson *Nat Chem Biol* **2022** 18, 999-. DOI: 10.1038/s41589-022-01076-6

[4] Fragment-linking peptide design yields a high-affinity ligand for microtubule-based transport JA Cross, MS Chegkazi, RA Steiner, DN Woolfson, MP Dodding *Cell Chem Biol* **2021** 28, 1347-1355. DOI:10.1016/j.chembiol.2021.03.010

[5] A *de novo* designed coiled coil-based switch regulates the microtubule motor kinesin-1 JA Cross, WM Dawson, SR Shukla, JF Weijman, J Mantell, MP Dodding, DN Woolfson *Nat Chem Biol* **2024** 20, 916–23 (2024). DOI: 10.1038/s41589-024-01640-2

LECTURE 18

Fluoropeptides as Biodegradable Biopolymers

Beate Koksch
Professor, Freie Universität Berlin

Fluorine as a substituent causes drastic changes in the biophysical properties of organic molecules and polymers. This results in highly appreciated properties of drugs and materials that are ubiquitous in our everyday lives today. However, the unique stability of the C–F bond can be problematic if this leads to poor biodegradability. Combining the special material properties of fluoropolymers with the biocompatibility of peptides could be an attractive solution.

The incorporation of fluorine substituents into otherwise hydrophobic amino acid side chains induces a polarity that no other functionality can generate in this form. This special polarity leads to drastic changes in the biophysical properties of peptides in terms of hydrophobicity, lipophilicity, solubility, as well as folding and proteolytic stability—criteria that are of utmost importance for biomolecular recognition, self-assembly, and thus the properties of peptide-based biomaterials.[1]

Generally, the combination of fluorine substituents, which lead to the much-described and widely used omniphobic properties in organic polymers, with the water-soluble properties of peptides, depending on their sequence and folding, promises new classes of compounds with extremely interesting properties. Fluoropeptides, which consist exclusively or to a large extent of fluorinated amino acids, offer a wide range of unexplored possibilities in terms of folding, self-assembly, and material properties. We have recently taken the first steps into this uncharted scientific territory.[2,3]

The lecture will present the first polyfluorinated peptides that consist of more than 50% of fluorinated amino acids. They represent an amphipathic peptide motif combining long stretches of fluorinated amino acids containing one to three fluorine substituents in the side chain of ethyl glycine with a short stretch of positively charged lysine residues that mediate solubility in aqueous medium. The structural and biomaterial properties of this new class of compounds are discussed here. This design is comparable to organic co-block polymers. The application of this class of fluoropeptide conjugates for the encapsulation of hydrophobic drugs and their specific delivery to their cellular targets is also presented. In addition, we are investigating how isolated enzymes and whole microorganisms are able to cleave C–F bonds of fluorinated peptides and amino acids. These results open up new avenues for the development of biomaterials containing fluorine.

[1] Berger A.A., Völler J.-S., Budisa N., Koksch B. *Acc. Chem. Res*. **2017**, 50(9), 2093.
[2] Chowdhary S., Schmidt R.F., Sahoo A.K., tom Dieck T., Hohmann T., Schade B., Brademann-Jock K., Thünemann A.F., Netz R.R., Gradzielski M., Koksch B. *RSC Nanoscale* **2022**, 14, 10176.
[3] Hohmann T., Chowdhary S., Ataka K., Er J., Dreyhsig G.H., Heberle J., Koksch B. *Chem. Eur. J*. **2023**, e202203860.
[4] Khan M.F., Chowdhary S., Koksch B., Murphy C.D. *Environ. Sci. Technol*. **2023**, 57, 9762.

LECTURE 19

Growth Mechanism of Coiled-Coil Peptide Nanocrystals and their Application for Intracellular Delivery of Proteins

Young Investigator Lecture 4

Ashutosh Agrahari
Graduate Student, Purdue University

Coiled-coil peptides have shown great promise as building blocks in developing hierarchical biomaterials. The GCN4 leucine zipper is one such motif that has previously been modified with the metal binding ligands, nitrilotriacetic acid and di-histidine at the N and C-terminus, respectively, to facilitate metal-promoted higher order assembly. The trimeric leucine zipper, **p2L** was reported to generate micron-scale hexagonal crystals with Zn(II) that encapsulate His-tagged proteins via His tag-metal interactions.[1,2] However, their larger size limited their applicability for cellular delivery of protein cargos, resulting in the need for the development of nanoscale higher-order materials.

Here we present the formation of nanocrystals with **p2L** in the presence of Ni(II) that are about 250 μm. Transmission electron microscopy, TEM, and small/wide angle x-ray scattering showed high ordering in the open-face hexagonal packing of coiled-coils within the nanocrystals. Furthermore, crystal growth was found to be more ordered throughout the P3 face in the presence of sodium salts, showing the influence of salts in preventing weaker ionic interactions. Temperature-dependent crystal growth experiments resulted in wider crystalline discs at lower temperature, 4 °C, whereas elevated temperature at 37 °C favored metal-ligand interactions over ionic interactions in the radial direction, leading to taller crystals.

After studies to probe the growth mechanism, the nanocrystals were utilized to incorporate His-tagged eGFP for intracellular delivery of the proteins. Confocal microscopy indicated that nanocrystals enter the cells via endocytosis and cellular accumulation was found to be concentration and time dependent.

[1] Nepal, M.; Sheedlo, M. J.; Das, C.; Chmielewski, J. *J. Am. Chem. Soc.* **2016**, 138 (34), 11051–11057.
[2] Curtis, R. W.; Scrudders, K. T.; Ulcickas, J. R. W.; Simpson, G. J.; Low-Nam, S. T.; Chmielewski, *J. ACS Biomater. Sci. Eng.* **2022**, 8 (5), 1860-1866.

LECTURE 20

Peptide-Based Nanomaterials: Progress from Structural Analysis to Design

Vincent P. Conticello
Professor, Emory University

Historically, structurally defined materials on the nanometer length-scale have been challenging to rationally construct and difficult to structurally analyze. Sequence-specific biomolecules, that is, peptides and nucleic acids, have advantages as design elements for construction of these types of nano-scale materials in that correlations can be drawn between sequence and higher order structure, potentially affording ordered assemblies in which functional properties can be controlled through the progression of structural hierarchy encoded at the molecular level.

However, the predictable design of self-assembled structures requires precise structural control of the interfaces between peptide subunits, protomers. In contrast to the robustness of protein tertiary structure, quaternary structure has been postulated to be labile with respect to mutagenesis of residues located at the protein-protein interface. Self-assembling peptide systems have been employed to interrogate the concept of quaternary structure designability within the structural context of synthetic filaments and nanotubes. These peptide systems provide an understanding of how minor sequence changes can translate into large changes in supramolecular structure.

The emergence of electron cryo-microscopy, cryo-EM, has enabled a revolution in the structural analysis of these peptide-based filaments at near-atomic resolution, which has provided significant evidence that the designability of protein interfaces is a critical consideration for control of supramolecular structure in self-assembling systems.

LECTURE 21

Peptide Hydrogels Control Neutrophil Extracellular Trap, NET, Formation *in Vivo* with Locoregional Precision

Tania Lopez-Silva
Postdoctoral Fellow, National Cancer Institute

Neutrophil extracellular traps, NETs, are DNA networks released by neutrophils in response to pathogens and have been associated with certain inflammatory diseases. Controlling NET formation with locoregional specificity in vivo would enable the study of this response in the context of disease and therapy with unprecedented control. We serendipitously discovered that positively charged peptide-gels rapidly induce NET formation *in vivo* whereas negatively charged gels do not. To our knowledge, the ability to induce NETosis using a gel's electrostatic charge has not been reported.

Based on these initial observations, we developed a materials platform comprising injectable peptide gels that can be implanted into tissue to provide site-specific anatomical control over NET formation. Further, microscale locoregional control of NET formation directly within a single implant can be accomplished by modulating the distribution of charge within the material. This hydrogel platform can also be used to finely tune the degree of NET formation *in vivo* by employing composites of oppositely charged gels. Thus, this chargebased peptide-material strategy can induce NET formation with locoregional control and precise tunability at a tissue and implant level.

LECTURE 22

Design of Out-of-Equilibrium Biomolecular Assemblies

Akif Tezcan
Professor of Chemistry and Biochemistry, University of California, San Diego

Life is characterized by a dynamic, non-equilibrium state of matter driven by constant energy flux and dissipation. In cells, such an active state of matter is maintained primarily by NTPases, N = A or G, which

transduce the energy stored in the phosphodiester bonds of ATP and GTP into other chemical or mechanical forms. For example, cytoskeletal assemblies such as actin filaments and microtubules create motion and mechanical force through NTP-fueled polymerization and depolymerization, enabling cells to rapidly respond to spatiotemporal changes in the environment and powering processes such as cell division and motility.

While there have been great advances in the design of complex protein architectures, the design of dynamic, non-equilibrium protein assemblies powered by external energy input has been largely out of reach. Toward addressing this challenge, we have taken two different routes: **1|** reengineering ATP-dependent enzymes into ATP-dependent self-assembly systems and **2|** leveraging synthetic, chemically tunable interactions to control protein self-assembly. In this presentation, I will summarize some of our recent efforts in this area.

LECTURE 24

Advancing Sustainable Peptide Synthesis: Green Strategies for Scalable and Efficient Automatised Solid-Phase Manufacturing

Anna Maria Papini
Professor, University of Florence

The transition to sustainable SPPS presents significant challenges, particularly in light of increasing regulatory restrictions. The European Commission's amendment of Annex XVII of REACH in 2023, banning the use of DMF due to its toxicity, has accelerated the search for greener alternatives. However, replacing DMF without sacrificing efficiency remains difficult. Investigations into non-hazardous solvent systems, particularly dimethyl sulfoxide-based binary mixtures, demonstrated their ability to maintain solubility and resin swelling. Automated solid-phase synthesis trials using controlled heating confirmed their effectiveness in synthesizing complex peptides, including ACP, 65–74, β-amyloid, 1–42, SARS-CoV-2 RBM, 436–507, and peptide-peptide nucleic acid hybrids targeting the SARS-CoV-2 Nucleocapsid Protein. Catch-and-release purification further reduced hazardous solvent use.

Beyond solvents, sustainable synthetic strategies were applied to pharmaceutical peptides like liraglutide and eptifibatide, and cosmetic peptides such as Serpin A1-based bio-peptides. The safer coupling reagent TBEC, 1-tert-butyl-3-ethylcarbodiimide, and eco-friendly solvents optimized two approaches: direct synthesis using lipidated lysine, 86% HPLC purity, and a catch-and-release lipidation strategy, >90% purity, reducing preparative HPLC dependency. Scalable, heat-enhanced SPPS methods addressed disulfide bond formation challenges, enabling a fully automated, cGMP-compliant process.

Additionally, these methods also facilitated the construction of side-chain-to-side-chain clicked antimicrobial peptides via copper-catalyzed azide-alkyne cycloaddition, underscoring the broader applicability of green strategies in medicinal and cosmetic ingredients syntheses. These advancements emphasize the need for continued innovation in sustainable peptide manufacturing, aligning with environmental responsibility while maintaining synthesis efficiency.

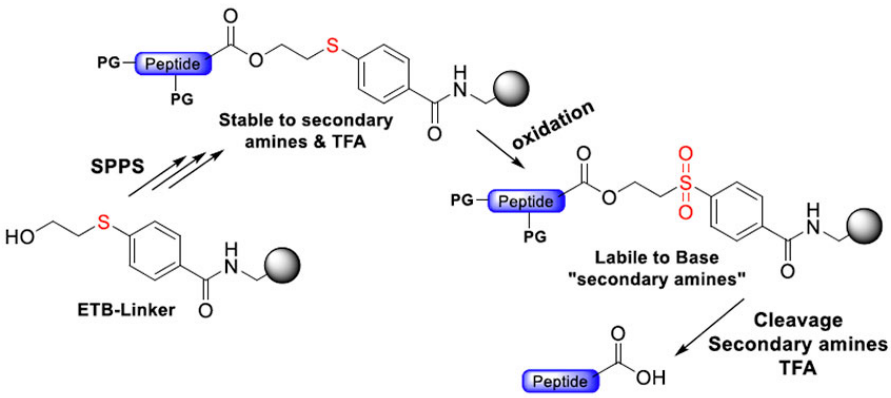
LECTURE 25

"Base Labile" Safety Catch Linker. Synthesis and Applications in SPPS in a Green Context

Sikabwe Noki
Postdoctoral Research Fellow, University of KwaZulu-Natal

Young Investigator Lecture 5

Safety-catch linkers and protecting groups, PGs play a significant role in peptide synthesis by allowing precise control of their stability: stable during the synthetic process in a broad range of chemical conditions and labile after chemical manipulation in one of the conditions that previously were stable. "Base-labile" safety-catch linkers and PGs offer a strategic advantage due to their stability under acidic, for example, TFA, and basic conditions, for example, piperidine, followed by selective cleavage under basic conditions, facilitating α-amino deprotection and final cleavage in solid phase peptides synthesis.



This study introduces a base labile safety catch linker, ETB Linker, based on a sulfinyl moiety, which allows peptide elongations using Fmoc chemistry. After the sulfinyl group is oxidated to sulfone, peptides are released via a β-elimination using a secondary amine, for example, diethylamine, piperidine. This ETB Linker was synthesized in a three-step and attached to aminomethyl resin to form ETB resin. Optimization of key reaction was achieved using a multi-detachable system, allowing precise control over linker and peptide release.

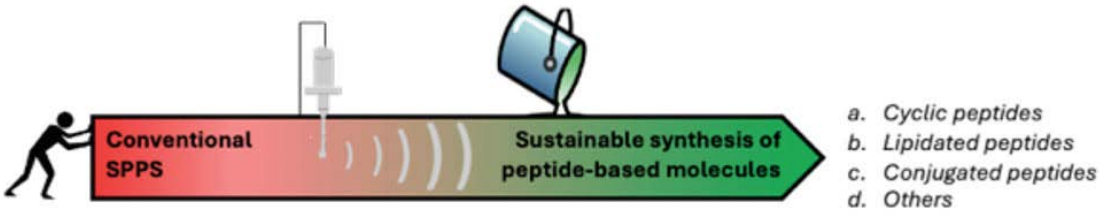
Traditional cleavage reagents like TFA, TFMSA, TFE pose sustainability challenges due to polyfluoroalkyl substance, PFAS, hazardous nature. ETB resin reduces dependence on these reagents, offering a greener alternative for the cleaving protected and unprotected peptides. The resin's efficacy was demonstrated in synthesizing longer peptides, such as peptide SAK and a 16-mer protected peptide, N-terminal sequence of liraglutide, proving its utility as a free-acid method for protected peptide preparation. Additionally, the ETB-linker was explored for substituting TFA in removing sidechain protecting groups, aligning with green chemistry context.

LECTURE 26

Ultrasound-Powered Sustainable Chemical Synthesis of Bioactive Peptides

Francesco Merlino
Assistant Professor, University of Naples Federico II

The rising demand for peptide-based therapeutics is compelling drug developers to address the sustainability challenges associated with their production.[1,2] Among chemical manufacturing peptide techniques, the Fmoc-based solid-phase peptide synthesis, SPPS, stands out as the most effective but expensive strategy. Since its inception, SPPS has undergone continuous refinement through both chemical innovations and technological advancements, including the application of alternative energy inputs to carry out the fundamental SPPS steps, such as microwave and continuous-flow systems. In recent years, the use of such energy sources has gained increasing relevance, driven by the need for more efficient, sustainable, and cost-effective SPPS synthetic methods.



Our recent findings have highlighted the potential of ultrasonication, US, in enhancing SPPS. We previously demonstrated significant reductions in both material consumption and reaction time when US was applied to assist crucial SPPS steps, such as amide bond formation and Fmoc deprotection.[3,4] In this presentation, we will introduce additional US-assisted methods for executing sustainable solid-phase reactions. Notably, we have explored reactions that lead to cyclic and conjugated peptides, among others, leveraging the synergy between US and green auxiliaries.

[1] Wang et al. *Signal Transduct. Target Ther.* **2022**, 7, 48.
[2] Muttenthaler et al. *Nat. Rev. Drug Discov.* **2021**, 20, 309–325.
[3] Del Bene et al. *Ultrason. Sonochem.* **2023**, 95, 106360.
[4] Merlino et al. *Org. Lett.* **2019**, 21, 6378–6382.

LECTURE 27

Retatrutide: A Novel Triagonist for Metabolic Disorders and CMC Advancements

Michael Kopach
Associate Vice President, Eli Lilly

Retatrutide, a novel triagonist, exemplifies the potential of incretin-based therapies for obesity and type 2 diabetes by targeting multiple metabolic pathways. Engineered with alpha and non-coded amino acids, Retatrutide demonstrates enhanced stability and efficacy, positioning it as a promising treatment to address the challenges of metabolic disorders.

LECTURE 28

Paradigms for the Discovery of Unknown Naturally Occurring GPCR Ligands in Chemical Neuroscience: Melanocortins and Opioids

Carrie Haskell-Luevano
Professor, University of Minnesota

G-protein coupled receptors, GPCRs, are fundamental proteins within the body that mediate a plethora of physiological conditions such as feeding, obesity, and energy homeostasis, melanocortins, and pain, opioids. Extensive work has been performed identifying and studying the classical endogenous ligands for de-orphaned GPCRs.

However, using mixture-based positional scanning libraries, it is possible to discover new endogenous peptide fragments that are able to stimulate and regulate the melanocortin and opioid GPCRs specifically. The paradigm presented offers the opportunity for the identification of undesirable drug "off-target" mechanisms, as well as new chemical space for the optimization of potential therapeutics through medicinal chemistry efforts.

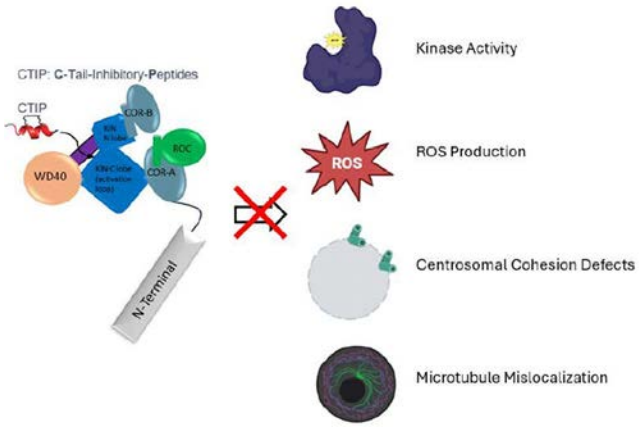
LECTURE 29

C-tail Mimics of LRRK2 Downregulate Kinase Activity in Parkinson's Disease

Young Investigator Lecture 6

Tiancheng Chen
Graduate Student, University of Georgia

Leucine-rich repeat kinase 2, LRRK2, is the most commonly mutated gene in Parkinson's disease, PD, a neurodegenerative disorder affecting over 10 million people worldwide. Pathogenic mutations in LRRK2 often result in hyperactivation, leading to detrimental effects such as centrosomal cohesion defects. Despite the recognized contribution of hyperactive LRRK2 in PD, the mechanisms by which multiple domains in LRRK2 regulate its activation and activity have not been well understood.



Here, we designed a library of constrained peptides, termed C-Tail-Inhibitory-Peptides, CTIPs, to mimic the C-terminal tail of LRRK2 to investigate its role in regulating LRRK2 activity. We demonstrate that

these cell-permeable peptides bind to LRRK2, downregulate its kinase activity, and suppress downstream effects, including substrate phosphorylation, reactive oxygen species, ROS, production, and centrosomal splitting. Unlike many ATP-competitive type I LRRK2 kinase inhibitors that induce toxicity and mislocalization of LRRK2 within cells, these constrained peptides do not disrupt LRRK2 localization. Our findings suggest that the C-tail of LRRK2 plays a critical role in its regulation and presents a promising alternative approach for targeted LRRK2 inhibition.

LECTURE 30

Designed Macrocyclic Peptides as Nanomolar Inhibitors of Self- and Cross-Seeded Amyloid Self-Assembly of Alpha-Synuclein

Aphrodite Kapurniotu
Professor, Technical University of Munich

Protein aggregation into cytotoxic oligomers and amyloid fibrils is linked to numerous devastating cell- or neurodegenerative diseases including Alzheimer's disease, AD, Parkinson's disease, PD, and type 2 diabetes, T2D. The key amyloid polypeptide in AD is the 40(42)-residue amyloid-β peptide, Aβ, in PD the 140-residue α-synuclein, αSyn, and in T2D the 37-residue islet amyloid polypeptide, IAPP, or amylin.

T2D is regarded as a major risk factor for PD. Cross-interactions between αSyn and IAPP might be a molecular link between T2D and PD. In fact, IAPP fibrils can act as "cross-seeds, thus accelerating amyloid self-assembly of αSyn, and IAPP and αSyn have been reported to co-localize in PD brains. Therefore, designing inhibitors of both self- and IAPP-cross-seeded αSyn amyloid self-assembly could be a useful approach to intervene with PD pathogenesis.

However, αSyn and IAPP are intrinsically disordered and their cross-interaction sites have been unknown, which hinders inhibitor design. Here we present our studies on macrocyclic peptides designed to mimic putative IAPP self-/cross-interaction sites. These peptides were found to be nanomolar inhibitors of both self- and IAPP-cross-seeded amyloid self-assembly of αSyn.[1] In addition, these peptides block Aβ-mediated cross-seeding of αSyn and inhibit amyloid self-assembly of IAPP and/or Aβ.[1,2]

Based on their broad-spectrum anti-amyloid function and additional drug-like features, these multi-targeting macrocyclic peptides are promising leads for multifunctional anti-amyloid drugs in PD, T2D, AD, and their comorbidities.

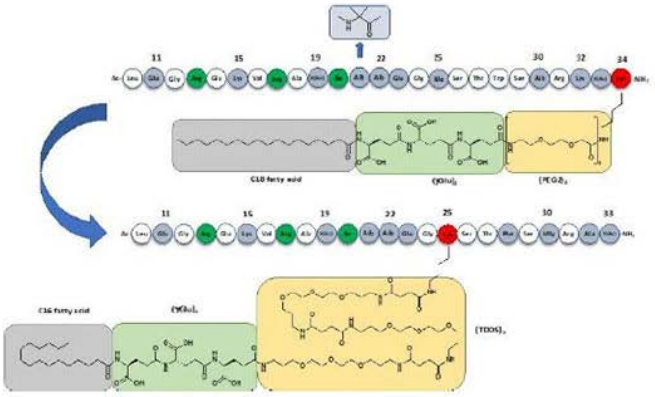
1 Hornung et al. *Angew. Chem. Int. Ed.* **2025,** in press.
2 Spanopoulou et al. *Angew. Chem. Int. Ed.* **2018**, 57, 14503–14508.

LECTURE 31

R2R01, a Potent Long-Acting RXFP1 Peptide Agonist as Clinical Candidate in Phase 2 Studies for Cardiovascular and Renal Diseases

Federica Tucci
Principal Research Scientist, IRBM

A C18 fatty acid single-chain analog based on the chain B of the relaxin-2 peptide hormone was recently identified as a potent, selective RXFP1 agonist with extended duration pharmacokinetics, PK.[1,2] Advanced PK profiling of this compound highlighted elevated levels of oxidative metabolism occurring in dogs and minipigs, which precluded further development.[3] Extensive optimization efforts to overcome these issues led to the design of new analogs with increased stability against metabolic oxidation while maintaining sub-nanomolar RXFP1 activity.



Key structural elements, including fatty acid chain length, attachment position, and linker structure were modified to improve PK parameters. Additionally, incorporation of α-methyl-Lys at position 30, together with other modifications, resulted in sub-nanomolar potency on RXFP1, improved duration of action, and reduced pseudo-allergic reactions due to mast cell activation.[4]

Our studies demonstrate that an integrated platform employing a combination of in vitro and in vivo approaches is needed for the development of peptide therapeutics and the identification of clinical candidates with improved probability of success. The R2R01 peptide from this series is undergoing Phase 2 clinical studies in heart failure and hepatorenal syndrome.[5]

[1] Mallart S. J. Med. Chem. 2021, 64(4):2139–2150.
2 Illiano S. Sci. Rep. 2022, 12(1):20435.
3 Esposito S. J. Pharm. Biomed. Anal. 2023, 227, 115256.
4 Mallart S. J. Med. Chem. 2025, in press.
5 Poirier B. Br. J. Pharmacol. 2024.

LECTURE 32

O-Linked Glycopeptides Derived from Endogenous Neurotransmitters as a Source of Brain-Penetrant CNS Drugs for the Treatment of Stroke, mTBI and Neurodegeneration

Robin Polt
Professor, The University of Arizona

Our studies with glycopeptides suggest that two conformational macrostates exist. **1|** a large water-soluble ensemble of structures, random coil state, and **2|** a much smaller amphipathic ensemble that is membrane bound. Most endogenous neuropeptides possess amphipathic character, which constrains them to the membrane compartment. Pioneering studies with enkephalins and endorphin/dynorphin analogues suggested that modulation of membrane affinity by glycosylation, or other water-soluble moieties, produces "*biousian glycopeptides"* that are systemically available and can cross the BBB.

Membranes are critical for peptide transport and binding events. The role of the membrane in transport is generally that of a barrier, but the role of the membrane in receptor binding is an enabling one. Schwyzer's "membrane compartment theory" can be useful in understanding the dynamics of peptide-receptor interactions. His contention is that the membrane should be viewed as an essential component in bringing together the receptor and ligand. Max Delbruck performed a theoretical study of receptor-ligand interactions in the context of membrane compartmentalization. He found that a 2D search for a receptor was much more efficient than a 3D search for a receptor, and suggested that the initial interaction was adsorption of the ligand to the membrane.

Our studies show that endogenous peptide neurotransmitters and hormones can be converted into glycopeptides related to enkephalin, endorphin, endomorphins, oxytocins, PACAP, and angiotensin$_{1-7}$ for the treatment of pain, addiction, and neurological conditions such as mTBI, Stroke and neurological issues. One glycopeptide, **PNA51 has now begun clinical studies for the treatment of vascular dementia**.

[1] Bernard K, Mota JA, et al. *Exp. Neurol*. **2024**, 381, 114926.

LECTURE 33

Inclusive Mentoring

Pascale Guiton
Assistant Professor, Santa Clara University

LECTURE 34

De Novo Protein Design of Functional Proteins

William DeGrado
Professor, University of California at San Francisco

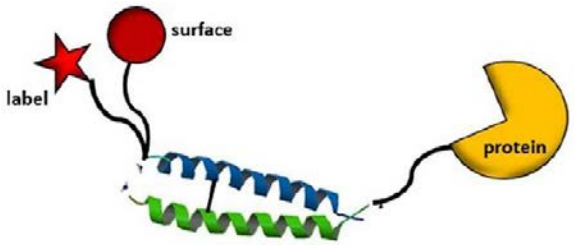
Not too long ago, the design of proteins from scratch that fold into predictable structures was considered an impossible task, but with advances in machine learning and artificial intelligence it is now increasingly routine. Given our ability to design protein structures the next challenge has been to design function. This talk will describe the design of proteins for the recognition and delivery of peptide and small molecule drugs, nucleotide sequencing, and environmentally friendly, efficient stereoselective catalysis of new-to-nature reactions.

LECTURE 35

Bind & Bite: Covalently Stabilized Heterodimeric Coiled-Coil Peptides for the Site-Selective Chemical Modification of Proteins

Jutta Eichler
Professor of Medicinal Chemistry, University of Erlangen-Nurnberg

Ensuring site-selectivity in covalent chemical modifications of proteins is one of the major challenges in chemical biology, medicinal chemistry, and related disciplines. We have modified a pair of heterodimeric coiled-coil peptides to enable the selective covalent stabilization of the dimer without using enzymes or cysteine moieties.



Fusion of one peptide to the protein of interest, in combination with linking the desired chemical modification to the complementary peptide, facilitates stable, site-selective attachment of the chemical moiety to the protein, through the formation of the covalently stabilized coiled-coil.[1] This ligation method was successfully used to selectively modify the HIV-1 envelope glycoprotein,[2] as well as to generate Fc fusion proteins of synthetic antibody mimetic peptides.

Furthermore, selectively addressing individual positions enabled the generation of mutually selective pairs of coiled-coil peptides,[1] for applications such as the concurrent chemical modification of more than one protein with more than one chemical entity, as well as the heterodimerization of proteins.

[1] Beutel J, Tannig P, Di Vincenzo R, Schumacher T, Überla K, Eichler J. *RSC Chem. Biol.* **2023**, 4, 794.
[2] Di Vincenzo R, Beutel J, Arnold P, Wang Y, Damm D, Tannig P, Lux A, Temchura V, Eichler J, Überla K. *Front. Immunol.* **2024**, 8, 1344346.

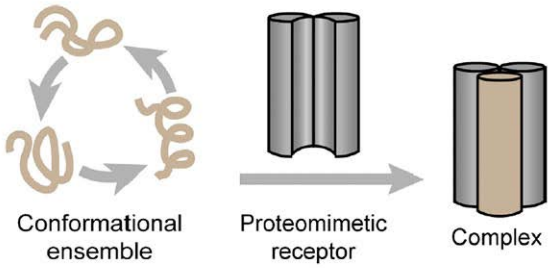
LECTURE 36

A Proteomimetic Strategy for Modulation of Intrinsically Disordered Protein MYC

Thy Nguyen
Ph.D. Candidate in Chemistry, New York University

Young Investigator Lecture 7

Traditional approaches to target proteins have relied on the paradigm that the unique three-dimensional folds of proteins provide ligandable binding sites. Conformationally dynamic proteins increase the level of difficulty in ligand design and the challenge is further exacerbated for proteins that are intrinsically disordered.



We hypothesized that one avenue for the development of binders for a disordered region would be to trap one of its thermodynamically accessible conformations with a receptor. Here we show the application of this approach to MYC, which represents a critical therapeutic target but has not yielded to small molecule inhibitors due to its conformationally dynamic nature. MYC adopts a helical configuration when it binds its cellular partner MAX.

We rationally designed a proteomimetic scaffold, termed Crosslinked Helix Dimers, CHDs, to trap this conformation. We show that MYC can be directly engaged both in biochemical and cellular assays. Overall, this work demonstrates a general method to capture and trap intrinsically disordered proteins with a propensity to adopt α-helical conformations.

LECTURE 37

A Peptide can Replace an Essential Enzyme in Yeast

Kira Podolsky
Postdoctoral Fellow, MIT

Young Investigator Lecture 8

Peptide catalysts conceivably facilitated the emergence and maintenance of cellular biology at the origins of life. Their functions may have been similar to large protein enzymes in modern cells.

Here, we report the first discovery of enzyme mimetic peptide catalysts that sustain eukaryotic life. We designed peptide libraries to complement the function of an essential enzyme, protein disulfide isomerase, PDI, in *Saccharomyces cerevisiae*. Two genetically encoded peptide catalysts, both 24 amino acids

long, maintain *S. cerevisiae* survival in the absence of PDI. Their capacity to keep pace with rapid cellular functions therefore implicates peptide catalysts as evolutionary precursors to enzymes.

LECTURE 38

Hydrocarbon-Stapled Peptidomimetic of TACC3 Disrupts CHC Interaction and Delays Mitotic Progression

Richard Bayliss
Professor of Molecular Medicine, University of Leeds

The complex formed by Transforming Acidic Coiled Coil 3, TACC3, and Clathrin Heavy Chain, CHC, enhances mitotic spindle stability by cross-linking k-fibres.[1] Previously, we elucidated the structural basis of the TACC3/CHC interaction, which is driven by hydrophobic residues on both proteins and the formation of a helix in TACC3 that docks into the helical repeats of CHC.[2] The interaction is also dependent on phosphorylation of TACC3 at S558 by Aurora-A. Here we find that this phosphorylation event plays an unusual role in a protein-protein interaction by overcoming the electrostatic repulsion between Lys507 of CHC and basic residues in TACC3.

Leveraging this insight, we optimized the sequence using peptide arrays to develop a hydrocarbon-stapled peptide, SP-TACC3, that binds CHC with over a hundred-fold higher affinity than the native peptide, effectively disrupting the interaction. The crystal structure of the SP-TACC3/CHC complex reveals the basis for the enhanced interaction and highlights the contribution of additional polar and hydrophobic interactions. SP-TACC3 efficiently penetrates cells and displaces TACC3 from the mitotic spindle, causing a delay in mitotic progression in two out of three cancer cell lines. This work showcases the novel application of hydrocarbon-stapled peptides to disrupt the TACC3-CHC protein-protein interaction in a cellular context, highlighting the potential of targeting this interface for future cancer therapies.

[1] Hood FE et al. *J. Cell Biol.* **2013** 202:463-78
[2] Burgess SG et al. *EMBO J. 2018* 37. e97902

LECTURE 39

Chemical Protein Synthesis as a Tool for Therapeutic Applications: The Development of Mirror-Image RaPID Technology & New Insulin Analogues

Norman Metanis
Professor, The Hebrew University of Jerusalem

Random nonstandard Peptides Integrated Discovery, RaPID, system is one of the most powerful methods for the selection of *de novo* macrocyclic peptide binders for proteins of interest by using a combination of flexible *in vitro* translation system, FIT, and mRNA display technology. Here, we show the development of mirror-image RaPID technology for the discovery of innate protease-resistant macrocyclic peptides that specifically bind to and inhibit Matrilysin, MMP7. MMP7 plays a crucial role in cancer metastasis and progression, making it an attractive target for therapeutic development. However, the development of potent and selective MMP7 inhibitors is challenging due to the conservation of active site across various MMPs.

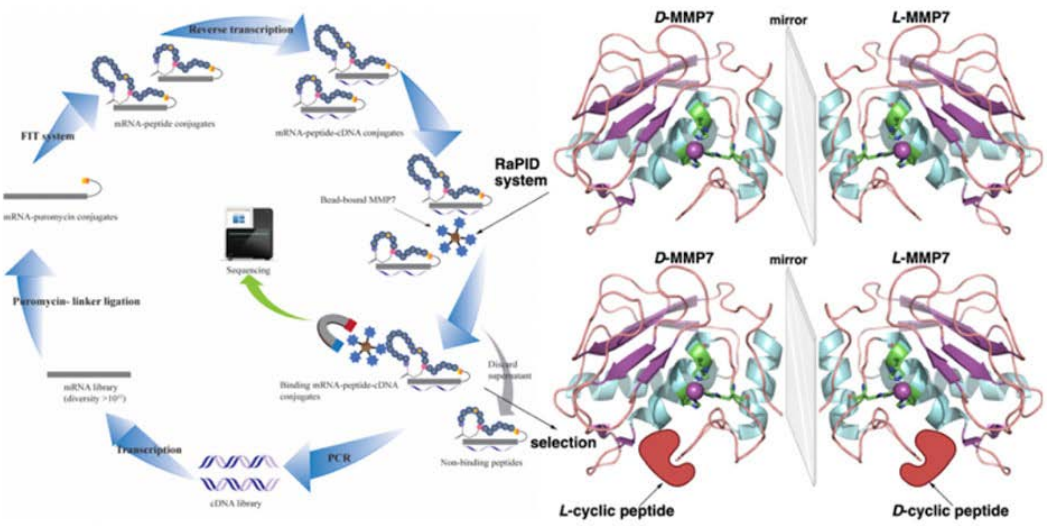


Figure 1. Mirror-image RaPID technology against MMP7.

We started by developing an approach for the chemical synthesis of the catalytic domain of MMP7, and upon optimization, we were able to synthesize both biotinylated *L*- and *D*-MMP7 in milligram quantities, which where both used in the RaPID system. One of the identified macrocyclic peptides against biotinylated *D*-MMP7, termed *D20*, was synthesized in its mirror-image form, *D'20*, consisting of twelve *D*-amino acids, one cyclic β-amino acid and a thioether bond. Notably, *D'20* potently inhibited the human MMP7 with IC_{50} of 90 nM, and showed selectivity over other MMPs tested in this study. Moreover, *D'20* inhibited the migration of pancreatic cell line CFPAC-1 while having no effect on the cell proliferation and viability. Additionally, *D'20* exhibited excellent stability in human serum, as well as in the simulated gastric and intestinal fluids. This study highlights that the mirror-image RaPID technology can be a powerful tool to develop *in vivo* stable macrocyclic peptides for therapeutic applications.

Furthermore, I will discuss our latest results for the preparation of new analogues of insulin, in which a disulfide 6-11 in chain A of insulin was replaced with diselenide, allowing us to prepare Se-insulin analogues in high yield. Further, we found that these insulins were more stable at room temperature than wt-insulin, and more resistant to fibrillation. These studies bring us closer to make more stable insulin analogues for diabetes melilotus.

LECTURE 40

Discovery and Characterization of Oncohistones

Tom Muir
Van Zandt Williams Jr. Class of 1965 Professor of Chemistry, Princeton University

LECTURE 41

Understanding and Manipulating Protein-Protein Interactions of Aurora-A Kinase Employing Intrinsically Disordered Regions

Andy Wilson
Professor, University of Birmingham

Intrinsically disordered regions, IDRs, are ubiquitous stretches of protein that do not adopt a stable structure, and a major class of protein structure found in all living organisms and viruses,[1] The contribution of IDRs to spatiotemporal control of cellular protein interactions and function remains poorly understood. This presentation will discuss our efforts to explore IDRs in regulating the cellular functions of a common factor, the protein kinase Aurora-A, an essential mitotic Ser/Thr kinase needed for cell-cycle progression, which, has received attention, but to date proven challenging, as an anticancer target.[2]

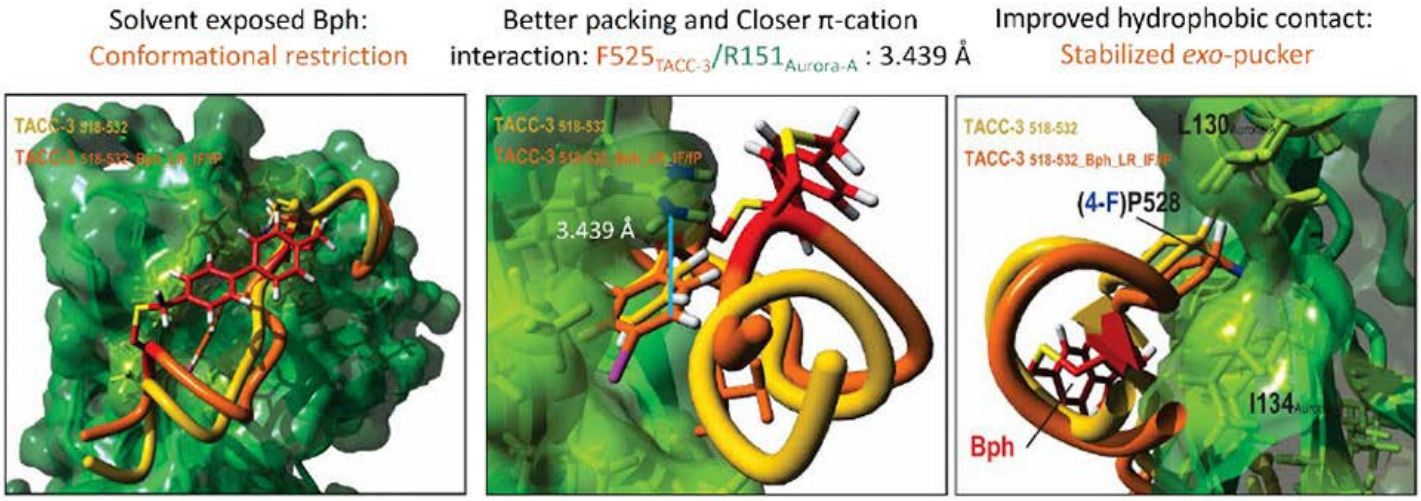


Figure 1 Aurora A binding TACC3 peptidomimetics: potent binding arises through a optimizing conformation effects and non-covalent contacts

Aurora-A is an incomplete kinase; its localization and activation are regulated through interaction with intrinsically disordered clients.[3] This presentation will discuss the structural and functional characterization of new Aurora-A PPIs,[4] alongside development of TACC3 peptidomimetics, Figure 1,[5] above, that bind to Aurora-A and inhibit its interaction with TACC3 without inhibiting its enzymatic function. Excitingly, these peptidomimetics also allosterically inhibit the N-Myc/Aurora-A interaction opening new opportunities for drug discovery.

[1] A. K. Dunker, et al., Intrinsically Disord. *Proteins*, **2013**, 1, e24157.
[2] J. Tischer, et al., *J. Cell. Biol.*, **2018**, 218, 10.
[3] S. G. Burgess, et al., *EMBO J.*, **2018**, 37, e97902.
[4] J. Holder, et al., *EMBO J.*, **2024**, 43, 5381.
[5] D. Gimenez, et al., *Chem. Sci.*, **2025**, 16, 354.

LECTURE 42

Design of a Covalent Protein-Protein Interaction Inhibitor of SRPKs to Suppress Angiogenesis and Invasion of Cancer Cells

Jacky C.K Ngo
Associate Professor, The Chinese University of Hong Kong

Serine–arginine, SR, proteins are splicing factors that play essential roles in both constitutive and alternative pre-mRNA splicing. Phosphorylation of their C-terminal RS domains by SR protein kinases, SRPKs, regulates their localization and diverse cellular activities. Dysregulation of phosphorylation has been implicated in many human diseases, including cancers.

Here, we report the development of a covalent protein–protein interaction inhibitor, C-DBS, that targets a lysine residue within the SRPK-specific docking groove to block the interaction and phosphorylation of the prototypic SR protein SRSF1. C-DBS exhibits high specificity and conjugation efficiency both *in vitro* and *in cellulo*. This self-cell-penetrating inhibitor attenuates the phosphorylation of endogenous SR proteins and subsequently inhibits the angiogenesis, migration, and invasion of cancer cells. These findings provide a new foundation for the development of covalent SRPK inhibitors for combatting diseases such as cancer and viral infections and overcoming the resistance encountered by ATP-competitive inhibitors.

LECTURE 43

Bicycle® Molecules as an Innovative and Unique Therapeutic Class

Sivaneswary Genapathy
Associate Director, Bicycle Therapeutics

Bicycle® molecules are a novel molecule formed by constraining short linear peptides into a stabilized bi-cyclic structure using a central chemical scaffold. Bicycle molecules have a unique structure that can deliver high precision to their chosen targets, while their size and surface area means they can potentially engage targets that have historically been resistant to conventional modalities.

Bicycle molecules can be readily conjugated to other payloads, such as Bicycle Drug Conjugates containing a selectively cleavable linker and a small molecule toxin payload, and Bicycle Radionuclide Conjugates® containing a stable linker-chelator system and a highly potent radioisotope.

They are designed to address significant unmet medical needs in patients.

LECTURE 44

Using Experimental Approaches and AI-Based Structural Modeling to Understand Kinase-Substrate Interactions

Laurie Parker
Professor, University of Minnesota

Protein phosphorylation is a crucial post-translational modification in all cells, carried out by kinase enzymes and reversed by phosphatase enzymes. It is regulated by a broad range of factors including protein-ligand and protein-protein interactions, scaffolding, and subcellular localization. Dysregulation of kinase activity leads to cellular abnormalities and disease, and thus kinases are a key target for drug discovery. Kinase inhibitor drug discovery depends on kinase assays, but in many cases, especially for understudied kinases, there is not enough information available about the substrate targets of a kinase to develop optimized assays. Optimization requires efficient substrates, appropriate reaction conditions and accessible detection methods.

The Parker lab employs both experimental and computational approaches to develop substrates and detection methods and implement them in kinase assays. We have established phosphoproteomics-based workflows for identifying substrate preferences and peptide sequences to use in tyrosine kinase and serine/threonine kinase assays. We are also using AI structure-based modeling, AlphaFold, to develop prediction criteria and hypotheses about kinase-substrate peptide interactions. While neither of these approaches, experimental or modeling-based, are ideal on their own for predicting novel kinase substrate peptide sequences or recognition selectivity, when used in combination they are valuable tools to streamline the assay design process. Implementing these approaches has also led us to new, and as-yet unanswered, questions about how kinases recognize peptides differentially to achieve selectivity in terms of reaction rates between similar kinase family members and/or substrate peptide sequences.

LECTURE 45

Computational Epitope Mapping and Chemical Engineering of Tick Proteins for Vaccine Development

Ingrid Dijkgraaf
Professor, Maastricht University

Tick-borne diseases impose severe burdens on healthcare systems and livestock industries, causing billions in economic losses worldwide. Climate change is expanding tick habitats, making novel tick control strategies more urgent than ever. Anti-tick vaccines, ATV, represent a promising approach, but their development is hindered by the low immunogenicity of tick proteins.

Here, we present a pipeline for ATV antigen, ATVA, design, integrating AlphaFold2 structure modeling, in silico antigenic epitope prediction, chemical remodeling, and multimerization. Tick salivary gland transcriptomic data were analyzed, secreted proteins identified via SignalP, and antigenic epitopes predicted using Discotope 3.0. Clustering based on secondary structure and cross-species conservation led to the selection of tick salivary lectin pathway inhibitor, TSLPI, from Ixodes scapularis, a key player in borreliosis transmission.

A 10-residue β-hairpin and a 9-residue linear epitope were synthesized using solid-phase peptide synthesis. The β-hairpin was cyclized via D-Pro-Gly turn using native chemical ligation to preserve its native structure, confirmed by NMR and CD spectroscopy. Both epitopes were tetramerized using a lysine wedge for immunization studies. ELISA revealed a 100-fold higher TSLPI-specific antibody response for tetrameric epitopes compared to monomers. Rabbit immunization followed by tick challenge showed higher TSLPI-specific antibodies, reduced tick weights, and lower egg hatching rates.

These findings highlight the potential of our ATVA pipeline for developing effective anti-tick vaccines, paving the way for novel strategies in tick control and disease prevention.

LECTURE 46

Integrating Molecular Dynamics Simulation & Machine Learning for Cyclic Peptide Structure Prediction

Yu-Shan Lin
Professor - Department Chair, Tufts University

A major obstacle to cyclic peptide development is that little structural information is available for these molecules, making it difficult to perform structure-based design or understand why different cyclic peptide sequences display different binding affinity, membrane permeability, and other properties. The lack of structural information is due to the fact that most cyclic peptides adopt multiple conformations in solution, existing as structural ensembles, which are very difficult to characterize using experimental techniques such as solution NMR spectroscopy.

In this talk, I will describe how we develop StrEAMM, Structural Ensembles Achieved by Molecular dynamics and Machine learning, models for cyclic peptides by combining molecular dynamics simulation and machine learning. We can now provide simulation-quality cyclic peptide structure predictions in seconds. We expect such a capability to rapidly predict cyclic peptide structures to enable researchers to understand the structural basis for the diverse properties of different cyclic peptides and greatly accelerate the development of this unique class of molecules.

LECTURE 47

AI-Driven Peptide Discovery: Unlocking the Potential of Peptide Arrays for Therapeutic Development

Ewa Lis
Chief Executive Officer, Koliber Biosciences

Peptide hit discovery methods such as phage display and mRNA display are widely used tools for identifying bioactive peptides. However, these approaches face significant limitations. Phage display often suffers from poor reproducibility and high false-positive rates, necessitating extensive validation. While mRNA display enables the incorporation of unnatural amino acids and can yield high-potency hits, it comes with substantial licensing costs and is prone to biases from non-uniform clone amplification. Additionally, hits identified through these methods are frequently hyperoptimized, making it challenging to introduce modifications that enhance developability without compromising potency. These constraints hinder the discovery of novel peptide therapeutics.

Peptide arrays, traditionally underutilized due to constraints in library size and accessibility, are emerging as a viable alternative. In this presentation, we will demonstrate how Koliber's machine learning technology, in collaboration with the peptide array platform developed by Robust Diagnostics, can overcome these challenges. We will show that large libraries are not essential for hit discovery and that initial hits can be rapidly optimized to nanomolar binding affinities using machine learning driven approaches.

Additionally, we will introduce visualization techniques to analyze binding modes, providing insights into peptide–target interactions. This approach paves the way for machine learning driven peptide array technologies to play a more prominent role in the discovery and development of novel peptide therapeutics.

LECTURE 48

Generative Deep Learning for Accurate *De Novo* Design of Macrocyclic Peptides

Gaurav Bhardwaj
Assistant Professor, University of Washington

The development of deep learning methods, such as AlphaFold and RoseTTAFold, has considerably improved the accuracy of structure prediction and design of larger proteins.[1,2] However, these deep learning methods typically do not apply or work well for small peptides with non-canonical amino acids. We previously described state-of-the-art "physics-based" methods for accurately designing hyperstable constrained peptides, macrocycles,[3,4] and membrane-traversing macrocycles.[5] To address the compute-related and functional limitations of those previous physics-based methods, we recently developed deep learning, DL, methods, AFCycDesign[6] and RFpeptides,[7] for highly accurate structure prediction, sequence design, and de novo generation of macrocyclic peptides.

These new DL tools outperform the traditional methods in their speed, accuracy, and overall success rates. We recently leveraged RFpeptides to successfully design macrocyclic peptide binders for multiple therapeutically-relevant protein targets, including targets with no available crystal or CryoEM structures.[7] In contrast to library-based approaches, we tested only 20 or fewer designs against each target and yet identified macrocycles with nanomolar, < 10 nM, binding affinities toward their targets. X-ray crystal structures of this protein-bound macrocycle match very closely, RMSD < 1.5 Å, with their computational models, confirming the designed structure and binding mode. In addition to applying these generative tools for designing new antibiotics, antivirals, and degraders for rare pediatric cancer targets, we are also expanding these DL methods to design precisely controlled oligomeric assemblies and incorporate the vast chemical diversity of non-canonical amino acids. Together, these new DL-guided methods enable custom and rapid design of peptides for diverse structures and functions.

[1] Jumper, J. et al. Highly accurate protein structure prediction with AlphaFold. *Nature* **2021** 596, 583–589
[2] Baek, M. et al. Accurate prediction of protein structures and interactions using a three-track neural network. *Science* **2021** 373, 871–876
[3] Bhardwaj, G. et al. Accurate *de novo* design of hyperstable constrained peptides. *Nature* **2016** 538, 329–335.
[4] Hosseinzadeh, P. et al. Comprehensive computational design of ordered peptide macrocycles. *Science* **2017** 8, 1461–1466.
[5] Bhardwaj, G. et al. Accurate *de novo* design of membrane-traversing macrocycles. *Cell* **2022** doi:10.1016/j.cell.2022.07.019.
[6] Rettie, S. A. et al. Cyclic peptide structure prediction and design using AlphaFold. *bioRxiv* **2023** doi:10.1101/2023.02.25.529956.
[7] Rettie, S. A. et al. Accurate *de novo* design of high-affinity protein binding macrocycles using deep learning. **bioRxiv 2024** doi:10.1101/2024.11.18.622547.

LECTURE 49

How Hsp70 Molecular Chaperones Bind Substrates with Selective Promiscuity

Lila Gierasch
Distinguished Professor, University of Massachusetts

Hsp70 molecular chaperones perform diverse roles in protein homeostasis, including facilitating of folding, inhibition of aggregation, targeting to organelles, and more. Their functions rely on their ability to bind to relatively short sequences in their substrates with a nucleotide-gated affinity. In general, Hsp70s bind short hydrophobic segments on their substrates; however, the detailed mechanism of client recognition is more complex, and the distinctions among modes of client recognition among Hsp70s are poorly understood.

While some Hsp70s bind proteins selectively, there are variations in the degree of client selectivity observed among Hsp70 isoforms. We previously observed that the *E. coli* Hsp70, DnaK, binds not infrequently to substrate sequences in a C-to-N, non-canonical orientation, as well as in an N-to-C canonical orientation, Clerico et al., *Proc. Natl. Acad. Sci. U.S.A.* **2021** 118, e2016962118. Of particular use in the study of synthetic peptide models bound to DnaK was NMR analysis of selectively δ1-methyl labeled, $^{1}H,^{13}C$, isoleucines that are near the substrate recognition cleft. The δ1-methyl resonance positions reported on the identity of the residue bound in the central pocket of the binding cleft, as well as the orientation of the bound peptide.

In recent work, we have compared the substrate-binding properties of the major cytoplasmic mammalian Hsp70s, Hsc70 and HspA1, to those of DnaK. Emerging from this work has been the working hypothesis that Hsc70s are less stringent in their binding than DnaK, with a greater diversity of binding modes, which may arise from the enlargement of the human proteome and wider array of physiological functions for these Hsp70s relative to that of *E. coli*.

LECTURE 50

Eradicating HIV-1 Latency Through the Development of Dual Inhibitors of HIV-1 Protease and Histone Deacetylase 3

Mark Lipton
Associate Professor, Purdue University

The eradication of human immunodeficiency virus type 1, HIV-1, from infected individuals remains a major medical challenge. Combined antiretroviral therapy, cART, leads to a decline in HIV-1 to imperceptible levels. However, discontinuing this treatment causes rapid viral rebound, arising from reservoirs of replication-competent proviruses in long-lived latently infected cells. These latent reservoirs represent a major obstacle for eradicating HIV-1.

Efforts to purge latently HIV-infected cells have used latency-reversing agents, such as histone deacetylase, HDAC, inhibitors, to force activation of proviruses in latently infected cells. Upon activation of latent HIV-1, the infected cells are then killed by the host immune response. However, this strategy must also be

used in concert with a separate treatment of cART, such as HIV-1 protease, PR, inhibitors, to prevent new rounds of infections by virions released from the stimulated cells. If HDAC and HIV PR inhibitor drugs are unable to simultaneously reach the same HIV-reservoir cells, new rounds of infections by virions released from the activated cells would unfortunately occur.

There is a substantial need, therefore, to develop agents that are dual inhibitors of HDAC3 and HIV-1 PR to eradicate HIV-1, by simultaneously activating HIV-1 latency within cells and preventing new infection. Through an extensive program consisting of molecular design, compound synthesis, enzymatic evaluation, and cell culture assays, we will describe the first peptidomimetic dual inhibitors of both the HDAC3 and HIV-1 PR enzymes, with low micromolar potency. Significantly, these agents also activate HIV-1 in latently infected human cells.

LECTURE 51

Unveiling the Mode of Aaction of SARS-CoV-2 Putative Fusion Peptides and their Exploitation as Antiviral Targets

Ana Salome Veiga
Assistant Professor, Gulbenkian Institute for Molecular Medicine

SARS-CoV-2 entry into host cells is mediated by the spike glycoprotein, S-protein, through a process that results in the fusion of the host and viral membranes. The fusion peptide, FP, is a key domain of the S-protein, known to insert into and disturb the host membrane. Once the FP anchors the virus to the host cell, the S-protein undergoes conformational changes allowing the completion of fusion. Despite its crucial role in viral entry, the region within the S-protein that corresponds to the FP is not yet fully clear. To shed light on this matter, we combined computational and experimental methods to characterize two previously proposed putative FPs, the N-terminal FP, nFP, and the internal FP, iFP.

Our results indicate that the iFP has a stronger affinity for membranes and exhibits higher hydrophobicity compared to the nFP, which tends to localize at the membrane-water interface. Moreover, the iFP causes higher membrane perturbation than the nFP, inducing lipid mixing and lipid vesicle content leakage. Furthermore, engineered spike-pseudotyped lentiviruses containing substitutions on the iFP region are unable to infect cells.

These findings suggest that the nFP may play a role in the initial interaction with the membrane, possibly facilitating the deeper insertion of the iFP and its role in promoting membrane fusion. The FP and other S-protein domains were used as targets to inhibit membrane fusion and block viral entry. Inhibitory small proteins were computationally designed resulting in a viral neutralization in the low micromolar range. Our study propose important insights to understand and inhibit the SARS-CoV-2 fusion machinery.

LECTURE 52

Mirror-Image Monobody Targeting MCP-1 Generated via mRNA Display and Peptide Ligation

Gosuke Hayashi
Associate Professor, Nagoya University

Engineered protein scaffolds function as binders against various target molecules with high affinity and specificity comparable to conventional IgG antibodies. However, biologically produced protein drugs are generally degraded by proteases and often exhibit immunogenicity. To increase protease resistance and decrease immunogenicity of peptides and proteins, mirror-image peptide/protein binders consisting of *D*-amino acids have been developed, so far mainly through the mirror-image phage display technique.

Here, we present a mirror-image protein binder derived from a monobody, one of the most promising protein scaffolds, using two notable technologies: chemical protein synthesis and TRAP, transcription-translation coupled with association of puromycin linker, display, an improved and streamlined version of mRNA display.[1] A sequential workflow of initial screening followed by affinity maturation, facilitated by TRAP display, generates an L-monobody with high affinity, K_D = 1.3 nM, against the pharmaceutically important monocyte chemoattractant protein-1, MCP-1, *D*-enantiomer.

By symmetry, the chemically synthesized *D*-monobody demonstrates strong and enantio-selective binding against L-MCP-1 and possesses pharmaceutically favorable properties such as resistance to proteolytic degradation, minimal immune response, and a potent inhibitory effect on MCP-1 binding to its cell membrane receptor. The production of a high-affinity mirror-image monobody elevates the value of mirror-image peptide/protein binders as a new modality in drug discovery.

[1] Hayashi G, Naito T, Miura S, Iwamoto N, Usui Y, Nonaka M, Oishi S, Murakami H. *Nat. Commun*. **2024**, 15, 10723.

LECTURE 53

Building Proteins from Scratch

Merrifield Award Lecture

Philip E. Dawson
Professor, The Scripps Research Institute

Proteins are the macromolecules through which biological function is mediated. Despite their central importance, synthetic chemists often consider them to be the targets of synthetic small molecules rather than the synthetic targets themselves. The repetitive chemical structure of many biological macromolecules suggests a chemical simplicity, yet in practice these molecules are deceptively difficult to assemble using the traditional organic synthesis toolkit.

One of the most significant advances in the assembly of these molecules has been the optimization of Merrifield's solid phase synthesis to produce highly pure, unprotected segments, followed by highly chemoselective, chemical ligation methods to assemble the segments and facilitate precise late-stage modification.

The development of the Native Chemical Ligation / Desulfurization approach for protein synthesis will be discussed and how it can be applied to the synthesis of complex macromolecular targets. In addition, a variety of non-native ligation chemistries have been explored through careful optimization of reaction rates top optimize utility and chemoselectivity. Reversible Absorption to a Solid Support, RASS, approach will be presented with applications to protein and nucleic acid targets including DNA encoded libraries. Together, these methods provide a robust toolkit for macromolecule synthesis that has been broadly utilized to advance peptide and protein science.

LECTURE 54

The Chemical Evolution of Peptides to Transform Human Health

Richard DiMarchi
Distinguished Professor of Chemical Biology and Gill Chair in Biomolecular Sciences, Indiana University

The advances in peptide-based treatment of adult-onset diabetes propelled the transformative management of obesity. The health benefit associated with weight reduction has been exhibited in a spectrum of comorbidities. As we search for new medicinal approaches to further optimize these breakthrough medicines the determination of what degree of improvement derives as an indirect benefit of lesser weight relative to directed biochemical action is a priority in continued
research.

What is inherently clear in a century of peptide sciences is that these miraculous endogenous peptides make pure poor drugs and at times misdirected what otherwise proved possible through chemical optimization. The advances in analytical, synthetic, and medicinal peptide chemistry continue to serve an indispensable role in the advancement of biochemistry, pharmacology, and therapeutic application of peptide hormones. In concert with multiple collaborators, we have championed the simultaneous recruitment of numerous physiological mechanisms optimized for pharmacological purposes to address the heterogeneity constituted by the multiple diseases associated with the metabolic syndrome.

We have integrated classical small and large molecule-based pharmacology across academic and commercial laboratories to achieve transformative therapeutic outcomes. The results have tangentially promoted the progression of medicinal approaches that restore health in overt disease, to those that can be used prophylactically to enhance strength, cognition, and longevity. The lecture will exemplify chemistry advancing our understanding of biology to promote enhanced health.

LECTURE 55

Novel Insights into Regulation of Energy and Glucose Metabolism by GIP and GIPR:GLP-1R Co-Agonists

Timo Müller
Professor, Ludwig Maximilians University München

The pharmacological management of obesity has long been regarded as a "mission impossible" due to limited effectiveness and poor tolerability. However, recent advancements in biochemical engineering have transformed the landscape, leading to the development of peptide-based therapies that simultane-

ously target receptors for glucagon-like peptide-1, GLP-1, glucose-dependent insulinotropic polypeptide, GIP, and/or glucagon.

These innovative treatments not only achieve unprecedented efficacy in reducing body weight and improving glycemic control in individuals with obesity and type 2 diabetes, but they also hold promise for treating neurodegenerative diseases, fatty liver disease, dyslipidemia, atherosclerosis, and cardiovascular conditions.

In my lecture, I will discuss the early development of these therapies, our discovery of GIP's role in central energy metabolism regulation, and recent insights into the effects of GIP receptor agonism and antagonism. Additionally, I will present new and unpublished data on a novel, highly effective quintuple polyagonist designed for the treatment of obesity and type 2 diabetes.

LECTURE 56

Machine Learning Guided Peptide Drug Discovery Speeds up Lead Identification as Demonstrated with Novel GLP-1R Agonists

Poanna Tran
Research Scientist, Gubra ApS

We have developed streaMLine, an innovative platform for peptide drug discovery that greatly shortens the time from initial hit to clinical drug candidate. The platform allows for high throughput synthesis and screening. Thousands of peptides are systematically screened in *in vitro* assays and on chemical- and physical parameters, whereby the streaMLine platform enables complete sequence exploration and simultaneous optimization of key parameters.

We employ a fully digitalized laboratory system where detailed information on all aspects of a sample lifetime is tracked. This enables accurate distinction of key chemical peptide modification from artefact background effects, using a machine learning approach. This unique strategy for peptide screening integrates with state-of-the-art **in vivo** pharmacology facilities, including advanced animal models and rapid determination of PK/PD relationships.

Using the streaMLine platform, we developed novel GLP-1R agonists, to demonstrate how high throughput screening peptide libraries and machine learning guided drug design can be applied to accelerate drug discovery. We systematically synthesized and screened a total of 2,688 peptides in a parallelized optimization workflow. Using this approach, we identified a vast chemical solution space for generating novel GLP-1R agonists based on an alternative peptide starting point, that is, the secretin backbone.

To validate the developed QSAR pipeline, we conducted an in-depth profiling of a developed GLP-1R agonist that showed high receptor selectivity, attractive physicochemical properties and pharmacokinetic profiling, and a potent weight-lowering *in vivo* efficacy.

LECTURE 57

Peptide-Based Therapeutics for Treatment of Cardiometabolic Diseases

Christoffer Clemmensen
Associate Professor, University of Copenhagen

The global obesity epidemic presents a critical challenge to human health, with weight regain following initial weight loss remaining a major hurdle in long-term obesity management. While incretin-based therapies have demonstrated substantial efficacy in weight reduction, sustaining these benefits requires innovative approaches targeting homeostatic weight regain mechanisms.

This presentation covers the current and emerging landscape of peptide-based therapeutics for weight management and introduces a novel peptide-drug conjugate designed to deliver small-molecule NMDA receptor antagonists via incretin hormones, enabling cell-specific modulation of neuroplasticity to amplify weight loss and metabolic benefits.

Our strategy harnesses the selectivity of incretin G protein-coupled receptors, GPCRs, expressed in hypothalamic and brainstem neurons to achieve targeted drug delivery. By coupling NMDA receptor antagonists to incretin-based peptides, our conjugates facilitate receptor-mediated uptake into key cell populations, thereby disrupting the maladaptive neuroplasticity associated with obesity.

This interdisciplinary effort bridges medicinal chemistry, neuroscience, and pharmacology to pioneer a multimodal pharmacotherapy targeting homeostatic weight regulatory mechanisms. By addressing a crucial unmet medical need, our peptide-based intervention has the potential to contribute to next-generation obesity treatments and reducing the burden of obesity-related comorbidities.

LECTURE 58

Two Bites at the Apple: Design and Repair of Peptide Therapeutics

Makineni Lecture

Krishna Kumar
Robinson Professor of Chemistry, Tufts University

The two incretin hormones GLP-1 and GIP are key regulators of glucose homeostasis and appetite signaling. The hormonal actions of these peptide ligands are mediated by binding and stimulation of the cognate receptors, GLP-1R and GIPR, followed by downstream signaling. Both peptides suffer from rapid inactivation by protease, DPP4, catalyzed hydrolysis. We have previously shown that N-terminal alkylation with a variety of chemical decorations can render the peptides refractory to enzyme action while simultaneously preserving receptor agonism.

Building on this molecular design strategy, we recently developed a new class of unimolecular tetra-agonists that engage four distinct receptors—GLP-1R, GIPR, glucagon receptor, GcgR, and neuropeptide Y2 receptor, Y2R, within a single peptide framework. This unprecedented integration of class B and class A GPCR targeting was achieved through rational engineering of chimeric sequences, leveraging struc-

ture-guided substitutions, C-terminal grafting of the PYY activation motif, and strategic lipidation. These tetra-agonists retain potent and balanced receptor activity, demonstrate biased signaling at GLP-1R favoring cAMP over β-arrestin recruitment, and exhibit robust resistance to DPP4-mediated degradation. The resulting scaffolds show therapeutic promise for enhancing glycemic control and inducing weight loss through synergistic hormonal modulation.

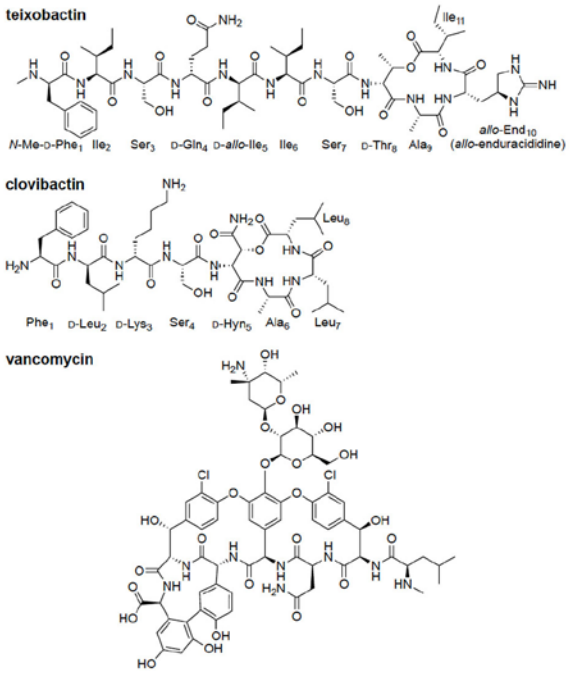
In addition, we will discuss the design of small molecule agents that can 'repair' the truncated inactive peptide derived from GLP-1 into a full-length functional compound. These studies present complementary strategies to modulate the glucose maintenance and weight loss through molecular design.

LECTURE 59

Potent Peptide Antibiotics Against Pernicious Pathogens

James Nowick
Distinguished Professor University of California, Irvine

New antibiotics of last resort are desperately needed to treat antibiotic-resistant infections from pathogens such as MRSA and VRE. This talk will describe how my research group has overcome problems associated with teixobactin and clovibactin, two promising new peptide antibiotics against Gram-positive bacteria that have not yet made it into the clinic.



The talk will also describe how we have overcome vancomycin resistance through the creation of vancomycin–teixobactin conjugates. Each of these projects has yielded potent new antibiotics with improved pharmacological properties over the naturally occurring antibiotics.

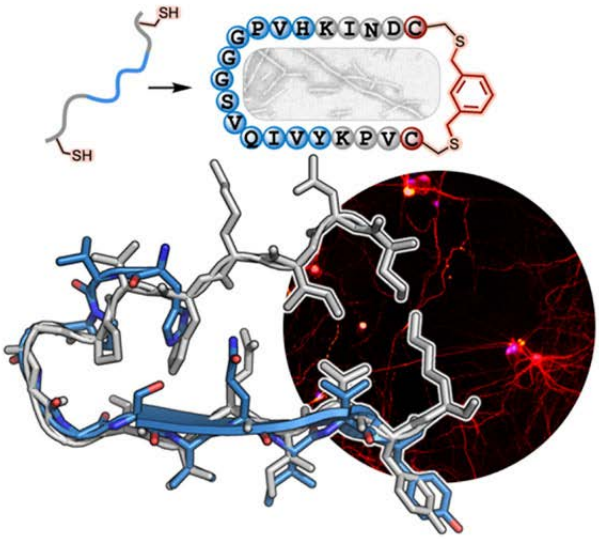
It is hoped that these studies will not only illuminate promising preclinical drug candidates but also shed light on three fascinating peptide antibiotics that achieve their remarkable mechanisms of action through molecular recognition and supramolecular assembly.

LECTURE 60

β-Arch Macrocycles as Functional Tau Proteomimetics

Juan R. Del Valle
W.K. Warren Family Professor, University of Notre Dame

The conformational plasticity of tau allows it to play a role in many important cellular processes; however, its misfolding and self-assembly into pathological amyloid filaments characterizes several neurodegenerative disorders. Disease-associated tau exhibits β-arch architecture, wherein protein monomers adopt β-arch folds that stack in register. The resulting filaments can seed the misfolding of soluble tau and propagate across neurons in a prion-like manner.



Recent structural data demonstrate that the conformations of tau protomers within filaments varies by disease, even when they are comprised of the same isoform and sequence. This raises the intriguing possibility of a link between conformational fold, seeding capacity, and disease progression. Current models of tau propagation based on co-factor-induced aggregation fail to capture the structural diversity of pathological tau folds. Access to homogenous, disease-relevant tau conformers is thus urgently needed.

Here, we describe the structure-based design and synthesis of peptide macrocycles that mimic the form and function of patient-derived tau aggregates. Diversity-oriented covalent stapling of core motifs observed in 4R tau folds from idiopathic disease affords cyclic β-arch peptides that self-assemble into amyloid filaments. A subset of these "mini-tau" macrocycles potently seed endogenous tau in engineered biosensor cells and primary neuronal cell cultures. Structural elucidation of our mini-tau filaments by cryo-EM reveals several conformational features congruent with those in pathological tau folds. Our studies provide a framework for the minimization of functional, disease-associated epitopes of amyloidogenic proteins that could find broad application in the development of vaccines and therapeutic antibodies.

LECTURE 61

Targeting Phosphatases with Peptides and Phosphomimetics

Maja Köhn
Professor, University of Bonn

Protein phosphatases play key roles in essential cellular processes. Their misregulation contributes to diseases such as cancer, immune diseases, and heart failure. Accordingly, there is a strong interest to study their mechanisms and druggability, as well as to validate them as disease targets.[1]

Often phosphatases are regulated by protein–protein interactions, PPIs, which cover large surfaces and are not amenable for small molecule binding.[1,2] Peptides and peptidomimetics as research tools and drug leads enable targeting large surface areas to inhibit PPIs, and natural interactions can be used as a starting point to design selective, stable, and cell-active peptidic PPI inhibitors.

Taking advantage of these properties, we develop peptide-based modulators for phosphatases, including their photo-induced activation and the incorporation of phosphomimetics.[3-5] I will discuss the development of bioactive peptides in the context of phosphatases, including the observation of rather unexpected findings concerning binding affinities and biochemical effects of those modulators.

[1] Köhn M. *ACS Cent. Sci.* **2020**, 6, 467–477.
[2] Kokot T, Köhn M. *J. Cell Sci*. **2022**, 135, jcs259618.
[3] Chatterjee J, Beullens M, Sukackaite R, Qian J, Lesage B, Hart D, Bollen M, Köhn M. **Angew. Chem. Int. Ed. 2012**, 51
[4] Trebacz M, Wang Y, Makotta L, Henschke L, Köhn M. **J. Org. Chem**. **2020**, 85, 1712–1717.
[5] Maller C, Marouda E, Köhn M. *ChemBioChem* **2024**, 25, e202400561.

LECTURE 62

Going with the Flow: Proton-Selective Transport through *De Novo* Designed Peptide Bundles

Huong Kratochvil
Assistant Professor, University of North Carolina at Chapel Hill

The precise transport of protons across cellular membranes is key for many biocatalytic and bioenergetic processes. Proton channels facilitate the selective and efficient movement of protons for all necessary functions while maintaining membrane fidelity and preventing ion leakage. The dynamics of interlumenal waters and pore-lining sidechains, among other features, define the ability of these channels to selectively and rapidly transport protons.

Through the *de novo* design of defined helical peptide bundles, we test the roles of specific sidechain chemistries and dynamics in the transport of protons. Our synthetic peptide channels reveal how specific protein–water interactions contribute to proton transport, providing new insights into the dynamics necessary for proton-selective function.

These studies not only uncover the physicochemical determinants of proton selectivity but also reveal key design principles in defining self-assembling membrane helical peptides. Further, these findings underscore the critical role of dynamic interactions in achieving both selectivity and efficiency, highlighting the need to explicitly consider these features in the design of selective peptide channels and other biomolecular systems for tailored functionality.

LECTURE 63

Semicarbazides as Amino Amide Surrogates in Peptide Mimicry for Treating Unmet Medical Conditions

William Lubell
Professor, Université de Montréal

Goodman Lecture

Substitution of nitrogen for the central alpha-carbon of an amino acid in a peptide has pertinent consequences on the conformation and activity of the resulting azapeptide.[1] The semicarbazide analog exhibits enhanced amide Brønsted acidity and Lewis basicity, favoring hydrogen bonding.[2,3] Moreover, pyramidalization of the alpha-amine in the aza-amino amide introduces nitrogen chirality with potential for dynamic inversion.[4]

Presenting such phenomena in the context of translational research, this talk will feature various advances in studying different semicarbazide motifs in peptide leads toward azapeptide candidates with therapeutic potential. Azapeptide analogs have been studied in the context of recognition and disruption of amyloid proteins for agents aimed at early detection and treatment of Alzheimer's disease.[2,3] In peptide ligands of the cluster of differentiation-36 receptor, CD36, semicarbazides have given high affinity and selectivity in azapeptide modulators of macrophage-driven inflammation with potential for treating diseases such as age-related macular degeneration and atherosclerosis.[4] Moreover, topological mimicry of peptide turn conformations using semicarbazide analogs has transformed peptide leads into peptide mimic modulators of the urotensin receptor, which mediates cardiovascular function.[5]

The utility of the semicarbazide motif in peptide science will be highlighted, with a focus on recent efforts in the synthesis and study of azapeptides.

[1] Chingle R, Proulx C, Lubell W.D. *Acc. Chem. Res.* **2017**, 50, 1541–1556.
[2] Habashi M et al. *J. Med. Chem.* **2023**, 66, 3058–3072.
[3] Habashi M et al. *Proc. Natl. Acad. Sci. U.S.A.* **2022**, 119(49), e2210766119.
[4] Proulx C et al. *Biomedicines* **2020**, 8(8), E241.
[5] Wei X et al. *J. Med. Chem.* **2023**, 66, 14241–14262.

LECTURE 64

Addressing the Challenge of Selective Inhibition of Histone Trimethyllysine Reader Proteins through Electrostatic Pocket Mapping

Marcey Waters
Glen H. Elder, Jr. Distinguished Professor, University of North Carolina at Chapel Hill

Post-translational modifications, PTMs, in histone proteins, including lysine methylation and acylation, regulate gene expression through recruitment of reader proteins to the nucleosome. Dysregulation of these events is prevalent in a wide range of diseases, such that there is much interest in developing inhibitors for these protein-protein interactions.

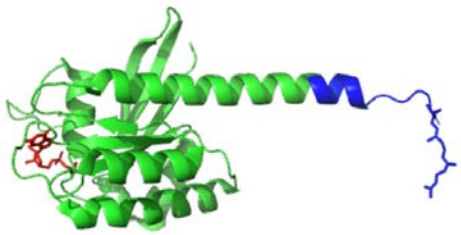
Nonetheless, each PTM typically has numerous reader proteins that bind it, making selective inhibition a challenge. To address this challenge, we have undertaken a campaign to map the diQerences in selectivity among reader proteins via pocket mapping, using a range of histone peptides containing a range of abiotic amino acid isosteres in place of the PTMs of interest. Results of these pocket mapping eQorts will be presented.

LECTURE 65

Synthetic Peptides and Modified Forms of Full-Length K-Ras4B, Prepared via Expressed Protein Ligation, Provide Insights into its Posttranslational Modification

Mark Distefano
Distinguished McKnight University Professor, University of Minnesota

Mutations in K-Ras4B are drivers in approximately 20% of all human cancers.[1] The protein undergoes post-translational modification steps including prenylation, proteolysis, and methylation near its C-terminus. To gain insight into this complex process, full-length forms of K-Ras4B containing various chemical probes have been prepared via expressed protein ligation between an N-terminal thioester, green, and a 15-residue C-terminal peptide, blue.[2]



Synthetic peptides containing residues incorporating non-natural dansyl groups were used to study the rate of prenylation while peptides incorporating diazirine-containing isoprenoids allowed interacting proteins to be identified. Confocal imaging of fluorescently labeled K-Ras4B allowed its localization to be followed in living cells. Incorporation of peptides containing orthogonal azide- and alkyne-containing isoprenoids allowed the assembly of a covalent dimer.

[1] Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. *Nat. Rev. Drug Discov.* **2014**, 13, 828–851.

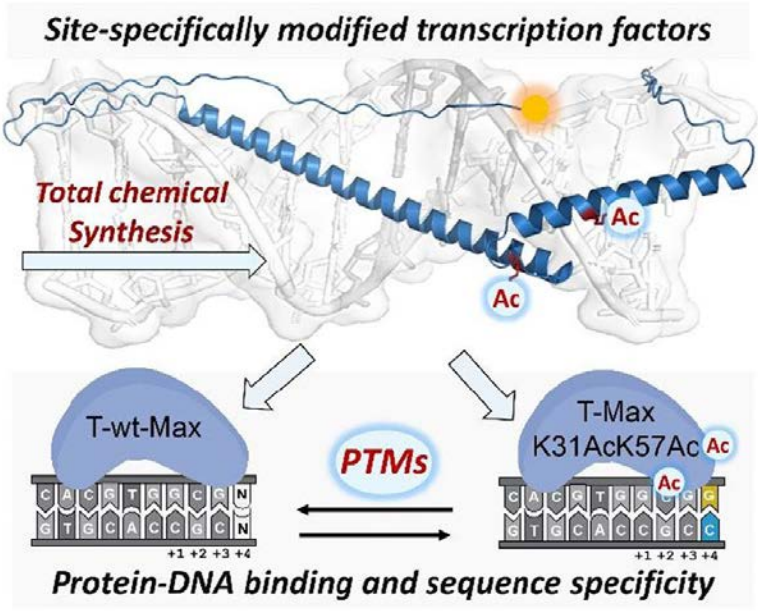
[2] Zhang SY, Sperlich S, Li FY, Al-Ayoubi S, Chen HX, Zhao YF, Li YM, Weise K, Winter R, Chen YX. *ACS Chem. Biol*. **2017**, 12

LECTURE 66

Cracking the Code of PTMs on Transcription Factor-DNA Interactions Using Synthetically Modified Proteins

Muhammad Jbara
Assistant Professor, Tel Aviv University

Chemical protein synthesis provides a powerful means to prepare novel modified proteins with precision down to the atomic level, enabling an unprecedented opportunity to understand fundamental biological processes.[1] Of particular interest is the process of gene expression, orchestrated through the interactions between transcription factors, TFs, and DNA. Here, we combined chemical protein synthesis and high-throughput screening technology to decipher the role of post-translational modifications, PTMs, for example, Lys-acetylation and Ser-phosphorylation, on the DNA binding activity of Max TF.



We synthesized a focused library of singly, doubly, and triply modified Max variants including site-specifically phosphorylated, acetylated, and fluorescently tagged analogs, for the first time.[2,3] The resulting synthetic analogs were employed to decipher the molecular role of Ser-phosphorylation and Lys-acetylation on the DNA binding activity and sequence specificity of Max.

We provide evidence that the acetylation sites at Lys-31 and Lys-57 and the phosphorylation at Ser-11 significantly inhibit the DNA binding activity of Max. Furthermore, we found that the acetylation mark can alter the binding specificities of Max toward certain sequences flanking its consensus binding sites. Our work provides insight into the hidden molecular code of PTM–TF and DNA interactions, paving the way to interpret gene expression regulation programs.[4]

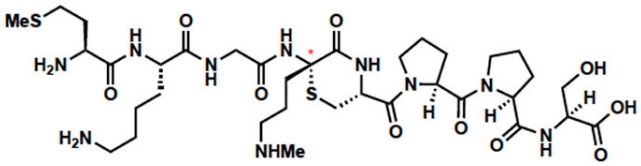
[1] Harel O, Jbara M. *Angew. Chem. Int. Ed.* **2023**, 62, e202217716.
[2] Nithun R, Yao Y, Lin X, Habiballah S, Afek A, Jbara M. *Angew. Chem. Int. Ed.* **2023**, 62, e202310913.
[3] Nithun R, Yao Y, Harel O, Habiballah S, Afek A, Jbara M. *ACS Cent. Sci.* **2024**, 10, 1295–1303.
[4] Lin X, Harel O, Jbara M. *Angew. Chem. Int. Ed.* **2024**, 63, e202317511.

LECTURE 67

Chemical Synthesis of Ribosomally Synthesized and Post-Translationally Modified Peptides, RiPPs

Chi Ting
Assistant Professor, Brandeis University

Ribosomally synthesized and post-translationally modified peptides, RiPPs, are a growing class of natural products, many of which possess antimicrobial activity. Sactipeptides are one subclass of RiPPs that are defined by thioaminoketal functional groups. In this presentation, thioaminoketals are assembled through the Markovnikov hydrothiolation of dehydroamino acids using a dithiophosphoric acid catalyst. This method results in the formation of the thioaminoketal directly from peptides containing dehydroamino acids and overrides the inherent reactivity of thiols to undergo conjugate addition.



enteropeptin A
[14 steps]
[stereochemical assignment]

Despite their therapeutic potential, many sactipeptides are stereochemically undefined, preventing their further development into antibiotics. Enteropeptin A is an antimicrobial sactipeptide with a highly unusual thioaminoketal embedded in a thiomorpholine ring. The stereochemical configuration at its thioaminoketal stereocenter was not assigned when the natural product was isolated.

In this presentation, the total synthesis of enteropeptin A is discussed. The synthesis of enteropeptin A and its diastereomer enabled the structural elucidation and the determination of the stereochemical configuration of enteropeptin A. In the synthesis, the Markovnikov hydrothiolation reaction was applied in a stereoselective cyclization to form the thiomorpholine ring. These results form a foundation and potential guidelines for the development of stereoselective peptide cyclization.

LECTURE 68

Identification of Lysine Acetoacetylation as a Novel Protein Post-Translational Modification

Y. George Zheng
Panoz Professor of Pharmacy, University of Georgia

Short chain fatty acylations establish connections between cell metabolism and regulatory pathways, serving as an adapted mechanism for cells when encountering variations in intracellular and environmental signaling cues. Lysine acetoacetylation, Kacac, was recently identified as a new post-translational modification, PTM, in histones. However, regulatory elements, substrate proteins, and epigenetic functions of Kacac remain unknown, hindering further in-depth functional understanding of ketone body modulated (patho)physiological processes.

Here, we introduce a chemo-immunological approach that enables rapid, straightforward, and reliable detection of Kacac, as well as its simultaneous comparison with lysine β-hydroxybutyrylation. Using this approach, we demonstrate that acetoacetate, rather than ketogenic amino acids, serves as the primary precursor for histone Kacac in HEK293T cells. We show that the histone acyltransferases GCN5, p300, and PCAF catalyze the enzymatic addition of the acetoacetyl motif from acetoacetyl-CoA to lysine, while histone deacetylase 3, HDAC3, enzymatically removes Kacac.

Furthermore, we establish acetoacetyl-CoA synthetase, AACS, is a major player in mediating cellular levels of Kacac in proteins. We conducted a comprehensive proteomic analysis of acetoacetylated proteins in human cells, and we identify 139 Kacac sites on 85 substrate proteins. Bioinformatics analysis of Kacac substrates and RNA-seq data reveal the broad impacts of Kacac on multifaceted cellular processes especially DNA/RNA/amino acid metabolism, gene expression, proliferation, and immune response.

These findings unveil pivotal functions and regulatory mechanisms for the acetoacetate-mediated Kacac pathway, opening a new avenue for further investigation into ketone body functions in various cellular processes and pathophysiological states.

LECTURE 69

Ubiquitin Signaling: Chemistry, Biology and Drug Discovery

Ashraf Brik
Professor, Technion-Israel Institute of Technology

du Vigneaud Lecture

Posttranslational modification of proteins by ubiquitin, Ub, such as ubiquitination, mediates various cellular processes, including protein homeostasis, cell cycle, DNA repair, and viral infections. Understanding the role of ubiquitination in these events is the basis for unraveling its precise role in health and disease. Chemical protein synthesis offers great opportunities to dissect the molecular basis of Ub signaling, as demonstrated in various examples.[1]

In this talk, I will highlight the progress of my laboratory in preparing unique ubiquitin conjugates to advance our understanding of this signal in detail.[2] As a showcase, I will present the chemical synthesis of tetra-ubiquitinated proteins that are differentially labeled at the Ub chain and the protein of interest to shed light on fundamental aspects of proteasomal degradation.[3-5] I will also present the novel chemistry that emerged from these synthetic efforts and its utility in peptide and protein synthesis in general.[6,7]

Finally, I will show how these methods, combined with the Random Non-Standard Peptides Integrated Discovery method, RaPID, allowed us to discover novel cyclic peptides that modulate Lys48- or Lys63-linked Ub chains selectively to interfere with their biological function, for example, proteasomal degradation and DNA repair, and bring forward potential new therapeutic modalities.[8,9]

1 *Nature Chemistry*, **2016**, 8, 407–418.
2 *Journal of American Chemical Society*, **2017**, 139, 4971–4986.
3 *Nature Communications*, **2021**, 12:6173.
4 *Proc. Natl. Acad. Sci*. **2013**, 110, 17726-17731.
5 *Accounts of Chemical Research*, **2019**, 52, 12, 3361-3371.
6 *Angew. Chem. Int. Ed.*, **2017**, 56, 10644-10655.
7 *Nature Communications*, **2018**, 9, 1-11.
8 *Nature Chemistry*, **2019**, 11, 644–652.
9 *Nature Communications*, **2022**, 13, 6174.

LECTURE 70

De Novo Discovery of Pseudo-Natural Products

Hiroaki Suga
Professor, University of Tokyo

Macrocyclic peptides possess a number of pharmacological characteristics distinct from other well-established therapeutic molecular classes, resulting in a versatile drug modality with a unique profile of advantages. Pseudo-natural macrocyclic peptides are accessible by not only chemical synthesis but also ribosomal synthesis. Particularly, recent inventions of the genetic code reprogramming integrated with an *in vitro* display format, referred to as RaPID, Random non-standard Peptides Integrated Discovery system, have enabled us to screen mass libraries over trillion members.

We have recently developed a method of *in vitro* synthesis of lactazole A, one of thiopeptides, using the FIT, flexible in-vitro translation, system integrated with six Laz enzymes, and demonstrated one-pot synthesis of its analogs. This has led to construct a mass library, ~10^{12}, of pseudo-natural lactazoles under reprogrammed genetic code, which was used for mRNA display to select bioactive species against proteins of interest. This lecture discusses the development of the technology and therapeutic potentials of pseudo-natural products.

LECTURE 71

Membrane-Permeable Cyclic Peptides Against Intracellular Targets and for Oral Delivery

Christian Heinis
Associate Professor, EPFL

Our laboratory is involved in the discovery and development of cyclic peptides for therapeutic applications. In recent years, we have begun to address the long-standing goal of developing target-specific peptides that are membrane-permeable and orally available. To this end, we are focusing on the generation of cyclic peptides that have a relatively small size, <1 kDa, and a limited polar surface area, so that they have a high chance of passively crossing membranes.

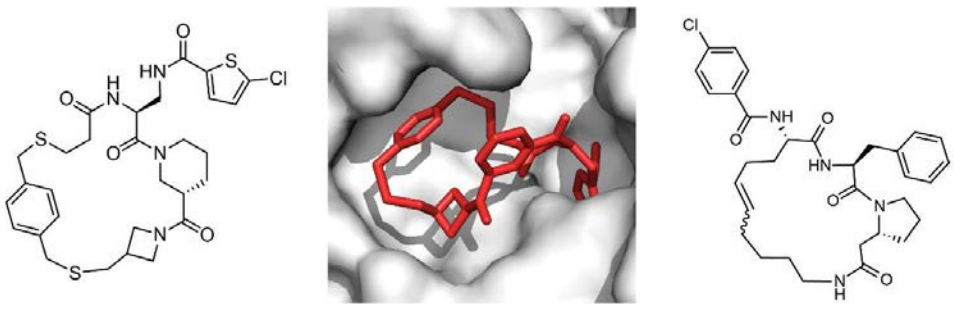


Figure: Structures of membrane permeable and orally available thrombin inhibitors identified by screening ten-thousands of random small synthetic peptides.

To generate sub-kilodalton cyclic peptides that bind to disease targets of interest, we have established an approach based on nanomole-scale cyclic peptide synthesis and high-throughput screening of crude products.[1,2] In short, we generate thousands of peptides by solid-phase peptide synthesis and combinatorially diversify them by reacting them with a myriad of chemical building blocks. In this approach, all reagents are transferred in nanolitre volumes by acoustic dispensing and reactions are performed at the nanomole scale, allowing tens of thousands of cyclic peptides to be synthesized and screened in a short time. Recently, we have shown that cyclic peptides developed using this approach can achieve good oral availability.[3]

In my talk, I will explain the approach to the synthesis and screening of cyclic peptide libraries, show examples of libraries and their screening, present nanomolar ligands we have developed against different proteins. In addition, I will present recent learnings about the structure-membrane permeability of cyclic peptides.

[1] S. Kale, et al., *Science Advances*. **2019**, 5, 8,
[2] S. Habeshian, et al., *Nature Communications*. **2022**, 13, 3823
[3] M.L. Merz, et al., *Nature Chemical Biology*. **2024**, 20, 5

LECTURE 72

A Cyclic Peptide Toolkit to Modulate Protein Citrullination

Louise Walport
Group Leader, Imperial College London and The Francis Crick Institute

Peptidyl arginine deiminase IV, PADI4, catalyses the citrullination of a wide range of substrates, with roles in neutrophil extracellular trap formation, chemokine signalling and establishment of pluripotency. Aberrant activity is implicated in a range of pathologies, including rheumatoid arthritis, multiple sclerosis, and various cancers, with emerging evidence suggesting PADI4 inhibition has significant therapeutic potential. Despite its clear therapeutic potential, key questions remain around its cellular regulation and activation.

To provide new tools to understand PADI4 function we have developed a set of cell permeable, potent and selective cyclic peptide modulators of PADI4. We employed the RaPID, Random non-standard Peptide Integrated Discovery, system to isolate binders of PADI4 from starting libraries of up to 10^{13} genetically-barcoded cyclic peptides. From three parallel screens designed to target different protein conformations, we identified a series of nanomolar PADI4 peptide binders, with diverse activities. In addition to a potent cell permeable PADI4 inhibitor, we identified first-in-class activators of PADI4 to low calcium conditions both in vitro and in cells. Structures of activator-bound PADI4 reveal an allosteric binding site that may also be important for physiological PADI activation.

We subsequently adapted the RaPID platform to identify covalent cyclic peptides, allowing identification of potent covalent cyclic peptide inhibitors of PADI4 and most recently, photoswitchable inhibitors of a related protein. Together, the peptides provide a powerful toolkit for further elucidation of the function of PADI4 in both normal development and disease, with potential to provide a basis for downstream drug development.

LECTURE 73

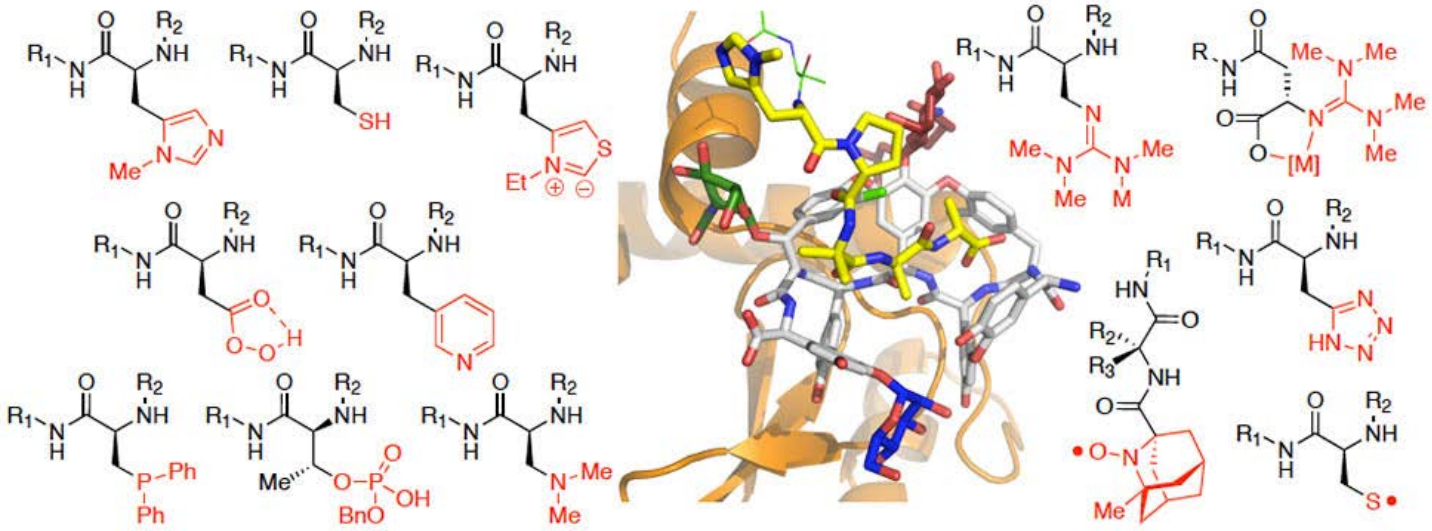
Peptide-Based Catalysis Version 2.0

Scott Miller
Sterling Professor of Chemistry, Yale University

Closing Keynote Lecture

This lecture will describe recent developments resulting from our efforts to develop peptide-based catalysts for asymmetric reactions, in particular for the preparation of densely functionalized, stereochemically complex structures. Over time, our foci have been on enantioselectivity, site-selectivity, and chemoselectivity.

In much of our current work, we are studying issues of enantioselectivity as a prelude to the extrapolation of catalysis concepts to more complex molecular settings where multiple issues are presented in a singular substrate. Complex natural products, for example, will be presented as quintessentially complex scaffolds for catalytic modification.



Mechanistic paradigms, and their associated ambiguities—especially in light of catalyst or substrate conformational dynamics—will figure strongly in the lecture. So too will questions related to the functional generality of catalysts as they confront various substrates, revealing occasional dichotomies of catalyst types that are better or worse suited for highly functionalized substrates.

Finally, several interesting collaborations—often unanticipated—will be discussed. A persistent theme in the lecture will be the extent to which small molecule-based catalysts and enzymatic catalysts share mechanistic analogies.

P-001

Structure-Activity Relationship Study of Tris-Benzamides as α-Helix Mimetics Targeting Estrogen Receptor for Treating Breast Cancer

Chia-Yuan Chen[1], Kara Kassees[1], Tae-Kyung Lee[1], Suryavathi Viswanadhapalli[2], Karla Parra[3], Ratna Vadlamudi[2], Jung-Mo Ahn[1]

[1]University of Texas at Dallas, Dallas, USA, [2]University of Texas Health Science Center at San Antonio, San Antonio, USA, [3]University of Texas Southwestern Medical Center at Dallas, Dallas, USA.

Estrogen receptor coregulator binding modulators, ERXs, represent a new class of compounds that are designed to disrupt the interaction between estrogen receptor α, ERα, and its coregulator proteins. This strategy holds promise as a potential solution to overcoming endocrine resistance in breast cancer.

We previously reported ERX-11 that is a tris-benzamide-based α-helix mimetic replicating the LXXLL motif.[1] ERX-11 was shown to inhibit ERα-positive breast cancer cell proliferation with a moderate potency, however the role of the substituents on ERX-11 has not been examined. Therefore, we conducted structure-activity relationship, SAR, studies via progressively substituting additional alkyl substituents at either the N- or C-terminus on ERX-11. From the SAR studies, a trans-4-phenylcyclohexyl group at the C-terminus showed greater than 10 times improvement in binding affinity and cell growth inhibition potency.

In this study, TK525 was identified to disrupt the ERα-coregulator interaction and suppress ERα-mediated transcriptional activity effectively.[2] It demonstrated strong antiproliferative effects on ERα- positive breast cancer cells both *in vitro* and *in vivo*, underscoring its promising potential as a therapeutic candidate for ERα-positive breast cancer.

[1] Raj, G. V.; Sareddy, G. R.; Ma, S.; Lee, T.-K.; Viswanadhapalli, S.; Li, R.; Liu, X.; Murakami, S.; Chen, C.-C.; Lee, W.- R.; Mann, M.; Krishnan, S. R.; Manandhar, B.; Gonugunta, V. K.; Strand, D.; Tekmal, R. R.; Ahn, J.-M.; Vadlamudi, R. K. *eLife* **2017**, 6, e26857.

[2] Lee, T.-K.; Kassees, K.; Chen, C.-Y.; Viswanadhapalli, S.; Parra, K.; Vadlamudi, R. K.; Ahn, J.-M. *ACS Pharmacol. Transl. Sci.* **2024**, 7, 2023-2043.

P-002

A Bi-Facial Tris-Benzamide ScaffoldMimicking Two Helical Surfaces

Chia-Yuan Chen, Jacob Michael Soares, Jung-Mo Ahn

University of Texas at Dallas, Dallas, USA

Over the past two decades, α-helical peptides have been widely used to study protein-protein interactions, PPIs, and to explore potential therapeutic candidates. However, natural peptide fragments often adopt poorly defined structures in solution, which makes them less likely to effectively bind to target proteins. Additionally, they tend to have significant drawbacks, such as susceptibility to enzymatic degradation, limited bioavailability, and poor membrane penetration. As a result, α-helical peptidomimetics has emerged as a more promising option for biomedical studies.

Previously, we reported a tris-benzamide scaffold that can place the side chains of three amino acid residues found at the i, i+4, and i+7 positions in a helix,[1] and ERX-11 was developed to inhibit estrogen receptor a for treating breast cancer.[2] To make improvements in the selectivity and potency as well as to gain a better understanding of the structure-activity relationship and potentially to be used in other hormone-sensitive cancers, we designed and synthesized a bi-facial 4-aminobenzoic acid. The tris-benzamide with this bi-facial unit allows to place four side chain functional groups found at the i-2, i, i+4, and i+7 positions of an ideal α-helix, reproducing two helical faces.

[1] Chen, C.-Y.; Elmore, S.; Lalami, I.; Neal, H.; Vadlamudi, R. K.; Raj, G. V.; Ahn, J.-M. *Method Enzymol.* **2024**, 698, 221-245.

[2] Raj, G. V.; Sareddy, G. R.; Ma, S.; Lee, T.-K.; Viswanadhapalli, S.; Li, R.; Liu, X.; Murakami, S.; Chen, C.-C.; Lee, W.- R.; Mann, M.; Krishnan, S. R.; Manandhar, B.; Gonugunta, V. K.; Strand, D.; Tekmal, R. R.; Ahn, J.-M.; Vadlamudi, R. K. *eLife* **2017**, 6, e26857.

P-003

Thio-Oxazole Modified Peptides Biosynthesized by Multinuclear Iron-Dependent Oxidative Enzymes, MNIOs

Mayuresh Gadgil, Douglas Mitchell

Vanderbilt University, Nashville, USA

Multinuclear iron-dependent oxidative, MNIO, enzymes are a class of peptide-modifying enzymes involved in the biosynthesis of ribosomally synthesized and post-translationally modified peptide, RiPP, natural products. A recently published report details how one member of this enzyme family transforms Cys residues in precursor peptides bearing -EGKCG- motifs into oxazolone/thioamide moieties.

By analyzing closely related homologs of this enzyme which also act on identical substrate motifs, we provide spectroscopic evidence to suggest that the formed post-translation modification is in fact a (5-thio)-oxazole rather than the oxazolone/thioamide structure. *In vitro* activity reconstitution of the purified MNIO enzyme, coupled with a cell-free biosynthesis platform for generating substrate peptide variants including unnatural amino acid incorporation by the flexizyme method, allowed for the elucidation of key enzyme-substrate recognition elements.

Mass spectrometry demonstrated that the (5-thio)-oxazoles effectively bind transition metals, thereby suggesting the functional utilization of these natural products as defense against metal-induced stress. We also provide evidence of the role of these (5-thio)-oxazole containing biosynthetic gene clusters in microbes for evasion from metal-mediated immune responses.

P-004

Bioengineering of Precursor Peptides to Develop Novel Hybrid RiPPs in Vitro

Songyi Xue, Wilfred van der Donk

University of Illinois Urbana-Champaign, Champaign, USA

Ribosomally synthesized and post-translationally modified peptides, RiPPs, are emerging as a large group of natural products. RiPPs are an attractive starting point for developing novel molecules with desired bioactivities, like antibiotics, antifungals, antivirals. Because of their conserved biosynthetic logic and genetic simplicity engineering changes to their structures are relatively facile.

In this work, our goal was to bioengineer RiPPs with novel structures. Combinatorial biosynthesis, which combines various enzymes from different biosynthetic pathways, is an appealing method for producing novel compounds with desirable structural properties. Our group has already created non-natural hybrid RiPPs products *in vivo* using combinatorial biosynthesis.

In this work, we present a new strategy for engineering RiPP precursor peptides *in vitro* to produce novel hybrid RiPPs, which could broaden the scope of combinatorial biosynthesis applications. With the new methods, we envision developing genetically encoded libraries in vitro to screen disease-related targets to identify novel peptide inhibitors.

Our genetically encoded library will provide a generic platform for the development of more potent and long-acting peptide modulators that inhibit a variety of disease-related protein-protein interactions, PPIs.

P-005

Loss-of-Function Mutations and *Helicobacter pylori* Pathoadaptation

Anamika Ghosh, Ankita Das, Debabani Ganguly, Sujay Chattopadhyay

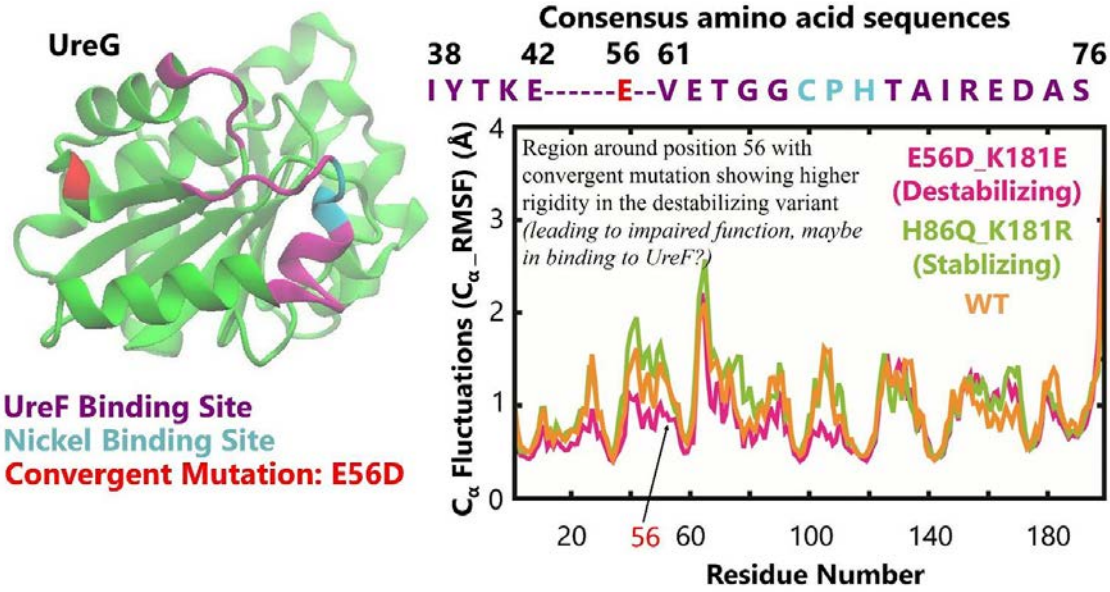
Centre for Health Science and Technology, JIS Institute of Advanced Studies and Research Kolkata, JIS University, West Bengal, India

One common mode of bacterial adaptive evolution is the inactivation of specific genes, functional presence of which may appear detrimental to the organism in some altered environmental conditions, following 'die-or-lose' dynamics, via accumulation of either non-synonymous mutations or truncation mutations, TnM, leading to a premature stop of the translation process.

We aimed to assess the potential pathoadaptive contribution of loss-of-function mutations in *Helicobacter pylori*, a rapidly evolving human-adapted organism known for its colonization in harsh acidic environments and association with gastritis, peptic ulcers, and gastric cancer. Our *in-silico* study on 346 complete *H. pylor*i genomes detected both TnM and convergent non-synonymous mutations, CM, in a set of genes.

While CM occurrence is thought to be a powerful adaptive marker, we hypothesized the overlap of CM and TnM to be an accumulation of potentially adaptive loss-of-function mutations in specific isolates.[1-3] Interestingly, one of these genes encodes UreG, essential for functional urease that helps *H. pylori* colonize and survive in the acidic gastric environment. Importantly, the CMs at position 56, flanked by two UreF-binding domains, were found to considerably destabilize the protein structure, showing significantly higher rigidity than the wild type in the molecular dynamics simulation analysis, similar to what detected for truncated variant.

If this lack of conformational stability and flexibility indicate non-functionality of the corresponding variants, we find that ~7% of our analysed isolates possibly harbour inactivated ureG gene, majority, ~65%, representing peptic ulcer patients. Future experimental studies are warranted to understand potential fitness impact of such inactivation events.



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[2] Robinson, S.M.; Rajachandran, V.; Majumdar, S.; Saha, S.; Das, S.; Chattopadhyay, S. *Microbiol Spectr*. **2022**, 10, e0196921.

[3] Gong, Y.; Zhai, K.; Sun, L.; He, L.; Wang, H.; Guo, Y.; Zhang, *J. Microbiol Spectr*. *2023*, 11, e0390322.

P-006

CyclicCAE: A Conformational Autoencoder for Effi cientHeterochiral Macrocyclic Backbone Sampling

Andrew Powers[1], P. Douglas Renfrew[2], Parisa Hosseinzadeh[1], Vikram Mulligan[2]

[1]University Of Oregon, Eugene, USA; [2]Flatiron Institute, New York, USA

Macrocycles are a promising therapeutic class. The incorporation of heterochiral and non-natural chemical building-blocks presents challenges for rational design, however.

With no existing machine learning methods tailored for heterochiral macrocycle design, we developed a novel convolutional autoencoder model to rapidly generate energetically favorable macrocycle backbones for heterochiral design and structure prediction.

Our approach surpasses the current state-of-the-art method, Generalized Kinematic loop closure, GenKIC, in the Rosetta software suite. Given the absence of large, available macrocycle datasets, we created a custom dataset in-house and *in silico*.

Our model, CyclicCAE, produces energetically stable backbones and designable structures more rapidly than GenKIC. It enables users to perform energy minimization, generate structurally similar or diverse inputs via MCMC, and conduct inpainting with fixed anchors or motifs. We propose that this novel method will accelerate the development of stable macrocycles, speeding up macrocycle drug design pipelines.

P-007

Computational Structure Prediction of Lanthipeptides with NMR Data Reveals Underappreciated Peptide Flexibility

Claiborne W. Tydings[1,2],Jens Meiler[1,2,3], Allison S. Walker[1,4]

[1]Department of Chemistry, Vanderbilt University, Nashville, TN, USA. Nashville, TN 37232, USA.
[2]Center for Structural Biology, Vanderbilt University, Nashville, TN 37232 USA
[3]Institute for Drug Discovery, Leipzig University Medical School, Leipzig, SAC 04103, Germany
[4]Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA. Nashville, TN 37232, USA.

Our inability to computationally model and design lanthipeptides in molecular modeling and design software such as Rosetta limits our ability to rationally design lanthipeptides for drug discovery campaigns.

Lanthipeptides are a class of thioether containing ribosomally synthesized and post-translationally modified peptide which often have antibiotic activity and interest in using lanthipeptides for bioengineering applications is growing. We propose that implementing support for the lanthionine rings and dehydrated amino acids found in lanthipeptides will enable accurate lanthipeptide modeling with Rosetta.

We find that when compared to the ensembles of lanthipeptides with NMR determined structures in the PDB, lanthipeptide ensembles generated with Rosetta have similar experimental agreement, lower Rosetta energy scores, and greater flexibility.

Our use of ensemble averaged NOE distances instead of requiring individual structures to satisfy all NOE restraints was key for revealing the flexibility of these peptides. Our Rosetta lanthipeptide ensembles show increased flexibility in non-cyclized peptide regions as well as increased lanthionine ring flexibility when internal hydrogen bonds are absent and glycine residues are present. Support for lanthipeptides in Rosetta enables the design and modeling of lanthipeptides in Rosetta for therapeutic development.

P-008

Late-Stage Reshaping of Phage-Displayed Libraries to Macrocyclic Landscapes for Ligand Discovery

Kejia Yan,[1] Christin Kossmann,[2] Fernando Bañales Meija,[3] Dustin J. Maly,[4,5] and Ratmir Derda[1]
[1]Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada
[2]Zealand Pharma, Søborg, Denmark.
[3]Graduate Program in Biological Physics, Structure and Design, University of Washington, Seattle, WA 98195, USA
[4]Department of Chemistry, University of Washington, Seattle, WA 98195, USA
[5]Department of Biochemistry, University of Washington, Seattle, WA 98195, USA

Foundational Genetically-encoded libraries, GEL, platforms like phage-, yeast-, mRNA-display construct libraries using 20 natural amino acids, 20AA. GEL can be expanded by unnatural amino acids, UAA, and chemical post-translational modification, cPTM. The standard procedure involves incorporating UAA or cPTM into a "naïve" library, followed by multiple rounds selection. However, these strategies do not leverage binding information previously obtained from 20AA selections. Whether libraries with such prior knowledge can offer an effective path for ligand discovery remains unresolved.

To explore this, we evaluated the feasibility of discovering macrocyclic peptide ligands from "non-zero knowledge" libraries by chemically reshaping pre-selected phage-displayed 20AA binders against the NS3a variant proteases. The re-shaping is performed first using 3,5-bis(bromomethyl)benzaldehyde, termed KYL. KYL diversified peptide libraries into bicyclic architecture and delineated 2 distinct sequence populations: **i|** peptides with HXDMT motif retained binding upon bicyclization, **ii|** peptides without HXDMT motif lost binding once chemically modified. The same HXDMT family can be found in selections starting from naïve KYL-modified library. Cross-comparison with alternative linchpin dibromomethylbenzene for reshaping further validated the sequencedependent nature of retention.

This study provides a case example of using pre-selected 20AA libraries to generate advanced macrocyclic peptide ligands and suggests that existing 20AAderived binders may serve as viable templates for further chemical diversification.

P-009

Predicting Cyclic Peptide Structural Ensembles with Diffusion Models

Nomindari Bayaraa, M. Secor, M.L. Descoteaux, and Y.-S. Lin

Tufts University, Medford, USA

Cyclic peptides have emerged as a promising drug modality, but the development of cyclic peptide drugs is hindered by limited structural information. Despite continuous advances in experimental techniques, characterizing cyclic peptide structures remains challenging. For instance, the characterization of cyclic peptides using solution NMR is difficult because cyclic peptides tend to form multiple conformations in solution. On the other hand, computational methods can provide the necessary temporal and spatial information, making techniques like molecular dynamics, MD, simulation an attractive alternative. Unfortunately, MD simulation is both computationally expensive and time-consuming, rendering it unsuitable for large-scale screening.

Our research focuses on designing and training diffusion models to predict structural ensembles of cyclic peptides efficiently and accurately. The diffusion models are directly trained on MD simulation data. Specifically, each frame of an MD simulation trajectory is used as a single training instance, and each frame is represented as sine and cosine values of backbone dihedral angles. The trained diffusion model can not only generate MD-quality structures of cyclic peptides, but also the generated structures follow the Boltzmann distribution sampled in the MD simulation. This approach of directly training on the backbone dihedral angles observed in MD simulations can accelerate the resource-intensive drug discovery process by allowing efficient computational design of cyclic peptides targeting biologically relevant systems. Ultimately, we aim to generalize to larger cyclic peptides, and diffusion models may help us achieve that goal.

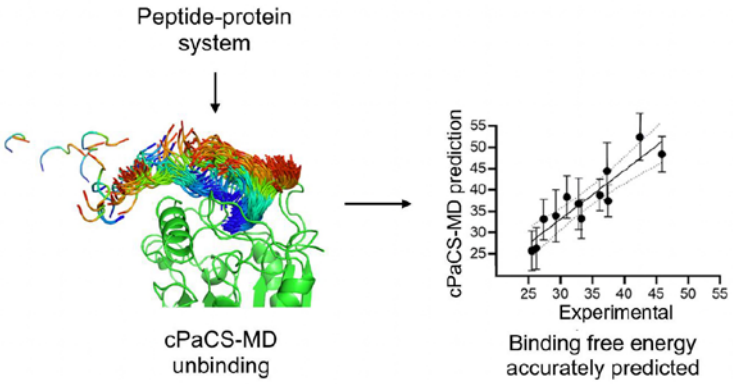
P-010

Dr PaCS-MD or How I Learned to Stop Worrying and Love *In Silico* Peptide Affinity Prediction

Viktor Prypoten,[1] Raymond S. Norton,[1,2] David K. Chalmers[1]

[1]Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia.
[2]ARC Centre for Fragment-Based Design, Monash University, Parkville, Victoria 3052, Australia

Peptide drugs make up a growing direction in the pharmaceutical market. However, computational prediction of how well peptides bind to their targets remains difficult, limiting the use of computational methods to assist peptide drug development. The large size and slow dynamics characteristic of peptides mean that typical computational approaches used to predict small molecule binding affinity struggle or fail entirely when used for peptides.



We have developed a novel method for modelling peptide unbinding, called contact Parallel Cascade Selection Molecular Dynamics, cPaCS-MD, that can accurately predict binding affinity in the range relevant to lead optimisation. We applied cPaCS-MD to a diverse set of twelve protein-peptide complexes and demonstrated superiority in both speed and accuracy over a widely used steered molecular dynamics-umbrella sampling, SMD-US, binding affinity prediction method.

cPaCS-MD predicts peptide binding affinity with a strong correlation, R^2=0.84, and high accuracy, mean absolute error of 2.7 kJ/mol, and is useful in a variety of peptide drug discovery applications, including assessment of docked structures.

P-011

Tumor-Associated O-Linked Glycopeptide Immunogen Design: The Glycosylated Amino Acid Affects Presentation and the Nature of Immune Response

Achyut Dahal, Caitlin Strain, Joseph Barchi

Chemical Biology Laboratory, Centre for Cancer Research, National Cancer Institute, NIH, Frederick, MD ,21702

Certain aberrant glycan structures covalently linked to proteins and/or lipids on the surface of tumor cells are called Tumor Associated Carbohydrate Antigens, TACAs. Many of these are O-linked glycans attached to serine, S, or threonine, T, hydroxyl groups in the long tandem repeat, TR, regions of large cell-surface glycoproteins called mucins. Of the 20 known mucins, Mucin-4, MUC4, was shown to be expressed in virtually all pancreatic ductal adenocarcinoma, PDAC, with no expression in the normal pancreas.

The MUC4 extracellular a-domain consists of repeats of 16 amino acid-long TRs, eg., TSSASTGHAT^{10}PLPVTD, rich in S and T, where most of these residues are O-glycosylated. Interestingly, various studies have shown that conformation around S versus T sites in mucin glycopeptides are strikingly different: Lectins may bind T-glycosylated sites over S-sites, and vice versa, and enzyme transfer of sugars to glycopeptides may prefer a T over an S site.

In our work, we found that glycosylation of MUC4-TR by GALNT-1, the enzyme that initiates O-linked glycan synthesis in humans, overwhelmingly preferred the T residue at position 10 of the MUC4-TR. This led us to synthesize 16-mer glycopeptides with either a wild type T or mutated S at position 10 with a specific TACA, the Thomsen-Friedenrech antigen, TFag, as the O-glycan. These glycopeptides were conjugated to Keyhole Limpet Hemocyanin, KLH, through our novel N-terminus thiol linker, mice were immunized, and a series of assays were carried out to assess differences in immune response.

We found that sera from both T10 and S10 glycopeptides bound antigens almost equally. However, flow-cytometry data showed that MUC4-T10TF polyclonal sera stained MUC4 positive cells much stronger compared to MUC4-S10TF sera. Our results thus far suggest comprehensive structural and immunological studies may help us to fully understand the differences in immune responses elicited by S versus T MUC4 glycopeptides and may facilitate the future design of highly selective tumor-specific MUC4-based immunotherapies.

P-012

Molecular Loops, Targeted Hits: Phage Display Unleashes the Power of Macrocyclic Peptides for Next-Generation Precision Therapeutics

Sebin Abraham, Wenshe Ray Liu

Texas A &b M University, College Station, TX, USA

BRD4, a key chromatin-binding protein, plays a crucial role in cancer progression by regulating transcriptional programs that drive tumor growth and survival. It has emerged as a promising therapeutic target in various malignancies, including acute myeloid leukemia, AML, BRD4-NUT fusion-driven carcinomas, and other solid tumors, as well as autoimmune and inflammatory diseases.[1,2]

This study utilizes phage display technology to identify high-affinity peptides targeting BRD4, leveraging the incorporation of the unnatural amino acid, UAA, acetyl-lysine, AcK, to mimic native post-translational modifications critical for BRD4 interactions. Using a cyclic amber-obligate 7-mer phage display library, we employed a robust system consisting of three plasmids: pADL-gIII, pCDF-AcKRS encoding AcK-specific tRNA/synthetase, and M13KO7TAA helper phage to ensure efficient display.[3]

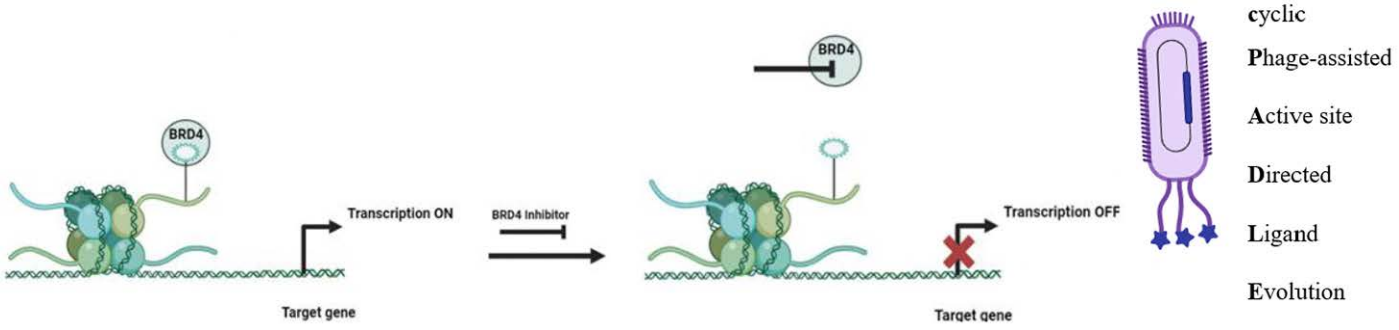


Fig 1: Schematic representation of Bromodomain 4 peptide inhibitor disrupting the translation process.

Next Generation Sequencing, NGS, was used to characterize library diversity, confirming optimal incorporation of AcK through amber codon suppression. Recombinantly expressed and biotinylated BRD4 bromodomains, BD1 and BD2 immobilized on streptavidin-coated beads facilitated iterative rounds of phage panning.

Notably, phage production increased 10- to 100- fold in the presence of AcK after three rounds of selection. The introduction of intensified selection pressures, such as reduced protein quantities and increased washing, further enriched high-affinity binders. Following phage selection, NGS identified top candidate peptides. Preliminary binding studies using AlphaScreen and Biolayer Interferometry, BLI, confirmed binding affinities, with early results showing highly

promising binding strengths, 920 nM. Building on the alanine scan results, a second-generation focused selection will prioritize key amino acids critical for binding within the 7mer sequence. This iterative approach aims to identify refined binders with enhanced low nanomolar affinities and improved selectivity for BRD4.

Prospects include detailed affinity maturation, structural validation via crystallography and cryo-EM, and evaluating these optimized peptides in advanced functional and therapeutic models for clinical translation. To validate cellular engagement, NanoBRET assays will assess peptide interactions with BRD4 in a live-cell context, offering insights into their specificity and efficacy under physiological conditions. Subsequent in vivo studies in disease-relevant animal models will evaluate therapeutic potential, focusing on cancer and autoimmune disease applications. This innovative approach leverages the chemical precision of UAAs to expand the functionality of peptide libraries, providing a powerful tool for targeting challenging proteins like BRD4.

Our results demonstrate the feasibility of isolating high-affinity binders with significant therapeutic promise, laying the groundwork for the development of next-generation drugs targeting BRD4 in diverse pathological contexts.

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[2] Barderas, R.; Benito-Peña, E. *Anal. Bioanal. Chem*. **2019**, 411 (12), 2475–2479.

[3] Tharp J.M., Hampton J.T., Reed C.A., Ehnbom A., Chen P.C., Morse J.S., Kurra Y., Perez L.M., Xu S., and Liu W.R., *Nat. Commun*. **2020**, 11,1392.

P-013

Aberrant Glycosylation and Antibodies in MOG-AD: Insights From Synthetic Glycopeptide- Based Assays for Patient Stratification

Silvia Bracci[1], Feliciana Real-Fernandez[2], Nicolas Collongues[3], Jérôme de Sèze[4], PaoloRovero[5], and Anna Maria Papini[1]

[1]Interdepartmental Laboratory of Peptide and Protein Chemistry and Biology, Department of Chemistry "Ugo Schiff," University of Florence, Sesto Fiorentino, Italy

[2]Institute of Chemistry of Organometallic Compounds, ICCOM, National Research Council of Italy, CNR, Sesto Fiorentino, Italy

[3]Biopathology of Myelin, Neuroprotection and TherapeuticStrategies, INSERM U1119, Federation of Translational Medicine of Strasbourg, University of Strasbourg; Department of Neurology, University Hospital of Strasbourg; Center for Clinical Investigation, INSERM U1434; Department of Pharmacology, Addictology, Toxicology, andTherapeutics, University of Strasbourg, Strasbourg, France

[4]Biopathology of Myelin, Neuroprotection and Therapeutic Strategies, INSERM U1119, Federation of Translational Medicine of Strasbourg, University of Strasbourg; Department of Neurology, University Hospital of Strasbourg; Center for Clinical Investigation, INSERM U1434, Strasbourg, France

[5]Interdepartmental Laboratory of Peptide and Protein Chemistry and Biology, Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence,Sesto Fiorentino, Italy

Myelin oligodendrocyte glycoprotein antibody-associated disease, MOG-AD, is a rare autoimmune demyelinating inflammatory disorder of the central nervous system, causing symptoms like vision loss, muscle weakness, paralysis, seizures, and headaches. These symptoms often overlap with conditions like multiple sclerosis, MS, and neuromyelitis optica spectrum disorder, NMOSD, complicating diagnosis. Serological evidence of anti-myelin oligodendrocyte glycoprotein, MOG, antibodies is a biomarker of MOG-AD.[1]

Cell-based assays are currently considered the gold standard for detecting anti-MOG antibodies but lack standardization and inter-laboratory reproducibility.[2] Alternative assays based on peptides are promising due to their ability to obtain site-specific modifications and efficient production, making them useful in studying how aberrant post-translational modifications, like glycosylation, contribute to immune recognition. These changes might also correlate MOG-AD to bacterial or viral infections.

We synthesized peptides based on the hMOG 25-55 fragment, including the immunodominant region 35-55, with glycosylation modifications at different sites, the native N31, N53, or both, with the idea in mind of possible genetic mutations. These synthetic peptides were tested using ELISA on serum samples from MOG-AD, MS, and NMOSD patients, alongside investigating cross-reactivity with hyperglucosylated nontypeable *Haemophilus influenzae* adhesin, previously linked to MS.[3]

The findings reveal differences in immune responses among patients and highlight the potential role of glycosylation and early bacterial infections in MOG-AD pathogenesis, aiding in patient stratification and advancing diagnostic approaches based on unique synthetic antigens.

[1]Marignier, R. et al. *The Lancet Neurology*. **2021** 20, 762–772.
[2]Banwell, B. et al. *The Lancet Neurology*. **2023** 22, 268–282.
[3]Quagliata, M. et al. *Journal of Peptide Science*. **2023** 29, e3475

P-014

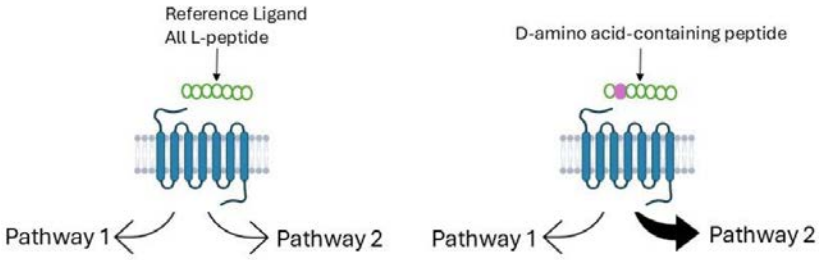
Progress Towards Evaluating the Functional Selectivity of Endogenous D-Amino Acid-Containing Neuropeptides

Alisha Doda[1], Baba M. Yussif[1,2], and James W. Checco[1,2]

[1]Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE, 68588
[2]Nebraska Center for Integrated Biomolecular Communication, NCIBC, University of Nebraska-Lincoln, Lincoln, NE, 68588

One of the understudied post-translational modifications that neuropeptides undergo is the conversion of L-amino acid residue to its corresponding D-amino acid residue. D-amino acid-containing peptides, DAACPs, have been shown to play significant biological roles, though these functions remain underexplored due to the lack of identified receptors for DAACPs.

Currently, only two receptor classes for DAACPs are known: the achatin receptor, found in both *Aplysia californica* and *Platynereis dumerilli*, and two allatotropin-related peptide, ATRP, receptors, apATRPR1 and apATRPR2, in *Aplysia californica*. Only the D-amino acid-containing analog of achatin activates the achatin receptor in both *Aplysia* and *Platynereis*, while both the all-L-analog of ATRP, all-L-ATRP, and its corresponding D-amino acid-containing analog, D2-ATRP, activate the *Aplysia* ATRP receptors.



We hypothesize that one diastereomer may differentially activate intracellular signaling pathways relative to all L-ATRP. In IP1 and cAMP assays performed with transiently transfected CHO-K1 cells, all-L-ATRP is a more potent activator of apATRPR1, whereas D2-ATRP activates apATRPR2 more effectively. However, apATRPR2 is more sensitive to D2-ATRP when signaling through $G_{\alpha s}$ pathway as compared to $G_{\alpha q}$ pathway. Similarly, apATRPR1 is more sensitive for all-L-ATRP when signaling through $G_{\alpha s}$ pathway as compared to $G_{\alpha q}$ pathway.

These results suggest that the isomerization of L-amino acid residue to D-amino acid residue leads to preferential activation of one signaling pathway over another. To further investigate this hypothesis, we are conducting IP1, cAMP, β-arrestin, and pERK assays for both apATRPR1 and apATRPR2. Ligand bias will be assessed by calculating the bias factors using an operational model of agonism.

This research will help us better understand the role of L- to D-residue isomerization in cellular signaling. Moreover, the presence of DAACPs across phyla suggests that cellular signaling involving DAACPs is not restricted to *Aplysia* or *Platynereis* and the knowledge of functional selectivity could be relevant to other systems.

P-015

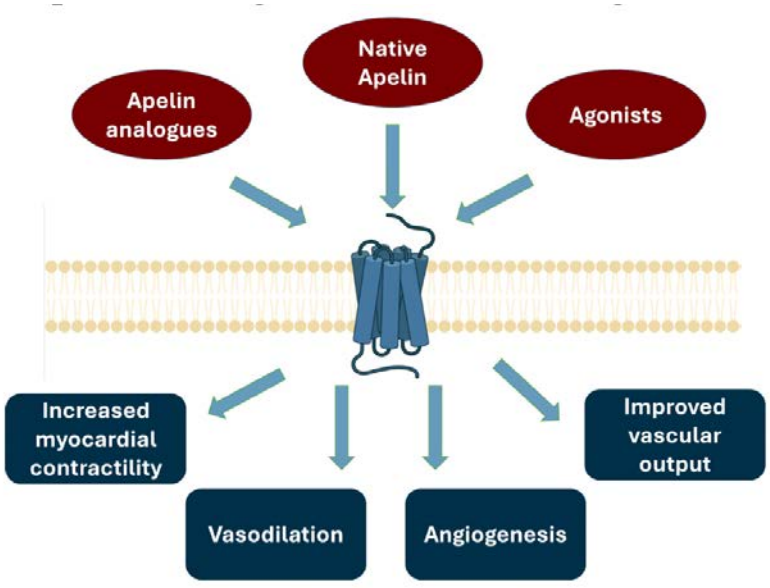
Investigating G-Protein Biased Signalling Pathways and Physiological Effects of Apelin Analogue Peptides and Small Molecule Agonists

Anjalee Wijewardane, Conrad Fischer, John Vederas

Department of Chemistry, University of Alberta, Canada

The leading cause of death worldwide, ischemic heart disease, arises from the formation of plaque in the coronary arteries, often resulting in heart attacks. While stents are commonly used to restore blood flow, their use can inadvertently worsen the damage, leading to myocardial ischemia-reperfusion injury, MIRI, a condition for which effective treatments are currently lacking.

In response to MIRI, the body activates the apelin-APJ receptor system. Apelin peptides bind to the APJ receptor, triggering a range of cardioprotective effects, such as vasodilation, angiogenesis, and myocardial contractility. However, the therapeutic potential of apelin is limited by its short half-life, <5 minutes, necessitating the development of more robust therapeutic solutions.[1]



Two main strategies that are being explored for MIRI are metabolically stable apelin analogues and small molecule agonists. These agonists for the APJ receptor can cause varying downstream physiological effects, which are likely due to the activation of different intracellular signaling cascades. Biased agonists hold potential as more precise medications by selectively activating specific therapeutic signaling pathways while minimizing the activation of unintended ones.

In this study, we test the G-biased signaling pathways of multiple apelin analogues and small molecule agonists of the APJ receptor. The poster will present the G-biased signaling profiles for various compounds, including our lead apelin-17 analogue, Cbz-PEG$_6$-NMeLeu-17A2,[1] a metabolically stable apelin-13 analogue, NMeLeu-13A2,[1] and a small molecule agonist, BMS-986224.[2] It will also offer insights into the physiological effects caused by the differential receptor binding of some of these agonists.

[1]C. Fischer et al., *J. Med. Chem.*, **2020**, 63, 12073–12082.

[2]J. James et al., *J. Med. Chem.*, **2021**, 64,3086-3099.

P-016

Activation of Class B GPCRs via Cell-Penetrable Membrane-Anchored Peptides

Haley M. Anchukaitis,[1] Nikita Bhat[1], Tristan C. Dinsmore[1], Martin Beinborn[1,4], and Krishna Kumar[1,2,3]

[1]Department of Chemistry, Tufts University, Medford, Massachusetts 02155, USA
[2]Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA
[3]Cancer Center, Tufts Medical Center, Boston, Massachusetts 02111
[4]Molecular Pharmacology Research Center, Tufts Medical Center, Boston, Massachusetts 02111, United States

Glucagon-like peptide-1 receptor, GLP-1R, and gastric inhibitory polypeptide receptor, GIPR, are class B1 G protein-coupled receptors, GPCRs, that regulate blood glucose and appetite suppression, and have inspired clinically useful compounds for type 2 diabetes and obesity. Parathyroid hormone 1 receptor, PTH1R, is another class B1 GPCR implicated in calcium homeostasis and bone development, and is a drug target for treating diseases such as osteoporosis and hypoparathyroidism. They are endogenously activated when their respective extracellular peptide hormones enter the orthosteric binding pocket.

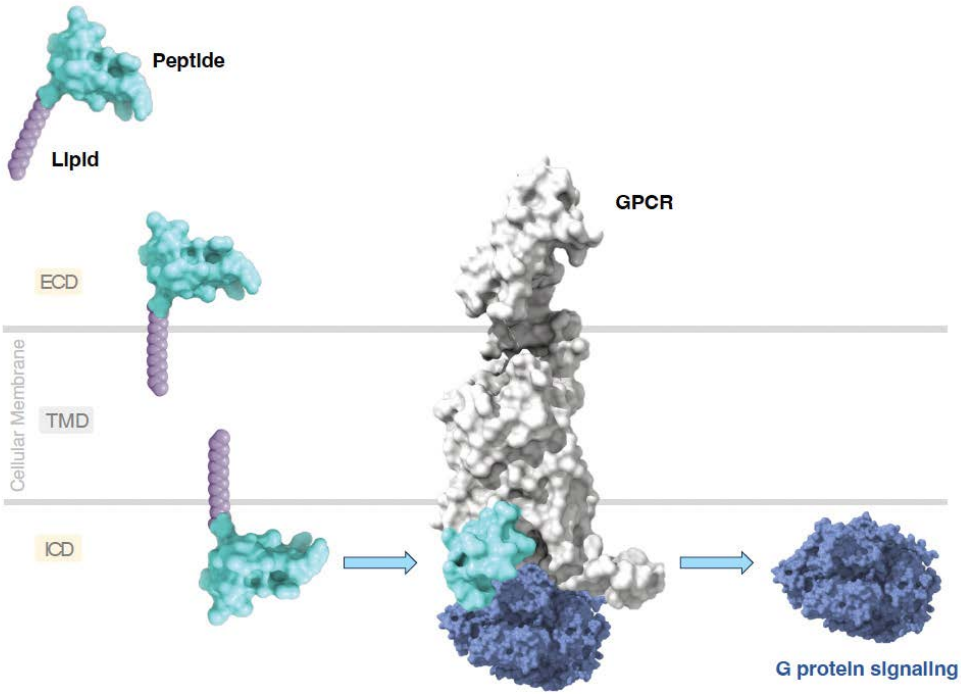


Figure 1. Mechanism of pepducin association with GPCRs and induced G protein signaling. Pepducins are lipidated peptides modeled after the intracellular region of a GPCR where the hydrophobic lipid portion, purple, allows for passage through the cell membrane and the peptide, cyan, region associates with the intracellular face of the GPCR, cyan or grey, or G protein, blue. Agonism or antagonism may result from this mechanism.

Within a metabolic disease state, the known high susceptible of these ligands to enzymatic degradation within the extracellular environment becomes a critical issue. Allosteric activation of these GPCRs can be achieved through pepducins, which are short peptides that associate with cell membranes via N-terminal lipidation and flip into the cytosol upon the tethering event. They selectively associate with their targeted receptor intracellularly via the mimicry of the receptor's intracellular loops, Fig.1. This class of compounds have been exclusively reported for class A GPCRs.

In this work, we have demonstrated the first reported agonists for class B1 GPCRs that act via a novel allosteric activation pathway. These rationally designed pepducins are potent in the low nanomolar range with a bias towards the productive G-protein signaling pathway over the β- arrestin-mediated internalization pathway. We have demonstrated translatable methods of increasing potency by perturbing hydrophobicity and N-terminal lipid length for optimal membrane-tethering. Expanding this technology across all GPCR families could discover new ligands and mechanisms of previously determined undruggable targets.

P-017

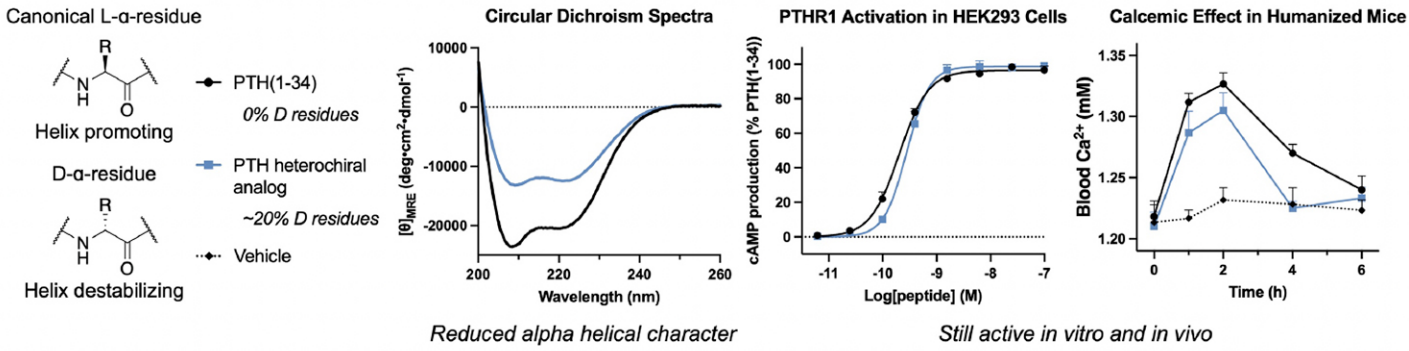
Potent and Biased Peptide Agonists of the Parathyroid Hormone Receptor-1 from a Heterochiral Design Strategy

Lauren My-Linh Tran, Jakob Hoeppner, Harald W. Jüppner, Thomas J. Gardella, and Samuel H. Gellman

University of Wisconsin at Madison

The role of dynamics in Class B GPCR activation is an increasing area of interest, and recent reports have highlighted the importance of peptide agonist flexibility in the glucagon family of receptors. We wanted to explore agonist dynamics in an unrelated Class B GPCR, the parathyroid hormone receptor-1, PTHR1. The PTHR1 regulates calcium homeostasis and bone remodeling and is a therapeutic target for treatment of osteoporosis.

We designed agonists of the PTHR1 with multiple L-to-D residue substitutions, generating "heterochiral" peptides with a mixture of L- and D-amino acids. D-amino acids reduce the α-helical character of peptides, thereby increasing flexibility. This heterochiral strategy was unexpected to yield potent agonists given the defined α-helix present in existing static agonist-bound structures of Class B GPCRs. Yet heterochiral analogs of parathyroid hormone, PTH, exhibited high potency at the PTHR1 despite lower α-helical character. We discovered heterochiral PTH analogs that manifested bias away from β-arrestin recruitment and receptor internalization signaling outcomes.



These functional biases may be beneficial in promoting bone formation over bone breakdown in osteoporosis treatment. Additionally, we demonstrated that our lead heterochiral peptide is active in vivo. This work challenges the existing paradigm in drug design to rigidify the ligand-bound structure, encourages the importance of dynamic structural information, and may serve as a potential framework for future therapeutic peptide applications.

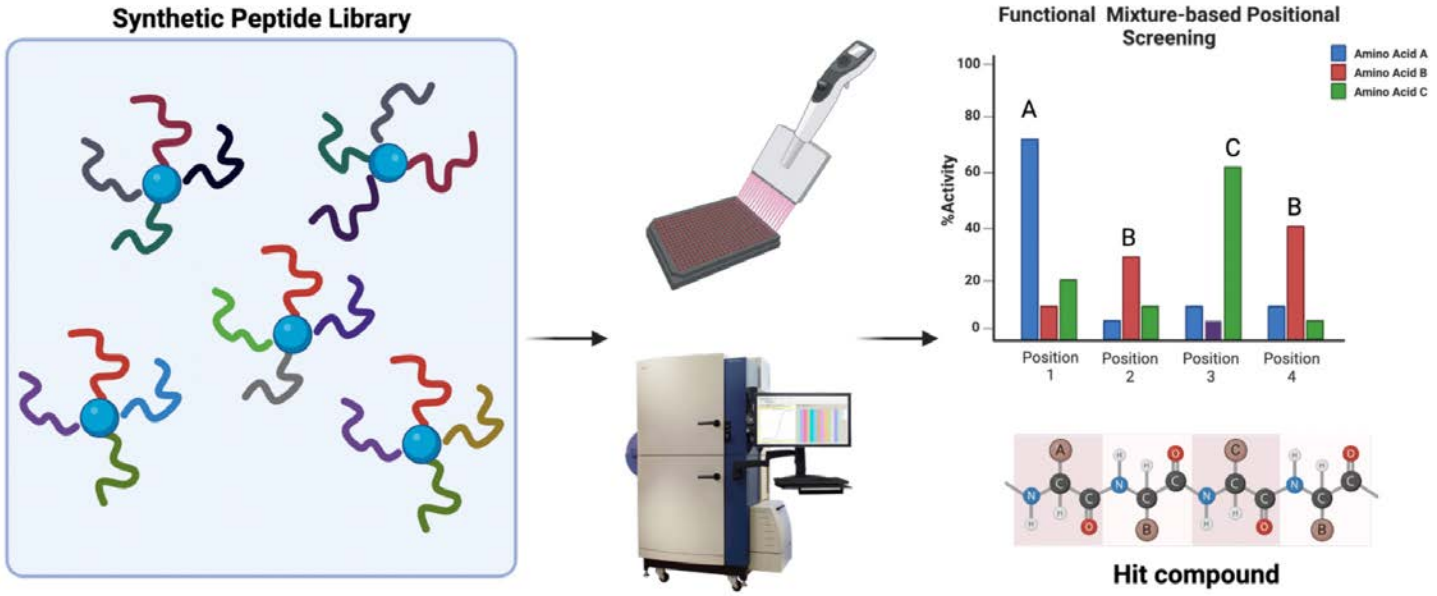
P-018

Calcium Mobilization Assay as a High Throughput Screening Assay for Mixture-Btased Positional Peptide Libraries on Opioid Receptors

Linh Tran[1], Clemencia Pinilla[1], Radleigh Santos[2], Carrie Haskell-Luevano[1]

[1]University of Minnesota, Twin Cities, Minneapolis, USA
2Nova Southeastern University, Fort Lauderdale, USA

The opioid crisis has impacted many individuals due to misuse and addiction to prescription and illicit opioid drugs. Opioid receptors are G protein-coupled receptors, GPCRs, that are expressed throughout the central nervous system and peripheral tissues. Opioid agonist ligands bind to the opioid receptors and stimulate downstream signaling cascades for various biological processes. The physiological function of opioid receptors prompted the development of agonists, antagonists, and biased ligands through structure-activity relationship, SAR, studies to treat multiple diseases, namely chronic pain, inflammation, and depression.



Herein, we described a pilot study using the Calcium mobilization assay with the HEK293 cells engineered to stably express the $G\alpha_{\Delta 6qi4myr}$ to screen a synthetic natural amino acid hexapeptide library. This mixture-based positional library of 122 mixtures with over 64 million compounds was screened on an automatic liquid-handling FLIPR device on human opioid receptors. This 384-well plate assay offers a valuable tool for high-throughput screening that can be applied to identify hit compounds in synthetic libraries for opioid receptors and possibly other GPCRs to treat different stages of diseases.

P-019

Protease Resistant Agonists of the Neuropeptide Y1 and Y2Receptors through Chemical Modification of Peptide YY

Marina Spenciner

Tufts University, Medford, MA, USA

Peptide YY, PYY, is a 36 amino acid agonist of the neuropeptide Y1 and Y2 receptors, Y1R and Y2R, that plays a balancing role in hunger and satiety signaling. Additionally, PYY stimulates anti-apoptotic and insulin regulatory pathways in the pancreas. A key hurdle that has prevented therapeutic translation is dipeptidyl peptidase-4, DPP4, truncation of the PYY N-terminus, resulting in diminished Y1R agonism.

In this work, N-terminal modifications were performed on PYY to generate a platform of DPP4-refractory agonists of the Y1R and Y2R. Unlike precedented methods that confer protection against DPP4-mediated degradation, this method retains receptor potency and efficacy equal to PYY. Further N-terminally directed chemical modalities were utilized to clarify Y1R agonism and a series of lipid diacid acylation positions were tested to introduce a protraction element to the DPP4 refractory agonists.

The use of N-terminal decorations demonstrates a superior method of conferring DPP4 resistance to receptor agonists of the Y1R. Meanwhile, substitutions to the PYY sequence elucidate a clearer picture of what is required by the Y1R and Y2R for agonist potency.

P-020

Waking a Sleeping Giant: Allosteric Modulators of the AdhesionReceptor ADGRL1

Raj Talukdar

Tufts University, Medford, USA

Adhesion G protein-coupled receptors, aGPCRs, represent a vastly unexploited class of membrane-bound receptors with tremendous pharmacological potential. Surprisingly, little is known about their characteristics and functionality in human physiology and disease. Additionally, there are no existing compounds that can selectively agonize aGPCRs.

Recently, the adhesion GPCR latrophilin 1, ADGRL1/LPHN1, was identified as a key regulator of food intake and energy balance. Deficiency of ADGRL1 in mice leads to increased food consumption, severe obesity, and dysregulation of glucose homeostasis. The rising prevalence of obesity and type 2 diabetes calls for an urgent need for novel therapeutics that stimulate weight loss and glycemic control, therefore this receptor class has garnered recent attraction.

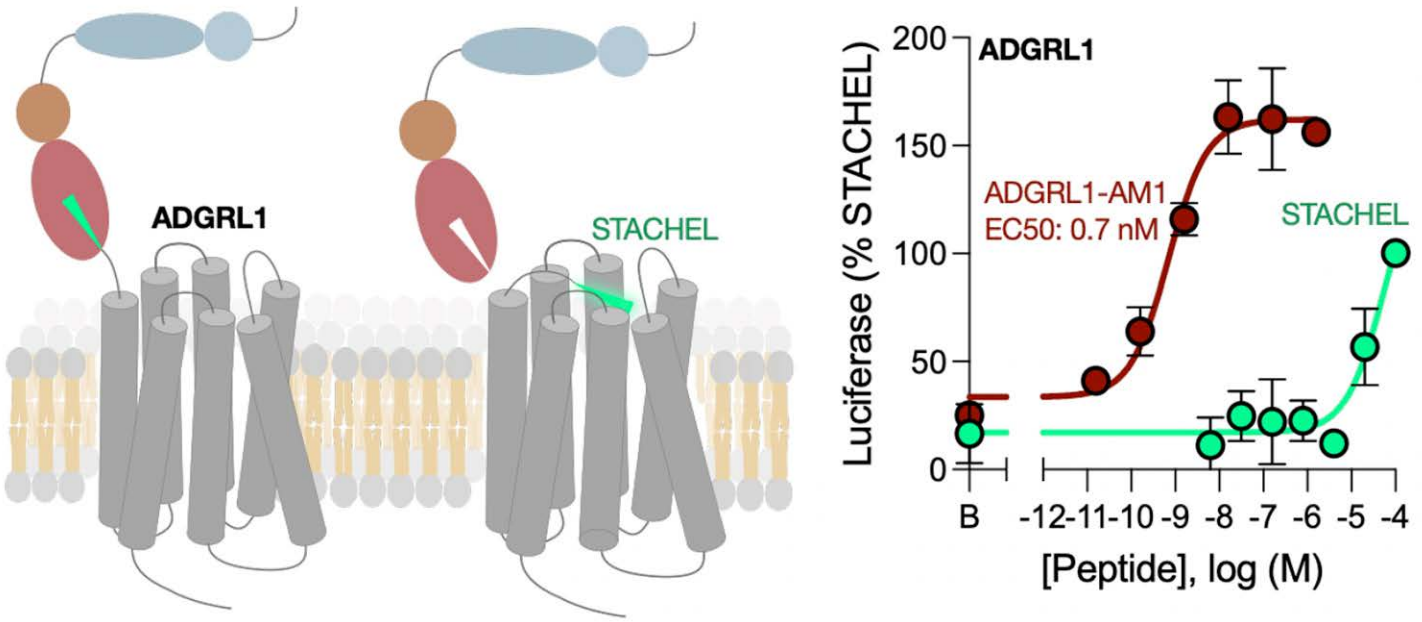


Figure 1. **a** ADGRL1 is an Adhesion G protein-coupled receptor that in its inactive state contains a large N-terminal extracellular region that upon an 'unmasking event' through autoproteolytic cleavage of the GAIN domain reveals the STACHEL agonist that is covalently fused to the remaining N-terminal fragment of the receptor. **b** The soluble STACHEL peptide sequence: TNFAVLMAHREIY-NH2, when incubated with the receptor only stimulates ADGRL1 at millimolar concentrations, the allosteric modulator "ADGRL1-AM1" developed in this work provides a greater sub-nanomolar potency in comparison.

The main reason for the lack of existing agonists of aGPCRs is due to the nature of their activation. In an autoproteolytic event within the N-terminal GAIN domain, a tethered agonist is revealed, termed STACHEL peptide, that agonizes the receptor partially facilitated through effective molarity, see Fig.1a. The key problem is that soluble STACHEL is not selective among aGPCRs and only agonizes these receptors in millimolar concentrations.

Due to the inaccessible nature of the orthosteric pocket for selective agonism, we report the development of an intracellular allosteric modulator. This agonist can agonize ADGRL1 with an EC50 of 0.7 nM, see Fig. 1b. Compounds of this nature are selective via their mimicry of intracellular loop sequences of the target receptor, which provides high therapeutic value to this class of receptors as these regions are often unique in sequence.

P-021

Peptide Ligand Tethering by Antibodies Enables Selective GPCRSignaling

Shivani Sachdev[1], Brendan Creemer[1], Thomas Gardella[2], and Ross Cheloha[1]

[1]Laboratory of Bioorganic Chemistry; National Institutes of Diabetes, Digestive, and Kidney Diseases; NIH, USA.
[2]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

G protein-coupled receptors, GPCRs, are the targets of 35% of approved drugs and control many important physiological processes. Studying GPCR function relies on ligands that selectively activate or block receptor activity. However, naturally occurring peptide ligand agonists often show promiscuity, activating multiple GPCRs and signaling pathways, complicating their use in mechanistic studies. Antibodies, Abs, are excellent tools for selectively targeting cell surface proteins, but developing GPCR-targeted Abs that induce receptor activation remains challenging.

To address this, we developed methodology to link GPCR-binding antibody fragments, nanobodies, Nbs, with peptide ligands to produce semi-synthetic conjugates that directly modulate GPCR function. Conjugates were prepared through enzymatic protein labeling, solid-phase peptide synthesis, and chemoselective conjugation chemistry. The activity of conjugates was assessed through cell-based pharmacological assays, cAMP production and β-arrestin translocation.

Linking weakly active peptides, PTH1-11, GLP1 mutant peptide, to Nbs that binds to the same receptor resulted in enhanced potency for signaling through the Gαs pathway. Surprisingly, these conjugates induced negligible β-arrestin recruitment, demonstrating unprecedented levels of pathway selective signaling activity, which offers exciting prospects for therapeutic development. Mechanistic studies revealed that the conjugates induce signaling through a mode that involves two receptor protomers, "activation in trans." We further tested whether analogous conjugates could target receptor heterodimeric complexes. Nb-ligand conjugates demonstrated activity only when both Nb- and ligand targets are co-expressed in a single cell, offering a path toward logic-gated functionality.

These tools provide new opportunities for mechanistic investigations of peptide-binding receptors and biomedical applications, with implications for therapies with fewer side effects.

P-022

A Rational Design of Unimolecular Tetra-Agonist Peptides of the GLP-1/GIP/Glucagon/Y2 Receptors for Metabolic Syndrome

Tristan Dinsmore, Jake Cortigiano, Siyuan Xiang, Martin Beinborn, Krishna Kumar

Tufts University, Medford, USA

The rising prevalence of obesity and type 2 diabetes creates an urgent need for novel therapeutics that stimulate weight loss and glycemic control. The metabolic regulation of these processes is partially mediated by peptide-receptor interactions. The gut-derived peptide hormone glucagon like peptide-1, GLP-1, plays important physiological roles including glucose homeostasis and appetite suppression. The success of GLP-1 in treating type 2 diabetes and obesity has prompted a new class of *unimolecular* dual and tri-agonists with expanded pharmacology to the peptide hormones GIP and glucagon through shared sequence homology.

These new agonists have been shown to produce greater weight loss in humans compared to GLP-1 receptor agonists alone. PYY is a co-secreted gut hormone alongside GLP-1, and its agonism of the Y2 receptor plays a stronger inhibitory role in food intake. This peptide shares no sequence homology to GLP-1, GIP, or glucagon, and therefore it has been considered a highly difficult element to incorporate in a unimolecular multi-agonist.

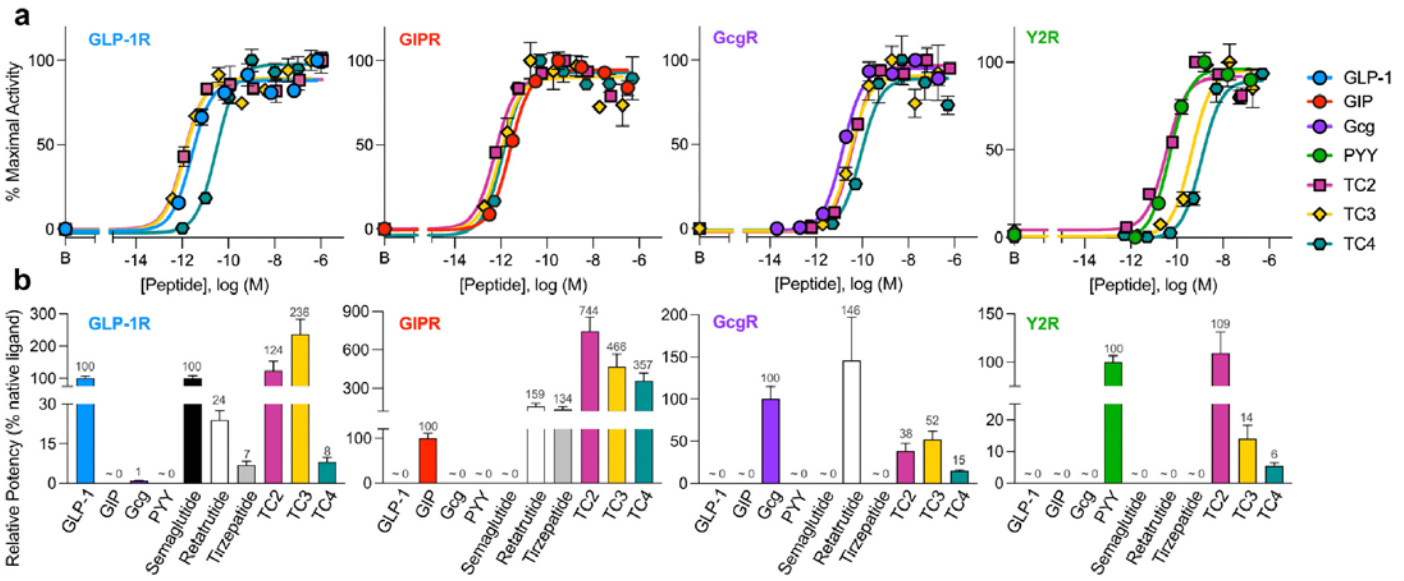


Figure 1. Potency of the tetra-receptor agonist chimeras TC2, TC3, and TC4 at the GLP-1R, GIPR, GlucagonR, GcgR, and Y2R. All 'TC' compounds are acylated with lipid side chains for improved half-lives *in vivo* and are protected against the serine protease dipeptidyl peptidase-4, which is a rapid degrader of GLP-1, GIP, glucagon, Gcg, and PYY. **a** A representative experiment illustrating concentration-response curves from the cAMP luciferase reporter assays for GLP-1R, GIPR, and GcgR, and the serum response element, SRE-luciferase reporter assay for Y2R. **b** A bar graph representation of the native receptor ligands, the FDA-approved semaglutide, tirzepatide, and retatrutide, and the TC2, TC3, and TC4 analogues. Relative % activity at each receptor = (native ligand EC_{50}/analogue EC_{50}) x 100. Errors represent ± SEM in **a**. Error represents relaJve standard error, RSE, to the mean relative potency value of greater than three experiments **b**.

Through a rational design process, a first-of-its-kind series of tetra-receptor agonist chimeras have been generated that are highly potent, efficacious, and accommodating to a series of lipid side chains with high therapeutic viability, see figure 1. Biased agonism was tested at the GLP-1 receptor to identify a variety of compounds that exhibited a favorable cAMP signaling profile while minimizing β-arrestin recruitment, thus likely reducing their rate of receptor-bound internalization. We also describe a method of potentially modulating β-arrestin recruitment without perturbing the potency the compound exhibits.

P-023

De Novo Designed Transmembrane Peptides, TM-Binders, targeting muOR Conformations

Weiyi Tang

Scripps Research, San Diego, USA

G protein-coupled receptors, GPCRs, undergo dynamic conformational changes within their transmembrane, TM, helical regions during ligand-induced signaling activation. These structural shifts are hypothesized to fine-tune the receptors' diverse functional responses to distinct ligands. The μ-opioid receptor, muOR, a key target in pain management, exemplifies this biased signaling, engaging the G-protein pathway for analgesia and the β-arrestin pathway, which is associated with adverse side effects.

Targeting to modulate protein conformations within the lipid bilayer is a lofty goal, seldom achieved, and is limited by our theory and practical ability to engineer stable and specific structured interactions targeting TM spans. We aim to address this gap and develop de novo designed TM-binders as a innovative chemical biology tools with distinct advantages from small molecule drugs and antibodies.

We tested a computational method to *de novo* design membrane-soluble peptides, termed TM-binders, designed to laterally interact with specific TM helices of muOR. By leveraging bioinformatic data, structural modeling, and pro-

tein design algorithms, we optimized peptide sequences for stability, specificity, and functional engagement from 42 designs spanning five structural poses were selected for testing. Our cell-based split-protein complementation assay demonstrates several synthetic TM-binders which form co-localized sequence-specific complexes with Opioid Receptors. Through integrated computational and experimental validation, we highlight one top TM-binder B10MP25, which selectively targets muOR in cell-based assays over a panel of membrane proteins and forms a stable complex in the membranes.

Collectively, TM-binders can serve as effective chemical biology tools and hints of therapeutic potential. The design principles and methodology established here extend beyond muOR, offering a versatile platform applicable to other GPCRs.

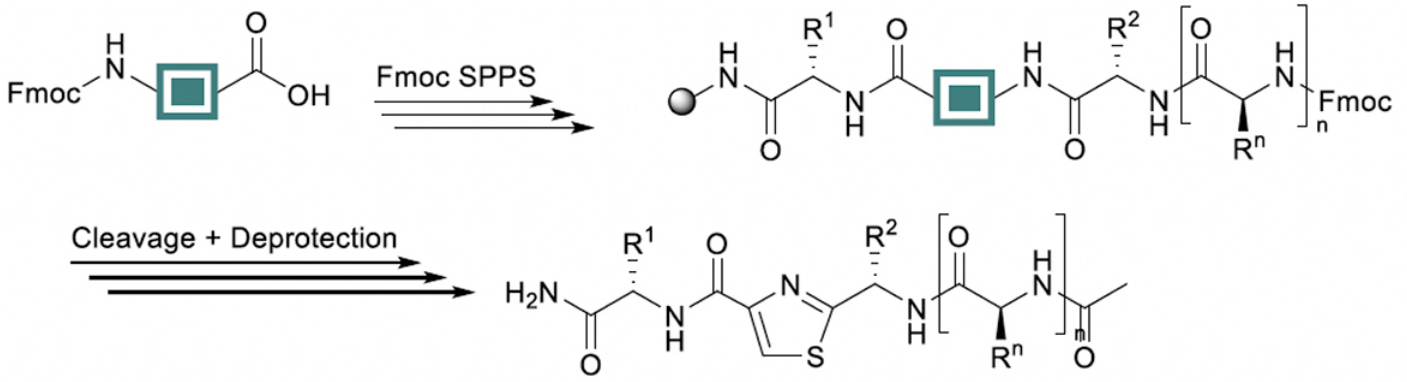
P-024

Novel Amino Acid Building Blocks for the Introduction of Azoles to Peptides

Ciaran McGrory, Eddie Myers

University of Galway, Galway, Ireland

Natural and non-natural azole-containing peptides are becoming increasingly prevalent in pharmaceutical research and development owing to their potent bioactivity relevant to several diseases.[1] However, the synthesis of these peptides is well below the level of efficiency of that demonstrated in nature by marine organisms. Although standard peptides, including those containing unnatural residues, can be routinely prepared by using automated solid phase peptide synthesis, SPPS, azole-containing peptides require additional manipulations in solution, including the use of azole dipeptides.[2] To progress the discovery and development of azole-containing peptide pharmaceuticals, more efficient synthetic routes are required.



We wish to report the development of an Fmoc-protected amino acid building block that allows the incorporation of thiazole motifs into the backbone of peptides by using standard automated SPPS protocols. This methodology will reduce synthesis time and the number of reactions required to produce thiazole-containing peptides, and promises to facilitate the preparation of large arrays of such peptides in parallel.

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P-025

Unlocking the Potential of Electroorganic Synthesis for Peptide Modifications

Dhanya Karipal Padinjare Veedu, Lara Malins

The Australian National University, Canberra, Australia

Despite the general appeal of electro-organic synthesis, electrochemistry is scarcely employed as a modification approach for peptides. Nonetheless, electro-organic methods, especially oxidation reactions, are highly desirable synthetic approaches which provide a means of avoiding toxic reagents, minimizing step count, and importantly, tuning conditions by varying the oxidation potential.

Implementing available electrochemical technologies, we disclose the development of an electroauxiliary-based anodic oxidation strategy for the functionalization of peptide substrates. The amidic side chains of glutamine residues, which are underexplored in the context of peptide modification chemistry, are functionalized with highly oxidizable, electro-active arylthioether auxiliaries. These residues are compatible with Fmoc-SPPS and can be further engaged with diverse nucleophiles under anodic oxidation conditions to access peptides possessing high-value functional handles. We also demonstrate the first proof-of-principle for peptide stapling via anodic oxidation and extend our method to the modification of dynorphin B, an endogenous opioid peptide. Finally, the selective, sequential modification of peptides bearing distinct glutamine auxiliaries with disparate oxidation potentials is readily accomplished.

In addition to side-chain electrochemical functionalizations, our group has recently showcased electrochemical peptide C-terminal modifications. Such transformations proceed via *N,O*-acetal intermediates and serve to expand the utility of oxidative decarboxylations pioneered by Seebach in the 1980s. Promising applications of this strategy for the synthesis of antibody drug conjugates, ADCs employed in targeted cancer therapy are currently underway.

Collectively, these examples highlight the reinvigoration of electroorganic synthesis as a tool for improving the sustainability and clinical relevance of peptide chemistry.

P-026

Conformational Modulation of Cyclic Peptides

Daniel Ou, Andrei Yudin

University of Toronto, Toronto, Canada

Despite the general appeal of electro-organic synthesis, electrochemistry is scarcely employed as a modification approach for peptides. Nonetheless, electro-organic methods, especially oxidation reactions, are highly desirable synthetic approaches which provide a means of avoiding toxic reagents, minimizing step count, and importantly, tuning conditions by varying the oxidation potential.

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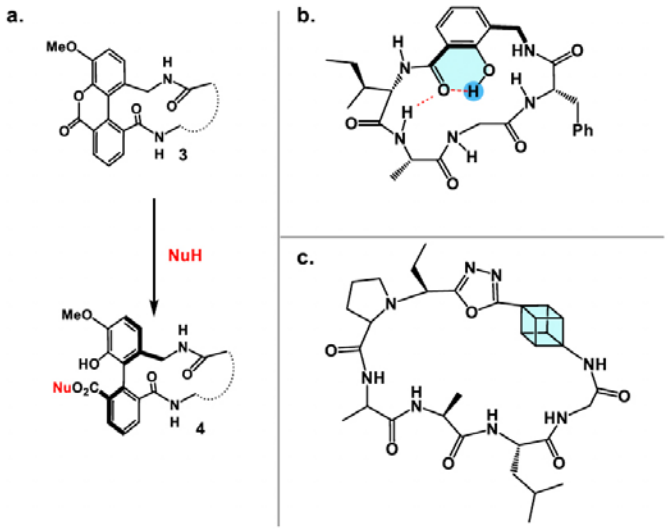

Figure: a. Bringmann lactone as a reactivity control in macrocycles. **b**. psedo-ring as the key structural elements **c**. bioisosteres replacement as a new tool for conformation modulation.

In addition to side-chain electrochemical functionalizations, our group has recently showcased electrochemical peptide C-terminal modifications. Such transformations proceed via *N,O*-acetal intermediates and serve to expand the utility of oxidative decarboxylations pioneered by Seebach in the 1980s. Promising applications of this strategy for the synthesis of antibody drug conjugates, ADCs employed in targeted cancer therapy are currently underway.

Collectively, these examples highlight the reinvigoration of electroorganic synthesis as a tool for improving the sustainability and clinical relevance of peptide chemistry.

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P-027

Development of Novel GLP-1 Peptide Semi-Synthesis Strategies

Diedra Shorty[1,2], Ryan Curtis[2], Stephanie Sandefur[1], Ankur Jalan[2] ,Nan Jia[1], Jenn Stockdill[2] ,Mike Kopach[2], and Chris Frye[1]

[1]Bioproduct Research and Development
[2]Synthetic Molecule Design and Development, Eli Lilly and Company, Indianapolis, Indiana

Incretin peptides such as semaglutide and tirzepatide have emerged as essential therapeutics for diabetes and weight management. However, their production via conventional solid-phase peptide synthesis, SPPS, is environmentally unsustainable, generating an estimated 13,000 kg of waste per 1 kg of peptide active pharmaceutical ingredient, API.[1] This inefficiency contributes to manufacturing bottlenecks and peptide supply shortages.

Here, we present a semi-synthetic strategy to produce glucagon-like peptide-1, GLP-1, that combines recombinant expression and chemoselective ligation, aiming to reduce waste and improve scalability. GLP-1 fragments are expressed in *E. coli* as inclusion bodies, enabling high-yield production of peptide segments. We employ native chemical ligation, NCL, to assemble these fragments, facilitated by a C-terminal cysteinyl–prolyl–leucine tag that allows conversion of recombinantly expressed peptides into thioester intermediates via carboxypeptidase Y-mediated hydrazinolysis.[2] The resulting peptide thioesters are ligated to produce full-length GLP-1 precursors, which are then enzymatically amidated at the C-terminus using peptidylglycine α-amidating monooxygenase, PAM, to yield native GLP-1(7–36) amide.

Preliminary results demonstrate the successful production of GLP-1 using this approach. By leveraging biosynthesis to supply peptide fragments in bulk and chemoselective chemistry to unite them, this platform offers a potentially more sustainable and scalable route for manufacturing GLP-1 analogues and related incretin therapeutics.

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P-028

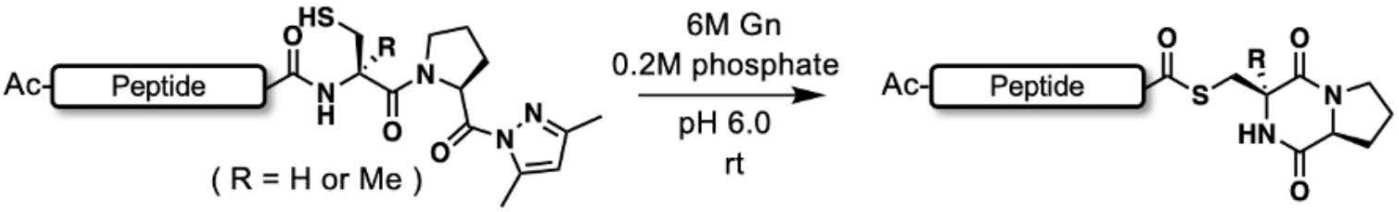
Accelerated Peptide Thioester Synthesis via Diketopiperazine Formation at α-Methylcysteine-Proline-Hydrazide Sequence

Genki Nakamura[1], Koki Nakatsu[2], Hiroshi Murakami[1], Gosuke Hayashi[1]

[1]Nagoya university, Aichi, Japan
[2]University of Cambridge, Cambridge, United Kingdom

Peptide thioesters are active species in native chemical ligation, NCL,[1] for chemical synthesis of bioactive or therapeutic proteins. We have previously developed peptide thioesterification strategies utilizing the N-S acyl shift at a cysteine residue and following irreversible diketopiperazine, DKP, formation, which proceed at the C-terminal Cys-Pro-LG, leaving group, sequence.[2,3] In this study, we aimed to accelerate the DKP formation-mediated peptide thioesterification process by replacing cysteine in the Cys-Pro sequence with α-methylcysteine, MeCys.

We began to synthesize model peptides containing C-terminal MeCys-Pro-hydrazide sequence by Fmoc solid-phase peptide synthesis, SPPS. Then, we evaluated the kinetics of DKP thioester formation of MeCys-Pro-hydrazide model peptides after conversion of the C-terminal hydrazide to acylpyrazole.[4] As a result, the DKP formation of MeCys-Pro-pyrazole peptide was dramatically accelerated compared to control Cys-Pro-pyrazole peptide. Interestingly, peptide DKP thioester formation of MeCys-Pro-pyrazole peptide proceeded via an unknown intermediate that has an identical mass of DKP thioester.



We have identified the structure of this intermediate and proposed a new reaction pathway. Furthermore, we demonstrated that the NCL rate of MeCys-derived DKP thioester was compatible with that of Cys-derived DKP thioester. Finally, we applied this strategy to chemical synthesis of Ubiquitin as a target protein, and succeeded to obtain Ubiquitin with a good yield, demonstrating the practicality of this method.

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[2]M. Yanase, K. Nakatsu, C. J. Cardos, Y. Konda, G. Hayashi, A. Okamoto, *Chem. Sci*. **2019**, 10, 5967-5975.
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P-029

Aqueous-Compatible Peptide Cyclization Strategies to Accesstyrosine-Linked Cyclic Antimicrobial Peptides

Jesus Sandres[1], Maxwell Austin[1], Tilly Dillon[1], Zach Nguyen[1], E. Dalles Keyes[1], Marcus C.Mifflin[1], Natalia Danilla[1], Ian Woodland[1], Georgia Brach[1], Aaron Puri[1], Joshua Price[2], Shelley Minteer[1], Andrew G. Roberts[1]

[1]University of Utah Department of Chemistry, Salt Lake City, USA
[2]Brigham Young University Department of Chemistry, Provo, USA

Peptide macrocyclization remains a powerful strategy to augment the metabolic stability and target affinity of bioactive peptides. Previously, we developed a tyrosine-selective cyclization method to prepare cyclic peptides with diverse, native side chain functionalities. The solution-phase oxidative cyclization method leverages the inherent nucleophilicity of phenol-bearing residues, for example, Tyr, Hpg, and the electrophilicity of *in situ* generated triazolinedione, TAD, peptides, akin to how nature forms phenolic cross-links in proteins and cycles in peptidic natural products, for example, arylomycins.

To make this chemistry user-friendly, we developed a novel solid-phase strategy to prepare TAD peptide precursors. To temper the reactive nature of *in situ* formed TAD peptides, we developed a simpler aqueouscompatible phosphate buffer that transiently forms TAD peptides and eliminates the need for specialized synthetic protocols. Now, the oxidative cyclization method can be performed chemically, using *N*-chlorosuccinimide, or electrochemically, in the same aqueous solvent system that facilitates more general substrate solubilization. These procedural refinements offer improved and reproducible access to tyrosine-linked cyclic peptides while showing better tolerance of oxidation-prone residues that may be incorporated in a desired peptide sequence, for example, Cys, Lys, Met, Trp.

These developments provide opportunities to design and incorporate Tyr-based conformational constraints and peptidomimetic character into cyclic peptide therapeutic leads. Advanced efforts toward cyclic peptide natural product mimics with antimicrobial activity and approaches toward peptide polycyclization will be presented.

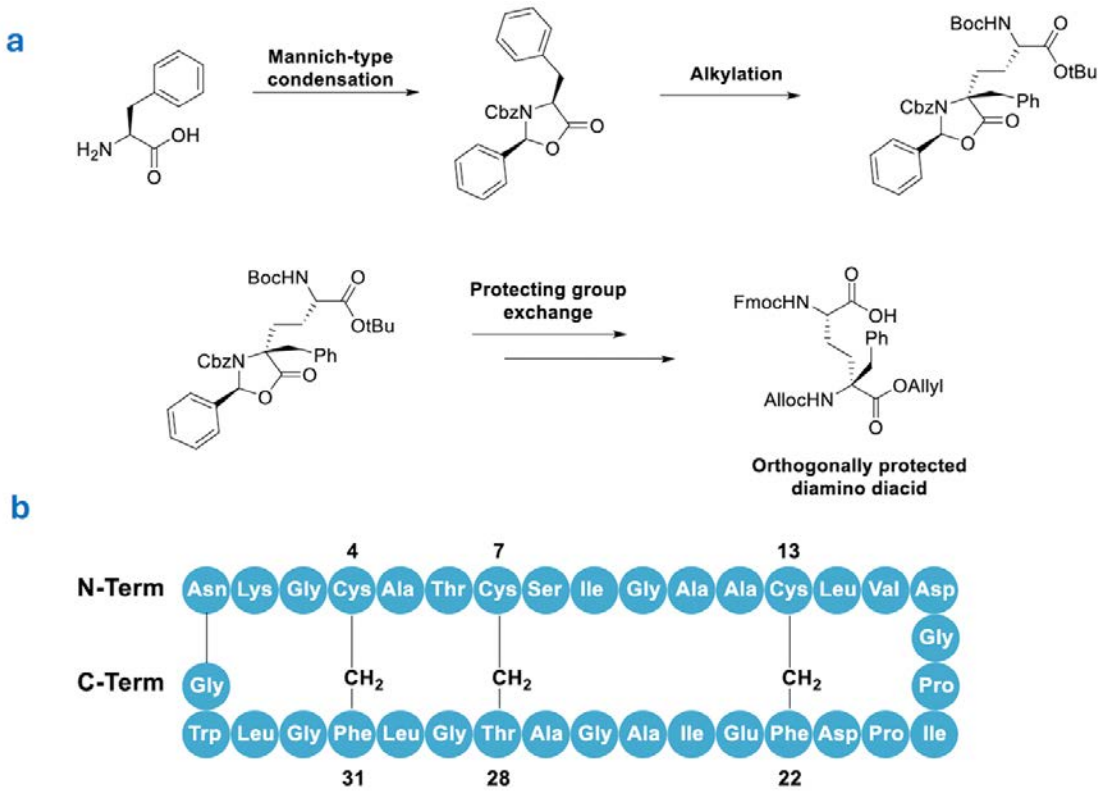
P-030

Synthesis of α-Quaternary Diamino Diacid Building Blocks for Developing Thioether Bridge Mimics of Sactibiotics

Karizza Catenza, Jose Abucay, and John Vederas

University of Alberta, Edmonton, Canada

Sactibiotics are a unique class of broad-spectrum bacteriocins characterized by sulphur-to-α-carbon bridges in the peptide chain. This unique structural characteristic gives them relatively high stability to proteolytic degradation, moderate heat, and acid treatment, thus offering an advantage over other bacteriocin classes as potential therapeutics. However, these thioether bridges make these bacteriocins more prone to hydrolysis under physiological pH and to inactivation by oxidation.



Scheme 1. Proposed synthetic route for orthogonally protected diamino diacids, **a,** for SPPS synthesis of carba analogues of subtilosin A **b**.

In this study, we report the synthesis of orthogonally protected α-quaternary diamino diacid building blocks to facilitate the synthesis of carba, S-to-CH_2, analogues of sactibiotics, with subtilosin A as our model compound. We employed a strategy known as selfregeneration of stereocentres, SRS, to achieve diastereoselective synthesis of α-quaternary diamino diacids using free amino acids as starting materials, see scheme **1a**. Stereopure oxazolidinone derivatives of *L*-Phe and *D*-Phe were obtained via a Mannich-type condensation reaction of their carboxylate salts with benzaldehyde and benzyl chloroformate.

These derivatives were then alkylated using protected (2*S*)-2-amino-4-iodobutanoic acid to access orthogonally protected α-quaternary diamino diacid precursors. Subsequent selective deprotection of the oxazolidinone moiety using hydrogenation, followed by protecting group installation, gave us access to orthogonally protected α-quaternary diamino diacids building blocks for solid-phase peptide synthesis of carba analogues of subtilosin A, see scheme **1b**.

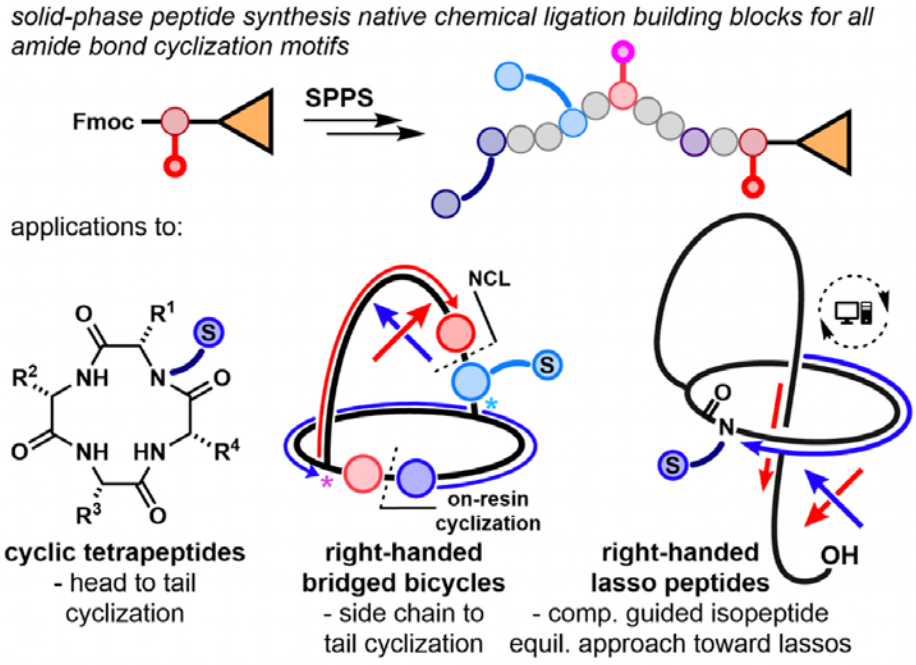
P-031

Development of Native Chemical Ligation Reagents for Thesynthesis of Cyclic and Bicyclic Peptides and a Reversi-blecyclization-Mediated Thermodynamic Approach Toward Lassopeptides

Marcus C. Mifflin, and Andrew G. Roberts

University of Utah, Salt Lake City, USA

Rigid and structurally complex peptides found in nature inspire the pursuit of novel strategies to access their promising therapeutic scaffolds. However, traditional cyclization strategies can lead to notable synthetic challenges and can require turn-inducing elements or noncanonical components to overcome unfavorable entropic costs.



To address these difficulties, we have developed a series of N-linked auxiliary, Aux, and C-linked cryptothioester building blocks for encoding native chemical ligation, NCL-based, cyclization of termini or side chains via solid-phase peptide synthesis. We have demonstrated that these reagents are readily accessible in multigram quantities and can undergo efficient Aux cleavage following NCL-based cyclization, thereby facilitating access to all possible amide bond-linked cyclization motifs.

We are applying these reagents for therapeutically promising systems, such as N→C cyclic tetrapeptides, which are a notable challenge due to the energy constraints of the cyclization step, and bridged bicyclic peptides that typically rely on noncanonical cyclization to generate one or both fused rings to circumvent lengthy syntheses. Additionally, we are developing a reversible NCL-cyclization approach as a potential strategy for synthetic lasso peptides. These are a particularly interesting class of natural products due to their unique threaded structure and array of bioactivities, but they are an elusive motif for synthetic access.

The streamlined workflow evaluates the thermodynamic stability of natural and Rosetta designed[1] lasso peptide sequences that have been energetically quantified using our established molecular dynamics simulation approaches[2] compared with experimental evaluation of potential lasso peptide chemical synthesis.

[1]Nguyen, J. D. M.; da Hora, G. C. A.; Mifflin, M. C.; Roberts, A. G.; Swanson, J. M. *J. Biophys. J.* **2025**, accepted for publication March 31, 2025.
[2]da Hora, G. C. A.; Oh, M.; Mifflin, M. C.; Digal, L.; Roberts, A. G.; Swanson, J. M. J. *J. Am. Chem. Soc.* **2024**, 146, 4444-4454.

P-032

An Amidino *C*-Terminal Hydrogen-Bond Surrogate for the Induction of α-Helicity in Short Peptides

Mohaddeseh Abbasi, Jeffrey A. Purslow, and Brett VanVeller

Iowa State University, Ames, IA, USA

Relatively small mimics of interface secondary structures have shown promise in disrupting protein-protein interactions, PPIs, which play critical roles in cell biology and medicinal chemistry. Many PPIs occur through structured interfaces, yet small peptides often adopt random coil conformations in solution. This conformational flexibility incurs a significant entropy penalty when binding protein receptors. To address this, constrained peptides mimicking interface secondary structures have been developed, offering enhanced binding affinities to PPI components.

Helices are particularly prevalent at PPI interfaces, and strategies for enforcing helicity in peptides have been widely explored. A notable approach is the hydrogen bond surrogate, HBS, strategy, which stabilizes the H-bond in the first helix turn at the *N*-terminus, *i* to *i*+4. While effective for *N*-terminal helicity, no robust HBS strategy exists for stabilizing helicity from the *C*-terminus. This is especially important as the unfolding process of helices is thought to preferentially begin at the *C*-terminus and propagate toward the *N*-terminus.

Developing effective methods to impose *C*-terminal helicity is, therefore, a promising avenue to enhance the stability of peptide helices and improve their efficacy in targeting PPI interfaces. By stabilizing helices at both termini, such approaches could mitigate entropy penalties and offer new opportunities to disrupt PPIs with high specificity. Advances in this area could have significant implications for therapeutic development and the study of protein interaction networks.

This work highlights the need for innovative *C*-terminal helicity strategies, an area ripe for exploration in peptide design and PPI modulation.

P-033

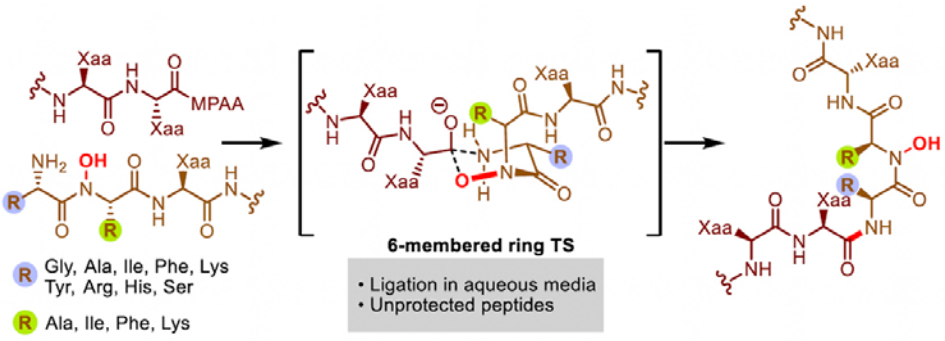
Chemical Ligation Using Backbone N-Hydroxylated Peptides

Natalia Cano-Sampaio, and Juan R. Del Valle

University of Notre Dame, Notre Dame, USA

Chemical ligation is a key technique in organic and biological chemistry for covalently linking peptides and proteins without enzymatic catalysts. Native chemical ligation, NCL, occurs in organic and aqueous media, using enforced proximity for an S-N acyl shift. While NCL typically relies on cysteine residues, expanding its scope requires often laborious syntheses of β- or γ-thiolated amino acids. Alternatives involving *O*-*N* acyl shifts are rare, mainly due to the challenge of selectively acylating oxygen over other competing nucleophiles. Our laboratory is addressing this by developing backbone *N*-hydroxy peptides that enable ligation of N-terminal residues whose thiolated forms are difficult to synthesize or entirely unexplored.

Here, we present a chemoselective ligation method that links peptide thioesters with unprotected peptides featuring backbone N-hydroxylation at the penultimate residue. Acylation of the hydroxamate is followed by *O*-*N* acyl transfer via a six-membered ring transition state to generate ligated products. Successful ligation occurred at both pH 7.0 and 8.0. Higher pH correlated with enhanced reaction rate, consistent with *O*-acylation as the rate-limiting step. Changing the identity of the N-terminal residue did not significantly influence rate or conversion. However, bulky *N*-hydroxylated residues lead to a decreased reaction rate. Successful removal of the *N*-hydroxyl group following ligation allowed us



to apply this method for the chemical synthesis of a folded miniprotein. Backbone hydroxamate ligation represents a novel strategy to form native peptide bonds and expands the toolkit available for synthesizing complex biomolecules.

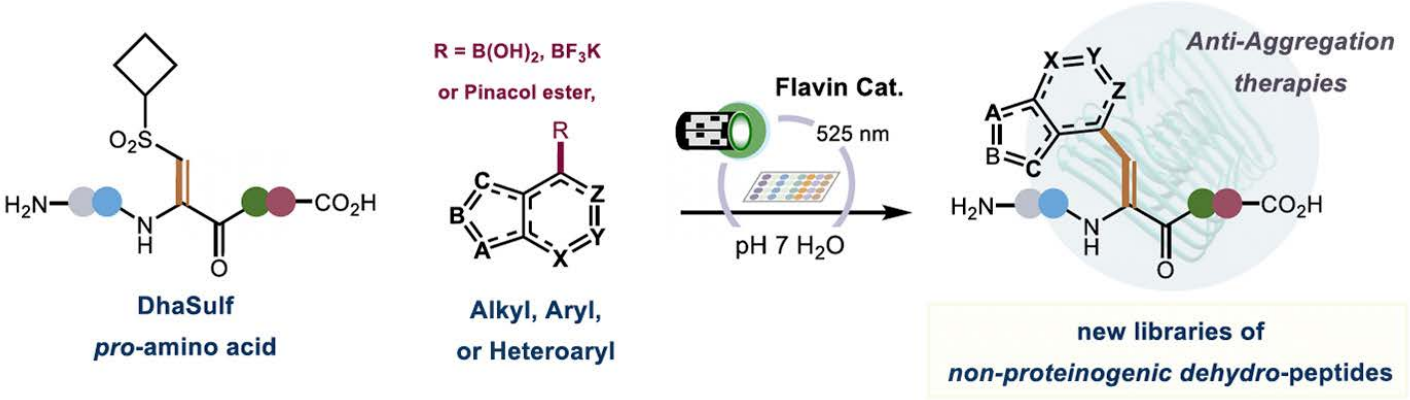
P-034

DhaSulf: A Latent Handle for Introducing Dehydroamino Acids intoPeptides & Applications for Anti-Aggregation Therapies

Pei Hsuan Chen, and and Steven Bloom

University of Kansas, Lawrence, USA

α, β-Dehydroamino acids, **dhAAs**, characterized by a double bond between their Cα - and Cβ-carbons, are integral to many bioactive peptides and biomedical materials. The rigid olefin locks the amino acid into one of two possible configurations, (E)- or (Z), each affecting key physicochemical properties such as molecular topology, hydrophobicity, and cellular permeability. But deducing which dhAA chemotype, aliphatic, aromatic, heteroaromatic, to use and which orientation, E or Z, to place it requires exhaustive medicinal chemistry, each dhAA variant being separately made and then incorporated into the nascent polypeptide through a long tedious chemical synthesis.



To streamline the discovery of optimal dhAA variants, we developed a novel AA, **DhaSulf** —essentially a dehydroalanine residue with a sulfonyl group appended to Cβ— that can be incorporated into peptides and then transformed into one of several dhAAs through reaction with a boronic acid and a flavin photocatalyst; the exact wavelength of light controlling the geometry and substitution pattern of the final dhAA. In this way, one DhaSulf peptide can become many dhAA analogs that can be tested for biological activity.

Applying this methodology, we prepared a series of dhAA peptides, namely, AcNH-Gly-Pro-**dhAA**-Phe-NH_2, that mimic the aggregation prone region of the Aβ42 peptide in Alzheimer's Disease, AD. We found that peptides with aliphatic dhAAs inhibited Aβ42 aggregation into mature amyloid plaques, establishing a set of lead structures for the development of new anti-aggregation therapies for AD.

P-035

Direct Installation of Amidines on Peptides

Rida Ibrahim, Jacob Byerly-Duke, XiFeng Wang, and Brett VanVeller

Iowa State University, Ames, IA, USA

Amidines are an under-explored isostere of the amide bond that are emerging as a promising candidate for peptide bond surrogates because they more closely approximate the properties of the native amide bond. Amidines have been reported in natural as well as artificially synthesized peptide backbones and have shown to possess potent antibiotic properties. Amidines modulate hydrogen bonding interactions and stabilize helical structure of peptides much like amides, reinforcing their significance as an ideal amide bond isostere. Despite the significance of amidines, their incorporation into linear peptides remains synthetically challenging.

To date, the sole reported strategy for installing amidines on linear peptides—via the attack of an amine on an electrophilic thioimidate—suffers from slow kinetics, undesired side reactions and difficult to monitor solid-phase steps, thereby limiting its broad applicability. We have developed strategies for rapid and direct installation of amidines into linear peptides which is compatible with the standard Fmoc solid phase peptide synthesis conditions. This work aims to overcome the critical roadblock of laborious amidine installation on linear peptides and enable their facile incorporation into peptide backbones.

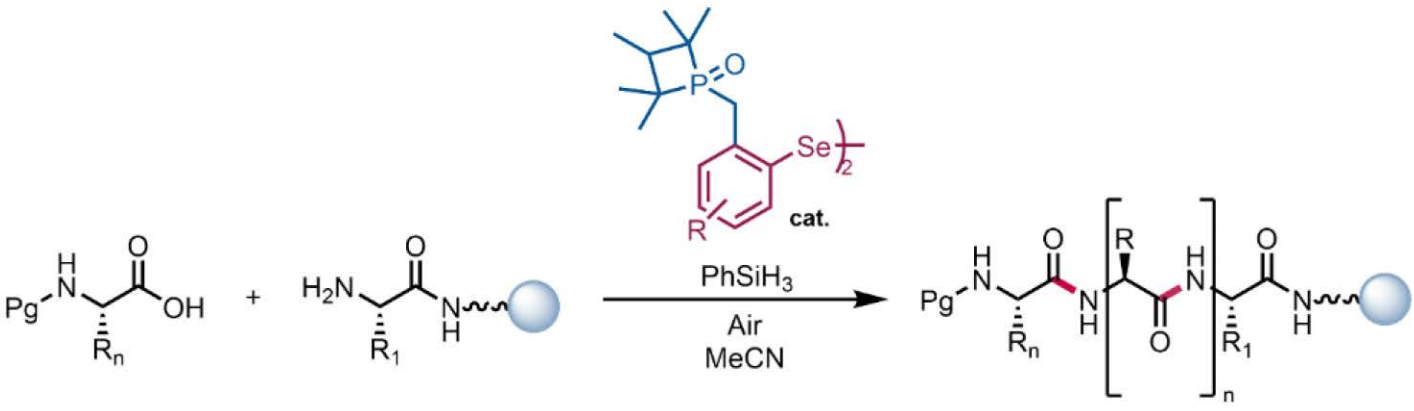
P-036

Direct Installation of Amidines on Peptides

Nihar R. Panigrahi, Shahrukh M. Khan, and Paramjit S. Arora

Department of Chemistry, New York University, New York, NY 10003, USA

In the age of peptide therapeutics, amide bond formation remains fundamental to constructing peptides and related pharmaceuticals. However, traditional methods often demand excessive reagents and generate substantial waste. Building on our lab's previous diselenide-based catalysts, *J. Am. Chem. Soc.* **2019**, 141, 15977 and *J. Am. Chem. Soc.* **2022**, 144, 8, 3637, we now report a third-generation system that unifies the aromatic diselenide and a recyclable phosphetane oxide into a single small-molecule scaffold.



This new catalyst was inspired by insights from earlier designs, with its mechanism further supported through NMR studies and computational, DFT analysis. Under optimized conditions, the catalyst promotes efficient formation of amide and peptide bonds in one to two hours. Importantly, the catalyst is compatible with solid-phase peptide synthesis and standard protecting groups. By integrating both practical improvements and mechanistic understanding, this system provides a promising route toward more sustainable peptide manufacturing.

P-037

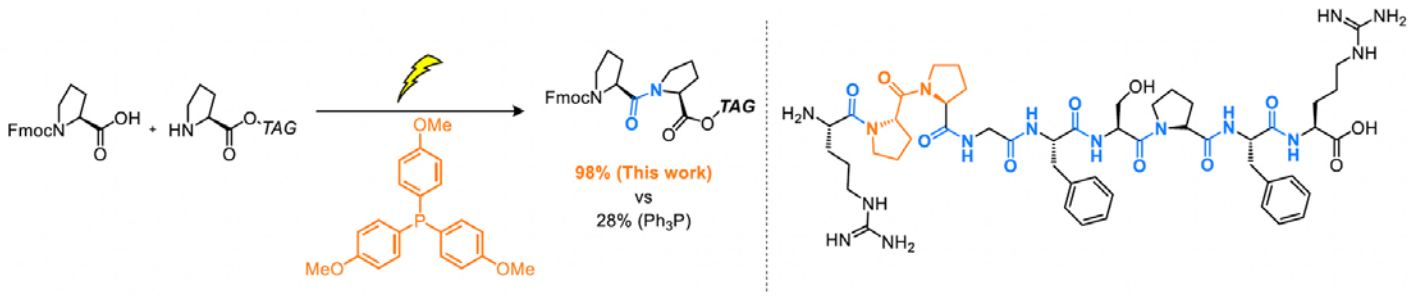
Electrochemical Peptide Synthesis Applicable to Sterically Hindered Amino Acids Enabled by Electron-Rich Triaryl Phosphine

Shingo Shinjo-Nagahara, Yohei Okada, Goki Hiratsuka, Yoshikazu Kitano, Kazuhiro Chiba

Tokyo University of Agriculture and Technology, Tokyo, Japan

As the remarkable growth has been made in peptide medicinal chemistry, the progress in synthetic method has been increasingly required.[1] The current peptide synthesis is estimated to produce 3,000-15,000 kg of waste per 1 kg of bioactive peptides, 10-50mer, which have been regarded as an urgent issue in green chemistry. One of the solutions is reducing the amount of waste from coupling reagents.[1] Although these reagents facilitate efficient peptide bond formations, byproducts are stoichiometrically generated and accumulated as waste.

To address this problem, we developed electrochemical peptide synthesis using triphenylphosphine, Ph_3P, as coupling reagents.[2] Triphenylphosphine oxide, Ph_3PO, is generated as a byproduct, but the reduction of Ph_3PO to Ph_3P has been achieved in various ways.[3] Therefore, Ph_3P can be a recyclable reagent to reduce the amount of waste in peptide synthesis. This method is applicable to all of canonical amino acids and oligopeptide synthesis.



When it comes to the electrochemical peptide couplings between sterically hindered amino acids such as proline, however, the reaction efficiency was lowered and starting material was recovered. Thus, we aimed to developing electrochemical peptide couplings applicable to sterically hindered amino acids. As a result of searching more suitable phosphines than Ph_3P, tris(4-$MeOC_6H_4$)$_3P$ was found to facilitate peptide bond formations even when sterically hindered substrates, for example, N-alkyl amino acids.[4]

Furthermore, oligopeptides which were difficult for our previous method was successfully synthesized. The superiority of the electron-rich phosphine was also examined by 18O labeling experiment, which suggested that the improvement of reaction efficiency is attributed to avoiding side reaction between phosphine cation and H_2O.

[1]M. C. Bryan et al., *Green Chem.*, **2018**, 20, 5082-5103
[2]S. Nagahara et al., *Chem. Sci.*, **2021**, 12, 12911-12917
[3]D. Hérault et al., *Chem. Soc. Rev.*, **2015**, 44, 2508–2528
[4]S. Nagahara, Y. Okada, G. Hiratsuka, Y. Kitano, K. Chiba, *Chem. Eur. J.* **2024**, 30, e202402552

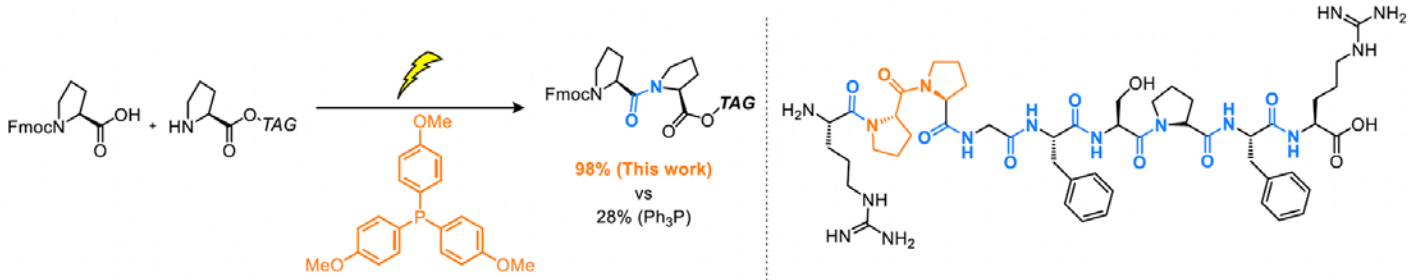
P-038

Soluble Tag Containing Unsaturated Hydrocarbon Chain Enables Highly Efficient Peptide Synthesis in Solution

Sota Adachi, Emiri Seta, Shingo Nagahara, Yoshikazu Kitano, Yohei Okada

Tokyo University of Agriculture and Technology, Tokyo, Japan

Liquid-phase peptide synthesis (LPPS) is a method of peptide elongation in solutions using a soluble tag, and it is possible to synthesize peptides on a large scale. In addition, compared to solidphase synthesis, LPPS does not require the excessive input of reagents and solvents, and is considered to be a greener method. In the previous report, most soluble tags contain saturated hydrocarbon chains.[1] However, they have poor solubility, and their use in peptide synthesis is limited to THF and halogenated solvents.



To improve the solubility, we have developed a soluble tag containing an unsaturated hydrocarbon chain. The unsaturated bonds in the tag dramatically change physical properties, and the tag dissolves in various organic solvents, including green solvents. The excellent solubility of this tag enables high-concentration peptide synthesis, up to 0.2 M, and suppresses peptide aggregation during the synthesis process.

[1]Sharma, A.; Kumar, A.; de la Torre, B. G.; Albericio, F. *Chem. Rev.* **2022**, 122, 13516–13546.

P-039

a,b-Dehydroamino-Acid-Containing Peptides Resist Proteolysis andInhibit Aggregation of a Tau-Derived Model Peptide

Stephanie Garcia[1], Huihua Xing[2], Aramis Pereira[2], Logan Knox[1], Julie Jimenez[1], Joshua Webster-Ford[1], Diego Moya[1], Joshua Payne[1], Courtney Dawes[1], Steven Castle[1], Martin Conda-Sheridan[2]

[1]Brigham Young University, Provo, USA
[2]University of Nebraska Medical Center, Omaha,USA

Abnormal function in the tau protein promotes self-assembly of unfolded monomers into amyloid aggregates such as Paired Helical Filaments, PHFs, and Neurofibrillary Tangles, NFTs. PHF6 is a hexapeptide segment of tau protein. It plays a dominant role in tau aggregation and is used as a model to design tau protein aggregation inhibitors. Segal and coworkers demonstrated that a substitution of proline at positions 2 or 3, P2 and P3, inhibits PHF6 aggregation and disassembles pre-formed aggregates. α,β-Dehydroamino acids, ΔAAs, induce β-turn formation and hence should share proline's ability to disrupt β-sheets. Previous studies have shown that ΔAAs increase the proteolytic stability of peptides that contain them. Inserting these residues at positions 2 and 3 of PHF6 could lead to a new class of proteolytically stable hexapeptide β-sheet breakers.

In this study we synthesized four ΔAA-containing PHF6 analogues with ΔAbu, derived from L-threonine, and ΔVal, derived from β-OH-Val, at position 2 or 3 using dehydrations and azlactone ring-openings as key reactions. We will present the proteolytic stability of these peptides as well as their activity as inhibitors of aggregation compared with Segal's P2 and P3 peptides and PHF6.

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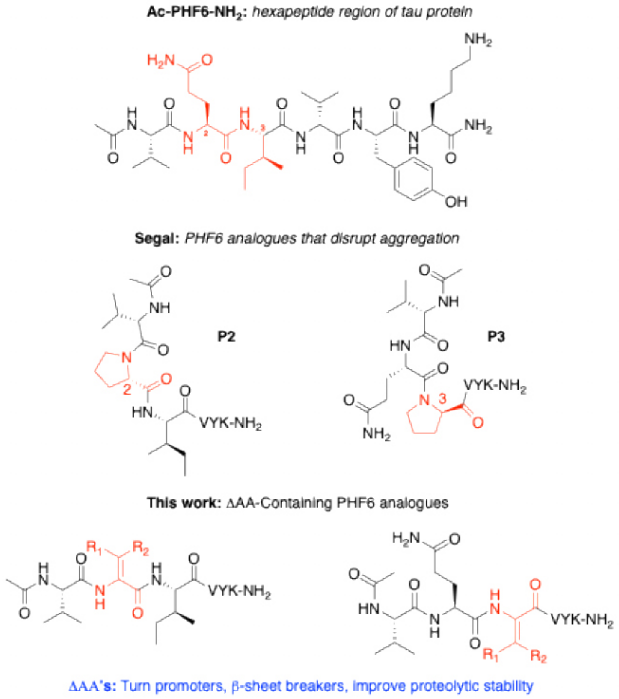


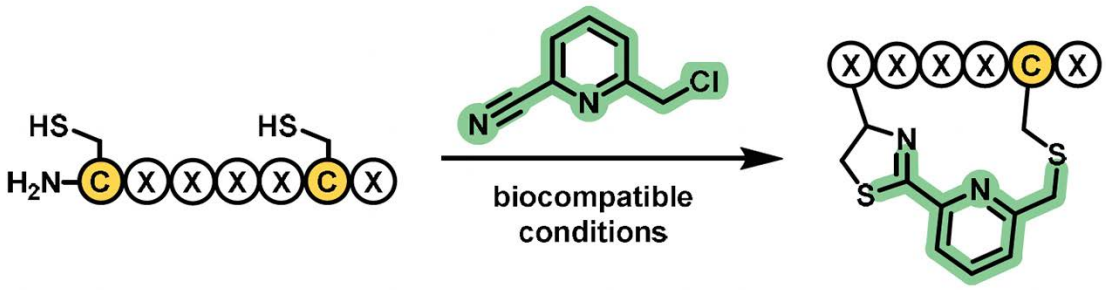
P-040

Non-Symmetric Cysteine Stapling in Native Peptides and Proteins

Sven Ullrich[1], Bishvanwesha Panda[2], Upamali Somathilake[2], Douglas J. Lawes[2], and Christoph Nitsche[2]

[1]University of Tokyo, Tokyo, Japan
[2]Australian National University, Canberra, Australia

Peptides have significant potential as therapeutics, but their pharmacokinetic limitations negatively impact their clinical potential.[1] Macrocyclisation strategies, including peptide stapling where amino acids are covalently crosslinked, offer ways to increase proteolytic resistance and target affinity.[2] We introduce 2-chloromethyl-6-cyanopyridine as a stapling reagent for the selective linkage of N-terminal and internal cysteines, creating non-symmetrically bridged macrocycles.[3]



The stapling reaction proceeds efficiently at physiological pH and generates diverse cyclic peptides with improved bioactivity.[3] Stapled peptides showed stronger binding affinity, inhibitory potency and stability against their intended biological targets.[3] Recombinantly expressed proteins can also be modified, as exemplified by the stapling of a protein fusion with an N-terminal and internal cysteine.[3]

[1]J. L. Lau and M. K. Dunn, *Bioorg. Med. Chem.*, **2018**, 26, 2700-2707
[2]H. C. Hayes, L. Y. P. Luk and Y.-H. Tsai, Org. *Biomol. Chem.*, **2021**, 19, 3983-4001.
[3]S. Ullrich, B. Panda, U. Somathilake, D. J. Lawes, C. Nitsche, *Chem. Commun.*, **2025**, 61, 933-936.

P-041

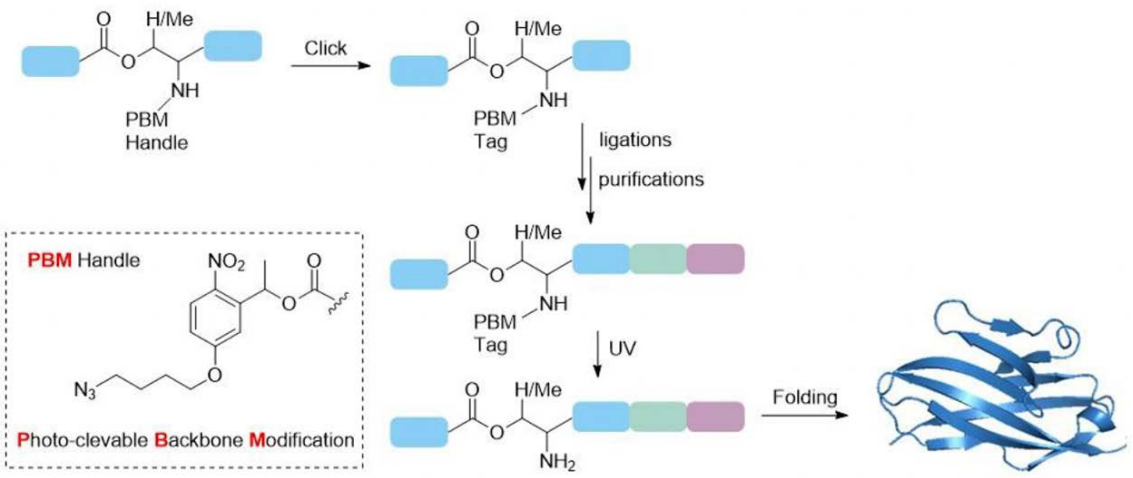
Photo-Cleavable Backbone Modification Strategy, PBM, for Difficult Peptide/Protein Synthesis *via* Modified Isopeptide

Yihui Cao, Xuechen Li

the University of Hong Kong, Pokfulam, Hong Kong

Chemical protein synthesis allows precise modifications of proteins, advancing new avenues in chemical biology fundament research and enabling innovation in therapeutic approaches. However, the challenges of purification loss and low reaction efficiency due to poor solubility continue to be significant obstacles in the chemical synthesis of proteins, especially those difficult proteins that are prone to form aggregation.

Herein, we introduce the photo-cleavable backbone modification, PBM, strategy to tackle above difficulties. In brief, an orthogonal photo-sensitive carbamate protecting group with a solubilizing tag was adopted at the alpha-amine of isopeptide, which could disrupt the peptide/protein aggregation and increase solubility. Meanwhile, the N-carbamate protection prevents the isoacyl depsipeptide linkage from the N-to-O acyl transfer under non-acidic pH, unlocking more potential applcations of isopeptide in protein synthesis.[1] Moreover, the PBM handle can be easily removed by light irradiation after ligation without the requirement of complicated additives and extra purification steps.[2,3]



This strategy combines the powerfulness of both isopeptide strategy and solubilizing tags method. The effectiveness of this approach has been demonstrated with a variety of peptide and protein substrates. This PBM strategy has been successfully applied in the chemical synthesis of T cell immunoreceptor with Ig and ITIM domains, TIGIT, offering the opportunity to obtain difficult peptide/protein with precise modifications.

[1]Hojo, H.; Takei, T.; Asahina, Y.; Okumura, N.; Takao, T.; So, M.; Suetake, I.; Sato, T.; Kawamoto, A.; Hirabayashi, Y. *Angew. Chem. Int. Ed*. **2021**, 60, 13900.
[2]Liu, X.; Liu, J.; Wu, Z.; Chen, L.; Wang, S.; Wang, P. *Chem. Sci.* **2019**, 10, 8694.
[3]Hu, X.; Shi, J.; Thomas, S. W. *Polym. Chem*. **2015**, 6, 4966.

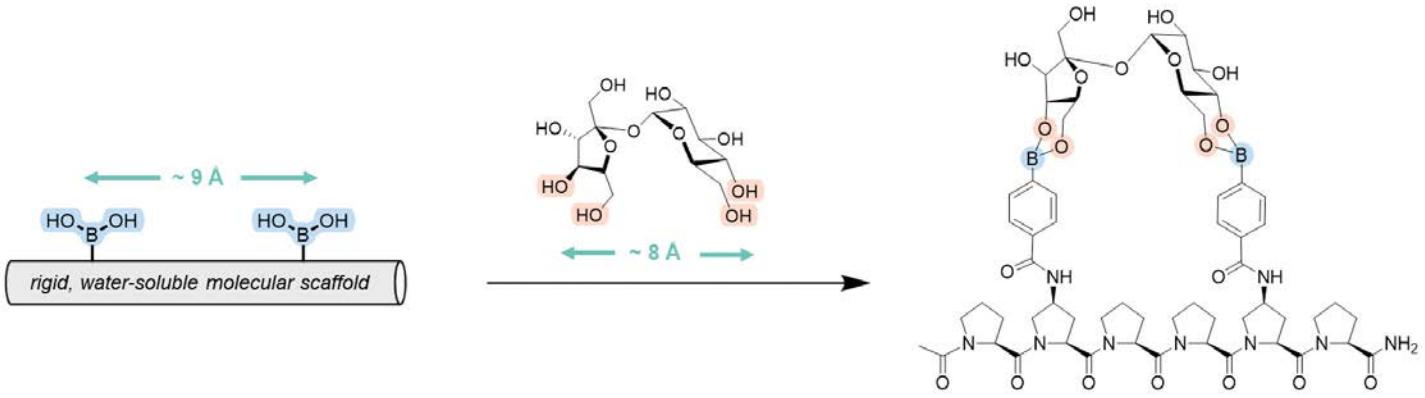
P-042

Selective Recognition of Sucrose by a Synthetic Peptide Receptor in Water

Lena Beiersdörfer, Mao Li, Dominik Zetschok, Linus Boll, Philipp Bittner, Helma Wennemers

ETH Zurich, Zurich, Switzerland

The selective recognition of saccharides in aqueous environments is a longstanding challenge due to their structural and functional similarity and the high solvation of carbohydrates in water. We recently developed an oligoproline-based receptor with two boronic acid moieties that binds the disaccharide sucrose with high selectivity and affinity in aqueous media, $K_a \approx 10'000\ M^{-1}$.



The conformationally well-defined proline hexamer allows for precise positioning of the recognition motifs in a distance of ≈ 9 Å. This spacing reflects the spatial orientation of the 1,3-diol moieties in sucrose. Reversible boronic ester formation between the boronic acid residues of the receptor and the diol groups of sucrose leads to a macrocyclic complex.

P-043

Analysis of BTM-P1 Antimicrobial Peptide by Alanine Scanning: Structural and Functional Insights

Mónica Aróstica[1], Thomas Dacal[1], César Segura[2], Fanny Guzmán[1], Constanza Cárdenas[1]

[1]Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[2]Universidad de Antioquia,Medellín, Colombia

Antimicrobial peptides, AMPs, constitute a primary defense mechanism of the innate immune system and are extensively studied as alternatives to conventional antibiotics, particularly against multidrug-resistant bacteria. BTM-P1 is a polycationic peptide of 26 residues derived from the N-terminal domain of the Cry11Ba toxin of *Bacillus thuringiensis* subsp. *Medellin*, exhibiting antimicrobial activity. Structurally, it has been described as an amphipathic, cationic α-helix capable of oligomerizing and forming pores in bacterial membranes, a property attributed to four hydrophobic residues in its N-terminal region. However, the precise factors governing its interaction with bacterial cells remain unclear.

In this study, an alanine-scanning approach was employed, systematically replacing each residue with alanine to assess its contribution to peptide function. Twenty synthetic analogs were characterized by mass spectrometry, HPLC, and circular dichroism and tested for antibacterial and hemolytic activity. Additionally, *in silico* analyses predicted toxicity and membrane-binding affinity. Alanine substitutions did not disrupt the peptide's helical structure but increased helicity in some analogs. Antibacterial assays revealed MIC values of 0.9–30 µM. Substituting lysine residues diminished antibacterial activity, highlighting the importance of cationicity, while replacing hydrophobic residues increased

MICs and reduced hemolysis. These findings provide valuable insights into BTM-P1's mechanism of action and factors influencing its antimicrobial properties.

Segura, C.; Guzmán, F.; Salazar, L.M.; Patarroyo, M.E.; Orduz, S.; Lemeshko, V. BTM-P1 Polycationic Peptide Biological Activity and 3D-Dimensional Structure. *Biochem. Biophys. Res. Commun*. **2007**, 353, 908–914, doi:10.1016/j.bbrc.2006.12.113.

Guzmán, F.; Aróstica, M.; Román, T.; Beltrán, D.; Gauna, A.; Albericio, F.; Cárdenas, C. Peptides, Solid-Phase Synthesis and Characterization: Tailor-Made Methodologies. Electron. *J. Biotechnol*. **2023**, 64, 27–33, doi:10.1016/j.ejbt.2023.01.005.

Carvajal-Rondanelli, P.; Aróstica, M.; Álvarez, C.A.; Ojeda, C.; Albericio, F.; Aguilar, L.F.; Marshall, S.H.; Guzmán, F. Understanding the Antimicrobial Properties/Activity of an 11-Residue Lys Homopeptide by Alanine and Proline Scan. *Amino Acids* **2018**, 50, 557–568, doi:10.1007/s00726-018-2542-6

P-044

Miniprotein-Driven IgG Oligomerization for Targeting Protein-Protein Interactions

Subhrodeep Saha, Jayanta Chatterjee

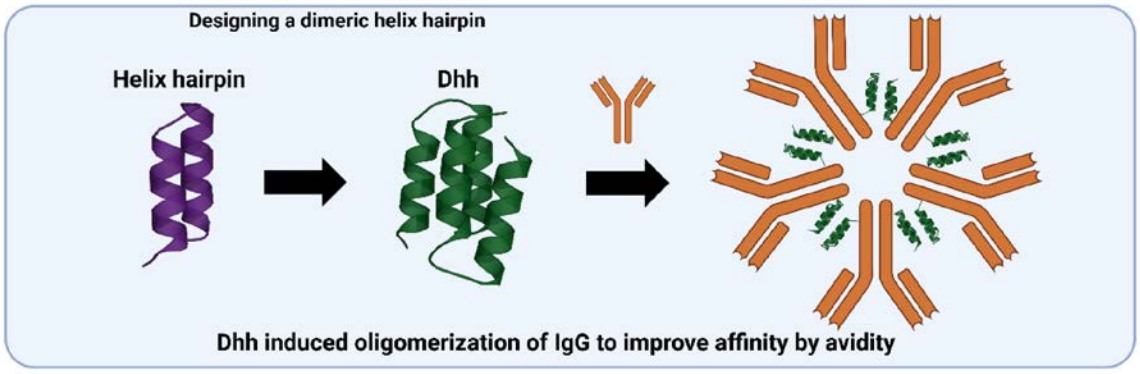
Indian Institute of Science, Bangalore, India

Protein-protein interactions, PPIs, play crucial roles in numerous biological processes and represent challenging yet promising therapeutic targets. The large interaction surfaces characteristic of PPIs are difficult to target with traditional small molecules, necessitating alternative approaches.

Here, we introduce a strategy employing miniprotein-driven oligomerization of immunoglobulin G, IgG, to enhance its efficacy in targeting PPIs through the avidity effect. This approach utilizes a dimeric variant, Dhh, of a helical hairpin peptide, hh, designed by us to bind to the Fc region of human IgG.[1]

Dhh, engineered by substituting hydrophobic residues at the solvent-exposed face of hh, induces IgG oligomerization upon binding to its Fc region resulting in a multivalent IgG. Multivalent interactions resulting from oligomerization would significantly enhance efficacy of binding to the target, thereby, causing the avidity effect.[2]

This study underscores the potential of Dhh as a versatile scaffold for antibody engineering and highlights the advantages of miniprotein-mediated IgG oligomerization in tackling large PPI surfaces. By exploiting the avidity effect of multivalent antibodies and the structural benefits of miniproteins, this platform provides a robust and scalable method for expanding the landscape of druggable PPIs.



[1]Khatri, B., Pramanick, I., Malladi, S.K. et al. A dimeric proteomimetic prevents SARS-CoV-2 infection by dimerizing the spike protein. *Nat Chem Biol* 18, 1046–1055, **2022**. https://doi.org/10.1038/s41589-022-01060-0

[2]Ku, Z., Xie, X., Hinton, P.R. et al. Nasal delivery of an IgM offers broad protection from SARS-CoV-2 variants. *Nature* 595, 718–723, **2021**. https://doi.org/10.1038/s41586-021-03673-2

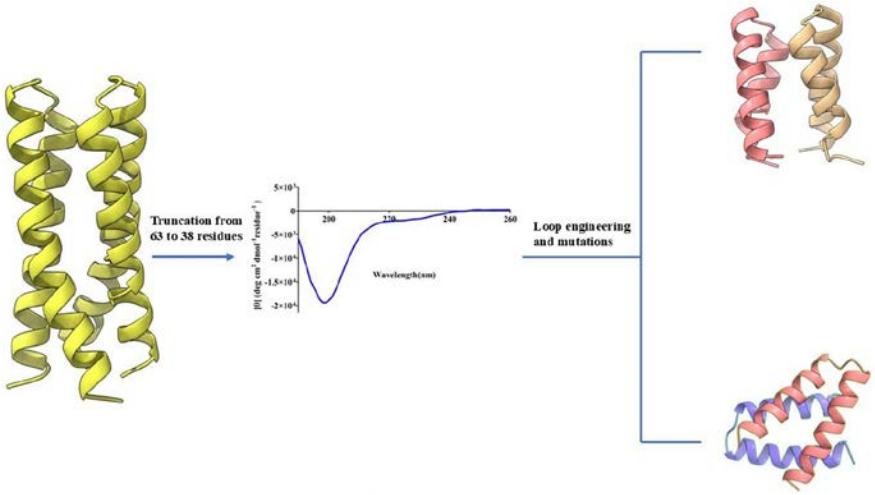
P-045

Design of Miniproteins: From Ultrastable α-Helical Hairpins to α-Helical Macrocycles

Subir Chatterjee, Ankur Singh, Jayanta Chatterjee

Indian Institute of Science, Bengaluru, India

Miniproteins are a specific class of proteins comprising less than 40 residues and can adopt a stable structure in aqueous conditions[1]. In the pursuit of miniprotein design, we aim to develop design strategies for designing α-helical hairpin motifs, which are significantly shorter than the naturally occurring ones. Truncation of longer α-helical hairpins compromises the hydrophobic interaction occurring along the length of the protein, which plays a significant role in the folding of the molecule and, thereby, maintaining the structural integrity of the protein.



We resorted to a combinatorial approach of loop engineering and incorporation of residues at specific sites, which led to the formation of thermally stable α-helical hairpins. Crystallographic evidence shows that the designed miniprotein exists as a dimer, and the choice of hydrophobic residues at specific sites also alters the topology of the dimeric assembly between *syn* and *anti*-orientations.

We extend the use of this specifically engineered loop to develop α-helical macrocyclic peptides, which have been synthesized by N-C backbone cyclization to use as inhibitors to target protein-protein interactions.

[1]Baker EG, Bartlett GJ, Porter Goff KL, Woolfson DN. *Acc. Chem. Res*. **2017**, 9, 2085-2092.

P-046

Photo-Stability of Peptides Exposed to LED, Fluorescent or UV Light in Liquid Formulations

Whitney Ong[1], Cheng Sun[1], Yushra Thanzeel[2], Kavisha Ulapane[3], Leonardo Allain[1], Olivier Mozziconacci[4], and Natalia Subelzu[1]

[1]Small Molecule Analytical Research and Development, Merck & Co., Inc., Rahway, New Jersey, USA
[2]Oral Formulation Sciences, Merck & Co., Inc., Rahway, New Jersey, USA
[3]Biologics Development and Pharmaceutics, Merck & Co., Inc., Rahway, New Jersey, USA
[4]Preclinical Development, Merck & Co., Inc., San Francisco, California, USA

The photo-stability of new drug candidates can influence the drug product quality through the formation of aggregates, degradation products, and chemical modifications in the drug substance. Although the light dose requirements are specified in the current version of International Council for Harmonization quality guideline, ICH Q1B, there is no differentiation between small molecules and macromolecules. Moreover, recent studies have demonstrated that com-

monly used excipients such as methionine and citric acid could be responsible for the light absorption and generation of reactive oxygen species in the formulation, resulting in the degradation of the active pharmaceutical ingredient.

In this study, we evaluated various light sources – ultraviolet, fluorescent, and different types of light emitting diodes, LED, with broad white and narrow, green and red, spectral distribution – on the formation of hydrogen peroxide and organic peroxides in liquid formulations containing commonly used excipients, such as citrate buffer. Using a model peptide compound methionine-enkephalin, MEn, the photo-stability was evaluated and compared under different light sources. The liquid formulations were prepared in citrate buffer, and iron chloride were exposed to different doses of light. The degradation products were evaluated with liquid chromatography and mass spectrometry.

Overall, the different light sources led to the peroxides formation albeit at varying levels. In all conditions, LED light sources provided the least damaging to liquid formulations where MEn degradation is low. The study provides valuable insights on the critical role of light sources and the impact of excipients in different drug formulation formulations on photo-stability. This holds significance for shaping stability testing protocols and should be considered in downstream processes such as manufacturing and storage conditions.

P-047

NMR-Guided Design of Heterogeneous-Backbone Mini-Metalloprotein Catalysts

Jacob Wolfe[1], W. Seth Horne[1,2]

[1]Molecular Biophysics and Structural Biology Program
[2]Department of Chemistry, University of Pittsburgh

Rational design of flexible regions of enzymes has been used to engineer functional properties including thermal stability, substrate specificity, and catalytic promiscuity. An alternate approach to traditional side-chain mutagenesis that can control protein properties is the incorporation of backbone modified amino acids into native protein sequences through total chemical synthesis: so-called "heterogeneous backbone substitution." Such backbone modifications have been shown to recreate diverse tertiary folds while fundamentally altering the chemical properties of the macromolecule chain. Less explored than protein structural mimicry using heterogeneous-backbone proteomimetics is the use of backbone alteration to rationally modulate protein function.

One property that can be readily tuned through judicious backbone changes but is less amenable to side-chain mutagenesis is conformational dynamics. The ability to mimic or maintain side-chain composition while site-specifically altering local backbone flexibility has the potential to enable the rational modulation of conformational dynamics in enzymes. To this end, we are exploring the application of sequence-specific backbone modification of catalytically active mini-metalloproteins. Here, we present progress towards development of design principles for locally increasing or decreasing chain rigidity in a targeted manner and the exploration of the effects of such changes on thermal stability and catalytic activity.

P-048

Development and Screening of the Hydrogen Bond Surrogate Enforced α-Helical and β-Hairpin mRNA Display Libraries

Amy Yang, Alex Nazzaro, Stefan Belić, Rumit Maini, Paramjit Arora

New York University, New York City, USA

mRNA display has emerged as a powerful platform for the discovery of high affinity peptide binders.1 The most common mRNA screens focus on macrocyclic peptides without defined secondary structures. Here we show that mRNA Display can be merged with the hydrogen bond surrogate (HBS) approach to develop α-helical and β-hairpin libraries.[2,3] We demonstrate the establishment of the method to incorporate the HBS crosslinks in peptides and screening of the platform against β-catenin and other protein targets.

This work is a proof-of-concept study for the identification of potentially potent HBS α-helical and β-hairpin binders for traditionally difficult-to-drug protein targets using an mRNA display platform.

[1]Huang, Y.; Wiedmann, M. M.; Suga, H. RNA Display Methods for the Discovery of Bioactive Macrocycles. *Chem. Rev.* **2019**, 119, 10360-10391.

[2]Sawyer, N.; Arora, P. S. Hydrogen Bond Surrogate Stabilization of beta-Hairpins. *ACS Chem. Biol.* **2018**, 13, 2027-2032.

[3]Sawyer, N.; Watkins, A. M.; Arora, P. S. Protein Domain Mimics as Modulators of Protein–Protein Interactions. *Acc. Chem. Res.* **2017**, 50, 1313-1322.

P-049

Design and Diversification of Genetically Encoded Bicyclic Peptides for Ligand Discovery

Andrew S. Hong[1] and Rudi Fasan[1,2]

[1]Department of Chemistry, University of Rochester, Rochester, NY 14627, USA
[2]Department of Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, TX 75080, USA

Bicyclic peptides constitute an attractive class of bioactive molecules and therapeutics for targeting protein-protein interactions and other "undruggable" biomolecular interactions. Despite the promise of this subset of macrocyclic peptides, strategies amenable to the high-throughput screening of bicyclic peptide libraries are limited mainly to either disulfide pairing or chemical crosslinking via exogenous, symmetric cyclization linkers.

Overcoming these restrictions, we report here a general and robust method for generating genetically encoded bicyclic peptides featuring two non-reducible and asymmetric thioether bridges. Through the incorporation of two cysteine-reactive non-canonical amino acids, ncAAs, a broad range of bicyclic macrocyclic organo-peptide hybrids, MOrPHs, can be produced spontaneously and chemoselectively in bacterial cells. Cyclization modules and connectivities were further diversified via the simultaneous incorporation of two distinct and orthogonal electrophilic ncAAs. Towards the selection of functional macrocyclic peptides, combinatorial libraries of phage-displayed bicyclic MOrPHs were designed and validated.

This work introduces a versatile approach for the generation of topologically diverse bicyclic scaffolds and expands opportunities for the discovery and evolution of cyclopeptide probes and drugs.

P-050

Ripping out the RiPP Leader: Formation of Leaderless Cyclicpeptides with a Radical SAM Maturase

Jacob Pedigo[1], Karsten Eastman[1,2], Vahe Bandarian[1,2]

[1]University of Utah, Salt Lake City, USA
[2]Sethera, Salt Lake City, USA

Ribosomally synthesized and post-translationally modified peptides, RiPPs, represent an emerging and diverse class of peptide-based natural products. RiPPs are genetically encoded and typically co-localized in biosynthetic gene clusters that also encode for maturase enzymes. These maturases introduce a broad range of post-translational modifications into the peptide precursor to generate the bioactive natural product. Most RiPP precursor peptides contain an N-terminal leader sequence, that is thought to aid in binding recognition by the maturase/s. Structural studies of RiPP biosynthetic enzymes generally show an N-terminal RiPP recognition element, RRE, that has been predicted to recognize the leader sequence. This poster focuses on a highly promiscuous radical S-adenosyl-L-methionine, rSAM, RiPP maturase, PapB, which has been previously shown to introduce thio(seleno)ether crosslinks into a variety of substrates. Our data demonstrates that PapB's promiscuity extends beyond substrate sequence to include the capacity to process peptides entirely lacking canonical leader sequences. These findings emphasize the potential of PapB, and related RiPP maturases, as broadly applicable biocatalysts for the enzymatic macrocyclization of structurally diverse peptides.

P-051

First-in-Class Non-Carbohydrate Inhibitors of Sialic Acid-Binding Immunomodulatory-Type Lectin-7, Siglec-7, Discovered From Genetically Encoded Bicyclic Peptide Libraries

Danial Yazdan[1], Michael Downey[1], Caishun Li[1], Edward Schmidt[1], Jeffrey Wong[1], Caleb Loo[1], Jaesoo Jung[1], Ryan Qiu[1], Ewa Lis[2], Lily Lindmeier[2], June Orbea[3], Matthew Macauley[1], Ratmir Derda[1]

[1]University of Alberta, Edmonton, Canada
[2]Koliber Biosciences Inc., San Diego, USA
[3]CICbioGUNE, Derio, Spain

Sialic acid-binding immunoglobulin-type lectins, Siglecs, are a class of immunoinhibitory cell signaling proteins with significant implications in cancer. Hypersialiation of cancer cells activates Siglecs, and this activation suppresses the immune recognition of these hyper-sialylated cells and promotes cancer-cell survival. Although Siglecs' natural substrates are gangliosides, a class primarily composed of glycoprotein with terminal sialic acid residues, Siglec proteins have a low affinity for these substrates. Hence, high-affinity inhibitors are a highly desirable focus of research.

Using genetically encoded libraries, we identified a group of bicyclic peptides with a strong affinity for Siglec-7 and Siglec-9. Specifically, we used bicyclic genetically encoded libraries modified by two-fold symmetric linkers, BiGEL2 to screen against these two targets, employing next-generation sequencing (NGS) analysis for hit nomination.

We synthesized approximately 100 peptides to explore their binding towards the Siglec targets using ELISA, BLI, and SPR. This study led to the discovery of low micromolar binders to Siglec-7 with IC_{50} & K_i <10 μM. Additionally, we preformed a preliminary structure-activity relationship profile using alanine scans to assist in the future development of highly potent, bioavailable, and immunosuppressive peptide binders of Siglec-7 and Siglec-9.

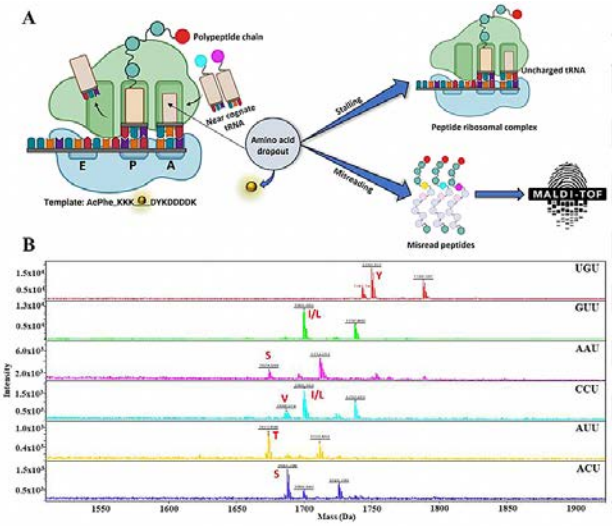
P-052

Avoiding Misreading During Genetic Reprogramming in mRNA Display

Gopal K. Dubey[1], Chase Chen[2], Bree Iskandar[2], Naga Garikiparthy[2], Julie Lee[2], Hratch J.Zokian[2], Adam Weinglass[2], Sanjay Adrian Saldanha[2], Kenneth K. Hallenbeck[2]

[1]Texas A&M University, College Station, USA
[2]Merck & Co., Rahway, USA

mRNA display is a powerful and increasingly accessible peptide discovery technology. It takes advantage of a reconstituted *in vitro* transcription and translation system to generate highly diverse affinity screening libraries. However, this process relies on the faithful translation of genetically encoded peptides, a conversion which is imperfect. Errors in translational decoding of mRNA can occur, decoupling the produced library from its genetic code.



Codon	Dropout	Misreading
UUU	F	I/L , Y
CCU	P	I/L , V
AUU	I	T
GUU	V	I/L
UCU	S	P
AGU	S	N
CUU	L	I, F
ACU	T	S
GCU	A	S
CAU	H	I/L
AAU	N	S
CGU	R	H
GGU	G	-
GAU	D	N, Q/K
UAU	Y	I/L, H, F
UGU	C	Y

Figure 1:
Misreading pattern for NNU codons with AA dropout.

A. Dropout experiment scheme with two potential outcomes: Stalling and Misreading.

B. MALDI-TOF spectra for 5 representative codons: UGU, red, GUU, green, AAU, pink, CCU, teal, AUU, yellow, and ACY, blue. The identity of peaks corresponding to misread masses are labeled in red. Theoretical masses are reported in SI Fig. 1.

C. Summary table with results for all NNU codons. For GGU, Gly was removed from the IVTT reaction but a mass corresponding to Gly incorporation was still observed.

This article contines on next page >

Because mRNA display affinity selections are commonly analyzed with sequencing of the encoding DNA, rather than direct detection of the peptides, misreading silently reduces library diversity and complicates analysis. In this study, please review our illustration on the previous page, we confirm the presence of significant translational misreading during the production of mRNA display libraries, develop best practices for genetic reprogramming, and deploy those rules to minimize the disconnect between genotype and phenotype in peptide affinity selections.

P-053

Site-Specific Peptide Arylation to Tune Protein-Protein Interaction Hot Spots

Jenna Cain, Nicholas Sawyer

Fordham University, Bronx, USA

Protein-protein interactions, PPIs, play key roles in most biological processes and are associated with diseases ranging from cancer to diabetes. A hallmark of many PPIs is the presence of "hot spots," residues at the protein interface that contribute disproportionately to the interaction energy. Among the twenty amino acids observed in proteins, two of the three aromatic amino acids, tyrosine and tryptophan, are frequently observed as PPI hot spots. The aryl side chains of both of these amino acids are electron-rich, and several studies have suggested that both steric and electronic factors contribute to tyrosine and tryptophan hot spots.

To dissect how electronic and steric factors contribute to interactions involving aromatic amino acids, we developed a new site-selective approach to introduce aryl groups with various steric and electronic properties into peptides. Our approach permits the parallel synthesis of many peptide variants from a single peptide batch by reacting different aryl groups with the peptides' nucleophilic side chains in a site-specific manner. Over 30 aryl groups with diverse chemical character have been incorporated into several distinct peptides using the same synthetic procedure, demonstrating compatibility with standard solid-phase synthesis and reagents. We envision that our approach will allow fine-tuning of steric and electronic factors of PPI hot spots for more efficient PPI targeting.

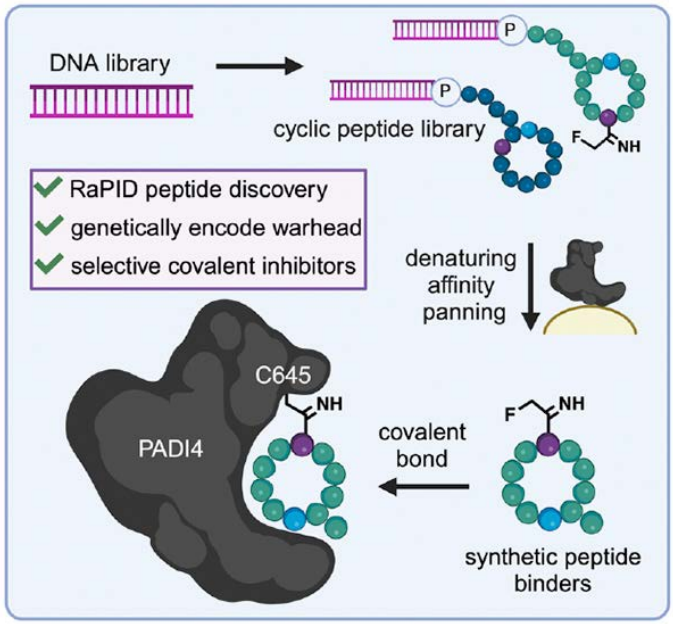
P-054

RaPID Discovery of Covalent Cyclic Peptide Binders

Isabel Mathiesen, Ewen Calder, Louise Walport

Francis Crick Institute, London, United Kingdom

Covalent inhibitors present a route to reducing dosing intervals and providing high potency against a target of interest. However, concerns remain about side-effects associated with off-target reactivity, hindering progress toward the clinic. Combining macrocyclic peptides with covalent warheads provides a solution to minimise off-target reactivity: the peptide provides tight binding with high specificity to the target, positioning a weakly reactive warhead proximal to a suitable residue in the target.



In this work we demonstrate the direct discovery of covalent cyclic peptides using encoded libraries containing a weakly electrophilic cysteine-reactive fluoroamidine warhead. We combine direct incorporation of the warhead into peptide libraries using the flexible in vitro translation system with a peptide selection approach that identifies only covalent target binders. Using this approach, we identify potent covalent inhibitors of the peptidyl arginine deiminase, PADI4, that re-

act exclusively at the active site cysteine.[1] PADI4 is an enzyme whose dysregulation is associated with rheumatoid arthritis and several cancers, and these peptides selectively inhibit PADI4 over the other PADI isoforms.[2] In the future, this platform will enable identification of further covalent peptide binders to a range of related enzymes and expansion to alternative warheads.

[1]Mathiesen, I. R., Calder, E. D. D., Kunzelmann, S. & Walport, L. J., *Commun. Chem.* **2024**, 7, 1–10
[2]Jones, J., Causey, C., Knuckley, B., Slack-Noyes, J. L. & Thompson, P. R., *Curr. Opin. Drug Discov.Devel.* **2009**, 12, 616–627

P-055

Expeditious Synthesis of Peptides with Multiple Glycosylations

Dror Ben Abba Amiel, Mattan Hurevich

The Hebrew University of Jerusalem, Jerusalem, Israel

Glycosylation is a posttranslational modification that plays a critical role in the proper structure and function of proteins, as over half of all mammalian proteins bear some form of glycosylation. Dysregulated glycosylation has been implicated in a number of disease states. As such, the synthesis of glycoproteins/glycopeptides and their derivatives is essential for understanding the connection between protein glycosylation and disease at the molecular level.

Herein, we review prior work and share current efforts toward synthesis of Fmoc-protected serine/threonine derivatives modified with N-acetylgalactosamine. These building blocks can be incorporated into general solid-phase peptide synthesis to yield corresponding glycopeptides, and we plan to synthesize mucin-like glycopeptides in future work.

P-056

Glycopeptide Phage Display for the Discovery of New Treatments Against Pathogenic Lectins

Liam Tucker[1], Hassan Boudjelal[1], Ethan Koland[1], Jasmyn Gooding[1], David Baker[2], Derek Warren[1], Robert Field[1], Taoyang Wu[1], María Marín[1], Chris Morris[3]

[1]University of East Anglia, Norwich, United Kingdom
[2]Quadram Institute Bioscience, Norwich, United Kingdom
[3]University College London, London, United Kingdom

Lectins play a key role in virulence for numerous pathogens, including the ESKAPE group member *Pseudomonas aeruginosa,* PA. The PA adhesins, LecA and LecB bind host cell glycans and play critical functions in host-cell attachment and biofilm formation.[1,2] These lectins typically bind these glycans with low monovalent affinity via shallow carbohydrate recognition domains, CRD. We aimed to increase binding affinity by selecting glycopeptides, GP, that concurrently bind CRD and nearby accessory binding pockets.[3]

We derivatised a monocyclic 7-mer peptide phage display library with reactive glycans to generate a GP library and combined this with an optimised DNA sequencing pipeline. The number of candidate GPs was reduced from thousands to tens through the inclusion of statistical filtering to remove non-specific binders. The optimised pipeline shows improved performance compared to publicly available pipelines, processing a 1 GB dataset within two minutes using multiprocessing and overlapping 8-bit encoding.

The LecA candidate GP pool was refined using ligand docking and molecular dynamics simulations, which identified three CRD vicinal accessory sites. Select candidates were synthesized, purified, and characterized using HPLC and LC-MS. Biolayer interferometry studies confirmed that select candidates displayed increased binding affinity in the nanomolar range. Ongoing studies are validating the activity of these GP candidates using microscale thermophoresis, surface plasmon resonance, haemagglutination and biofilm assays. In conclusion, our phage display platform shows strong potential to deliver rapid and precise discovery of glycopeptide-based ligands for a range of lectin targets.

[1]Wentworth JS, Austin FE, Garber N, Gilboa-Garber N, Paterson CA, Doyle RJ. Cytoplasmic lectins contribute to the adhesion of

Pseudomonas aeruginosa. *Biofouling*. **1991**;4(1-3):99-104.
[2]Diggle SP, Stacey RE, Dodd C, Cámara M, Williams P, Winzer K. The galactophilic lectin, LecA, contributes to biofilm development in *Pseudomonas aeruginosa*. *Environmental Microbiology*. **2006**;8(6):1095-104.

[3]Ng S, Lin E, Kitov PI, Tjhung KF, Gerlits OO, Deng L, et al. Genetically Encoded Fragment-Based Discovery of Glycopeptide Ligands for Carbohydrate-Binding Proteins. *Journal of the American Chemical Society*. **2015**;137(16):5248-51.

P-057

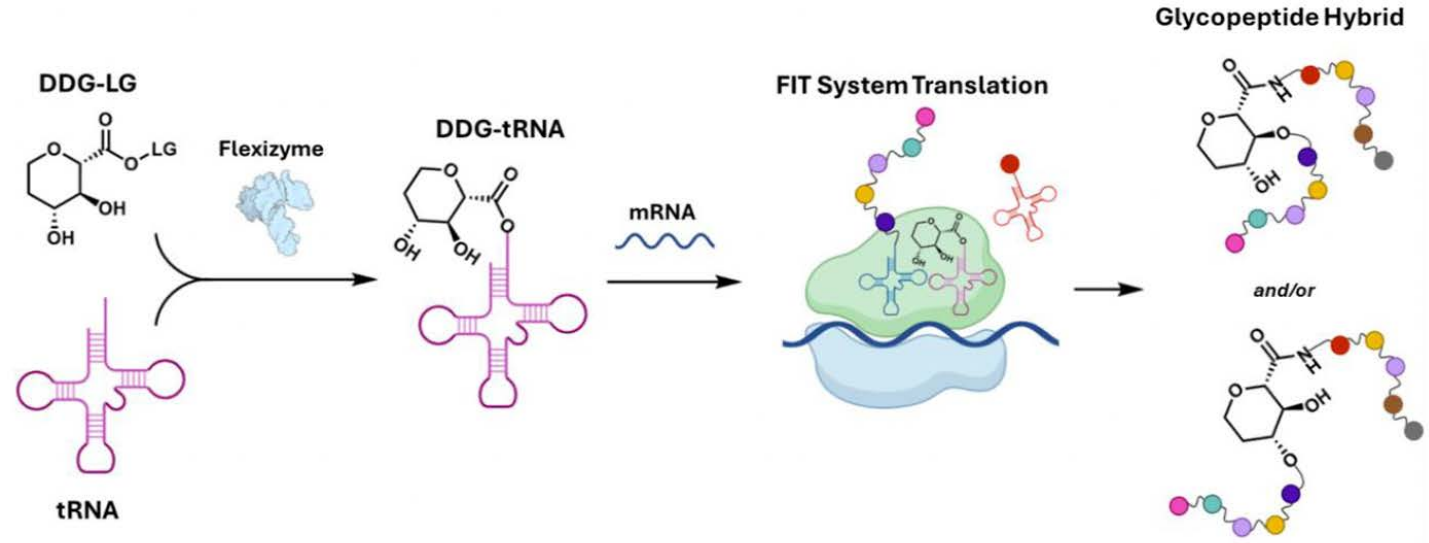
Flexizyme-Enabled Ribosomal Incorporation of Carbohydrate-Based Monomers

Luca Danieli, Hiroaki Suga

The University of Tokyo, Tokyo, Japan

Expanding the chemical space accessible to ribosomal translation is a key objective in synthetic and chemical biology. The in vitro reconstitution of pseudo-natural translation systems has significantly broadened the repertoire of monomers that can be ribosomally incorporated, extending well beyond the canonical 20 amino acids. Advances such as engineered aminoacyl-tRNA synthetases and flexizymes have enabled the incorporation of diverse non-canonical structures, including cyclic β-amino acids[1] and α-hydroxy acids[2].

Here, we report the ribosomal incorporation of a carbohydrate-derived monomer into a peptide chain. Specifically, we synthesized an activated ester of the β-hydroxy carboxylic acid 1,2-dideoxy-D-glucuronic acid, DDG, and loaded it onto an engineered proline tRNA using a flexizyme. This DDG-charged tRNA was then successfully used in an *in vitro* reconstituted *E. coli* translation system to direct incorporation of the carbohydrate monomer at both the initiator and elongator positions.



To our knowledge, this represents the first example of the ribosomal incorporation of a carbohydrate-like moiety as a peptide backbone elongator. This work not only highlights the broad versatility of flexizymes but also opens the door to the exploration of peptides with novel properties conferred by carbohydrate-based monomers.

[1]Katoh, T., Sengoku, T., Hirata, K. et al. *Nat. Chem.* **2020**, 12, 1081–1088

[2]Ohta, A., Murakami, H., Higashimura, E. et al. *Chem. Biol.* **2007**, 14, 1315-1322

P-058

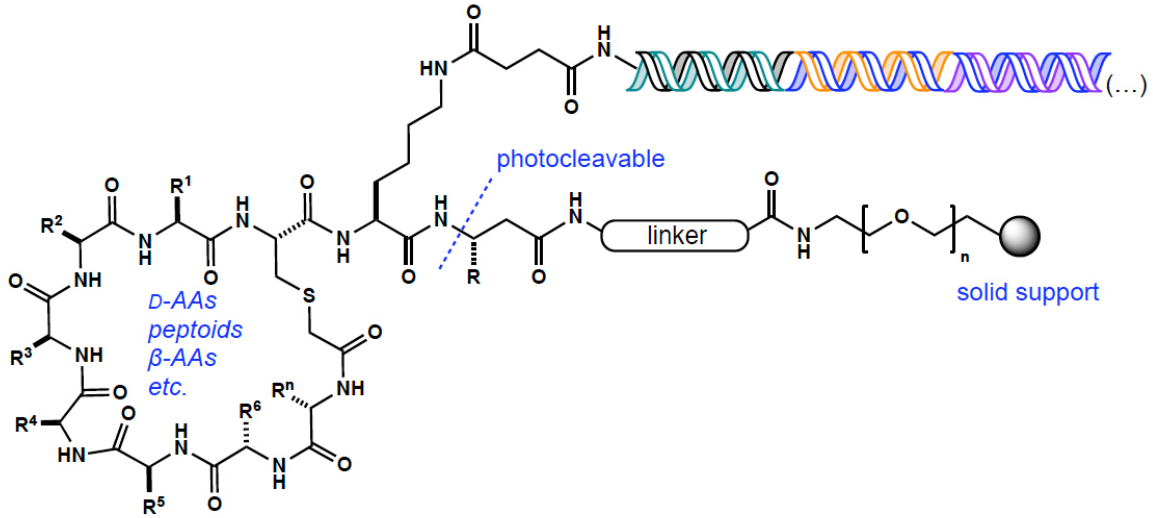
Discovery of Insulin Receptor Agonists by DNA-Encoded Libraries, DEL

Maxwell Austin, Danny Chou

Stanford University, Stanford, USA

DNA-encoded libraries, DELs, are powerful combinatorial platforms for peptide synthesis that endow the user with total synthetic control, allowing for use of entirely unnatural building blocks. Our method expands the chemical space of peptide DELs to larger, more complex peptides as part of our pursuit of insulin replacements. Despite extensive pharmaceutical engineering efforts, diabetes management is still dependent on injectable insulin formulations and would greatly benefit from an oral insulin replacement.

S597 is a phage display-derived peptide with high insulin receptor, IR, binding affinity but suboptimal activation ability. In a binding mechanism distinct from human insulin, S597 activates the IR by engaging two distinct binding sites. We posit that separate site 1 and 2 binders can be designed and connected by a linker to activate the IR, opening the possibility to incorporate peptidomimetics and unnatural structures using S597 as an initial lead.



We aim to discover unnatural site 1 and 2 ligands by developing DEL methods for library synthesis of cyclic peptides and helical mimetics. Traditionally, peptide DELs are limited to five steps, due to repeated use of coupling conditions without washing. To increase the number of efficient reaction cycles, we are developing a DEL design with DNA conjugated directly to resin-bound peptides, allowing for longer syntheses with similar iterative coupling steps to solid-phase peptide synthesis, each tagged with a unique DNA barcode. Intact peptide-DNA conjugates are photochemically cleaved from the resin for IR binding selections followed by PCR analysis. This new DEL methodology provides a new platform to discover peptidomimetic binders of target proteins.

P-059

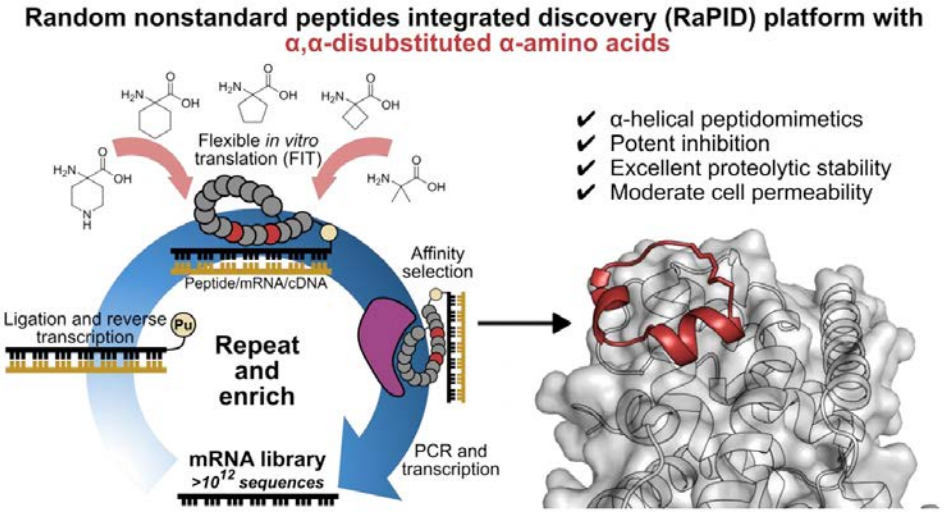
De Novo Discovery of α-Helical Peptides Containing α,α-Disubstituted α-Amino Acids

Maxwell Sigal[1], Markus Egner[2], Chikako Okada[3], Daniel Merk[2], Toru Sengoku[3], Takayuki Katoh[1], Hiroaki Suga[1]

[1]The University of Tokyo, Tokyo, Japan
[2]Ludwig Maximilian University of Munich, Munich,Germany
[3]Yokohama City University, Yokohama, Japan

α-helical peptidomimetics have been widely utilized as powerful tools to regulate α-helical protein-protein interactions, PPIs.[1] Critical to their success are α,α-disubstituted α-amino acids, dαAAs, which induce helicity and can increase binding affinity, proteasomal resistance, and cell permeability.[2] Currently, there is a critical need to develop

a high throughput, *de novo* screening platform which can effectively employ dαAAs. Modern high throughput, cell-based display technologies are restricted to L-α-amino acids, while chemically synthesized libraries containing dαAAs are low-throughput and low diversity, leading to weaker binding peptides.



Herein, we overcame these obstacles by employing the *in vitro* mRNA display platform known as Random non-standard Peptide Integrated Discovery, RaPID.[3] To the best of our knowledge, we report the first ribosomally synthesized peptide libraries based on dαAAs. Diverse linear and thioether-closed macrocyclic peptide libraries comprising of over 10^{12} members were created. Subsequently, these libraries were applied to affinity selection against peroxisome proliferator-activated receptor gamma, PPARɣ, a nuclear receptor implicated in diabetes and various cancers. Many hit peptides were potent *in vitro* inhibitors, IC_{50} 0.5 – 50 nM), highly serum stable, half-life up to 100 h, and moderately cell permeable with *in cellulo* activity in the low μM range.

Finally, co-crystallization revealed that peptides bound at the α-helical PPI interface via a helix-turn-helix conformation. These data validate the dαAA-based RaPID platform, providing access to the *de novo* discovery of dαAA-containing α-helical peptidomimetics.

[1]Li, X.; Chen, S.; Zhang, W.-D.; Hu, H.-G. *Chem. Rev.* **2020**, 120 (18), 10079-10144.
[2]Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers,* Peptide Sci. **2001**, 60, 396-419.
[3]Passioura, T.; Suga, H. *Chem. Commun.* **2017**, 53 (12), 1931-1940.

P-060

Efforts Towards the Synthesis of Cyclic Z-Domain Peptide Libraries

Noelle Waltenberg, James Checco

University of Nebraska-Lincoln, Lincoln, NE, USA

Chemical synthesis of proteins using solid-phase peptide synthesis has been an effective technique for synthesizing proteins that would otherwise be difficult in gene expression systems. The addition of unnatural amino acids and chemoselective peptide ligation are some benefits of chemical synthesis. A proof-of-concept study is currently underway developing a new synthetic approach to creating small helical peptide libraries using multiple ligation techniques. An ideal model system is the well-studied Z-domain. This small, three-helix protein is derived from staphylococcal protein A and binds to human IgG. Studies using phage display have shown that Z-domain maintains binding ability to human IgG when truncated.

Additional work, also utilizing phage display, has engineered the Z-domain sequence to recognize proteins beyond human IgG. One disadvantage of phage display is its limitation to naturally occurring amino acids. Our study aims

create a new approach for creating Z-domain combinatorial libraries using unnatural amino acids in combination with chemoselective ligation techniques. To expand off the work that has been previously completed using the Z-domain system, individual helices of Z-domain are synthesized using solid-phase peptide synthesis and ligated together using multiple sequential chemical ligation reactions. This study uses two different Z-domain analogues – one designed to bind human IgG, Z-IgG, and the other to VEGF, Z-VEGF.

Current work has included the successful synthesis of individual helices for Z-IgG and Z-VEGF, reaction optimization for azidealkyne cycloaddition, ongoing optimization for achieving dibromo-xylene cyclization, and preliminary binding assays to study these interactions post-ligation. These assays will be used to test the capability of the ligated helices using IgG, VEGF, or other Z-domain binding proteins. The value of this study would allow for the ability to create Z-domain combinatorial libraries that can be utilized to rapidly identify protein-binding Z-domain analogues bearing non-natural amino acid residues.

P-062

Fluorescence-Quenching Screen for Discovering Potent Protein–Protein Interaction Inhibitors

Seungyoon Jang[1], Minhyeon Shin[2], Hyun-suk Lim[1]

[1]POSTECH, Pohang, South Korea
[2]Daegu National University of Education, Daegu, South Korea

Protein–protein interactions, PPIs, are involved in wide range of biological processes, and aberrant PPIs are implicated in many complex diseases such as cancer and immunerelated disease. Hence, there is a significant interest in developing molecules that can modulate disease-related PPIs. However, targeting PPIs is one of the most difficult challenges in drug discovery due to the lack of efficient high throughput screening, HTS, method. The conventional screening methods used for identifying modulators of PPI such as surface plasmon resonance, FRET, and enzyme-linked immunosorbent assays are usually based on multi-well plate format.

On-bead screen methods have inherent false positive problems. The high ligand density on the bead surface enables the target protein to bind with multiple ligands on bead surface, avidity effect, and the hit compounds isolated from screening may not act as inhibitors of PPI, as expected. In this this study, fluorescence-quenching on-bead screen method was firstly developed for discovery of direct inhibitors of target PPI. By utilizing fluorescence-quenching on-bead screen, we successfully discovered the direct inhibitors of NCOA1 and STAT6 interaction. Taken together, fluorescence-quenching on-bead screen will serve as a novel HTS method for discovering direct inhibitors of PPI excluding false positive issues in typical affinity-based screening.

P-063

Systematic Assessment of Backbone N-Methylation on Peptide Permeation Across the Mycomembrane in Live Mycobacteria

Zichen Liu[1], Irene Lepori[2], Sloan Seigrist[2], Marco Pires[1]

[1]University of Virginia, Charlottesville, USA
[2]University of Massachusetts, Amherst, USA

Mycobacteria process an outer membrane like gram-negative bacteria but have a more complex cell wall envelope, consisting of a highly hydrophobic mycomembrane on the outside, and an arabinogalactan layer covalently attached to its peptidoglycan, PG. The presence of mycomembrane provides a drug penetration barrier for the antibiotics to reach their target, which has been long hypothesized to be one of the major reasons for the failure of antibiotics in mycobacteria.

Recently, the discovery of ClpC1P1P2 complex targeting antimycobacterial peptides highlighted the essentiality for the peptides to be cyclic with backbone N-methylation to cross the mycobacterial cell envelop and reach their intracellular targets.[1] While it has been demonstrated beneficial for peptide drugs to be N-methylated to cross the mammalian cell membrane,[2] there is limited information about whether the N-methylation method can help the peptides readily penetrate the mycomembrane.

Herein, we show an assay developed in our lab that can assess the relative permeability of small azido molecules, including peptides, through the mycobacterial cell envelope.[3] The assay involves metabolic incorporation of a dibenzocyclooctyne, DBCO, conjugated tetra peptide-based probe on the PG of *Mycobacterium smegmatis, M. smegmatis*, and the incubation of the bacterial cells with azido-tagged compounds. The rest of DBCO sites on the PG were determined by incubation with azido-fluorescein, which can give cellular fluorescent signal reversely related to small molecule permeability.

Bacterial cells tested with small molecules with high permeability to the PG were expected to show low fluorescent intensity and vice versa. A series of backbone *N*-methyl and azido-tagged short peptides were designed to systematically assess the effect of *N*-methylation on peptide permeability through the mycomembrane.[4] Additionally, the permeability of a series of analogues of an antimicrobial peptide were found to be enhanced by *N*-methylation, corresponding to their increase in antimycobacterial activity.[4]

Although only a library of short peptides was tested, we project that the assay provided a platform for better understanding of the permeability of peptide libraries crossing the mycomembrane, facilitating the development of novel antimycobacterial peptides.

[1]Hoi, D. M. et al. Clp-targeting BacPROTACs impair mycobacterial proteostasis and survival. *Cell* **2023**, 186 (10), 2176-2192.e22.
[2]Ovadia, O. et al. The Effect of Multiple N-Methylation on Intestinal Permeability of Cyclic Hexapeptides. *Molecular Pharmaceutics 2011*, 8 (2), 479-487.
[3]Liu, Z. et al. A Metabolic-Tag-Based Method for Assessing the Permeation of Small Molecules Across the Mycomembrane in Live Mycobacteria. *Angew. Chem. Int. Ed.* **2023**, e202217777.
[4]Dash, R. et al, Systematic Determination of the Impact of Structural Edits on Accumulation into Mycobacteria. *bioRxiv* **2025** Jan. doi: 10.1101/2025.01.17.633618.

P-064

Antimicrobial Coating Based on Mussel Adhesive Proteins and Silver Nanoparticle-Binding Sequences for Surface Modification of Titanium

Adriana Gauna[1], Ítalo Moglia[2], David Vásquez[3], Freddy Celis[4], Fanny Guzmán[5], Fredys Sánchez[6], Maycol Huerta[7], Carlos Álamos[1], Eyleen Araya[7], Marcelo Kogan[1]

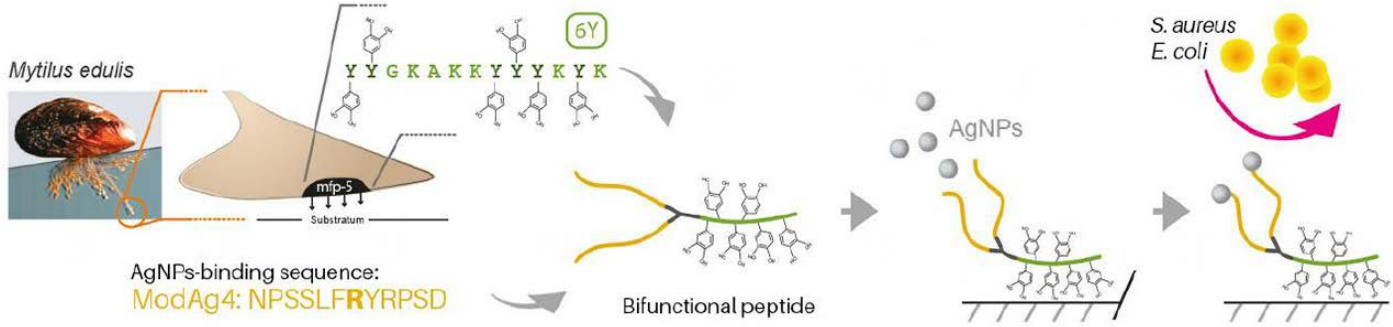
[1]Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile., Santiago, Chile
[2]Departamento de Química, Facultadde Ciencias Naturales, Matemáticas y del Medioambiente. Universidad TecnológicaMetropolitana., Santiago, Chile
[3]Departamento de Química Farmacológica y Toxicológica,Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
[4]Laboratorio de Procesos Fotónicos y Electroquímicos, Facultad de Ciencias Naturales yExactas, Universidad de Playa Ancha, Valparaíso, Chile
[5]Núcleo de Biotecnología deCurauma (NBC), Pontifi cia Universidad Católica de Valparaíso, Valparaíso, Chile
[6]Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas yFarmacéuticas, Universidad de Chile, Santiago, Chile
[7]Departamento de Ciencias Quimicas,Facultad de Ciencias Exactas, Universidad Andres Bello., Santiago, Chile

Titanium alloys are widely used in biomedical devices; however, they are prone to bacterial infections.[1] Silver nanoparticles, AgNPs, are well-known for their broad-spectrum antimicrobial properties[2], but their use is limited by the cytotoxicity associated with high silver ion release. Therefore, developing a stable coating that allows controlled ion release is critical.

In this study, we synthesized a bifunctional peptide by combining a mussel-derived adhesive sequence rich in dihydroxyphenylalanine[3] with an AgNP-binding sequence.[4] This peptide retains both adhesive and antimicrobial properties, facilitating effective integration with titanium surfaces and AgNPs.

Surface characterization techniques included: **1.** Quartz Crystal Microbalance, QCM, analysis to assess stable adhesion of the peptide and AgNPs; **2.** contact angle measurements to determine surface wettability; **3.** X-ray photoelectron

spectroscopy, XPS, to confirm the presence of peptide and AgNPs on the surface; **4.** scanning electron microscopy, SEM, to evaluate particle density, shape, and size; **5.** atomic force microscopy, AFM, to observe surface roughness changes due to AgNP adsorption; and **6.** Raman spectroscopy, which provided insights into the orientation of the peptide on the titanium and nanoparticle surface.



The AgNP-coated surface demonstrated a controlled release of silver ions and achieved 100% bacterial killing of *S. aureus* and *E. coli* after 24 and 3 hours of exposure, respectively, with no cytotoxic effects on fibroblasts. This approach may offer effective protection against common pathogens in surgical implants. Moreover, the use of synthetic peptides allows flexibility in future modifications, such as the incorporation of D-amino acids or additional functional sequences.

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P-065

Peptide Nanonet Coating to Prevent Catheter-Associated UrinaryTract Infection

Dhanya Mahalakshmi Murali[1], Jiaqi Ge[1], Nhan Dai Thien Tram[1], Tsung Wen Chong[2], Rachel Pui Lai Ee[1]

[1]National University of Singapore, Singapore, Singapore
[2]Singapore General Hospital,Singapore, Singapore

Catheter-associated urinary tract infections, CAUTIs, pose a significant challenge in healthcare, making up around 40% of hospital-acquired infections due to the introduction of uropathogenic bacteria through urinary catheters.[1] The primary pathogens linked to CAUTIs include Uropathogenic *E. coli,* UPEC, *K. pneumoniae* Carbapenemase Producers, and *P. mirabilis*.[2] Current preventive measures, such as antimicrobial catheters, have seen limited success due to poor biocompatibility, short-lived effectiveness, and the growing problem of antibiotic resistance.[3] In our previous work, we developed a collection of β-hairpin antimicrobial peptides, AMP, that can form nanonets in the presence of bacteria, enabling simultaneous trapping and killing of pathogens.[4,5]

In this study, we covalently conjugated one of these peptides, BTT1-3A, onto a polyurethane substrate, which serves as a model polymer that can be applied for urinary catheter development. Over a four-week period, the AMP coating exhibited antifouling ability against uropathogens. Scanning Electron Microscopy revealed the formation of

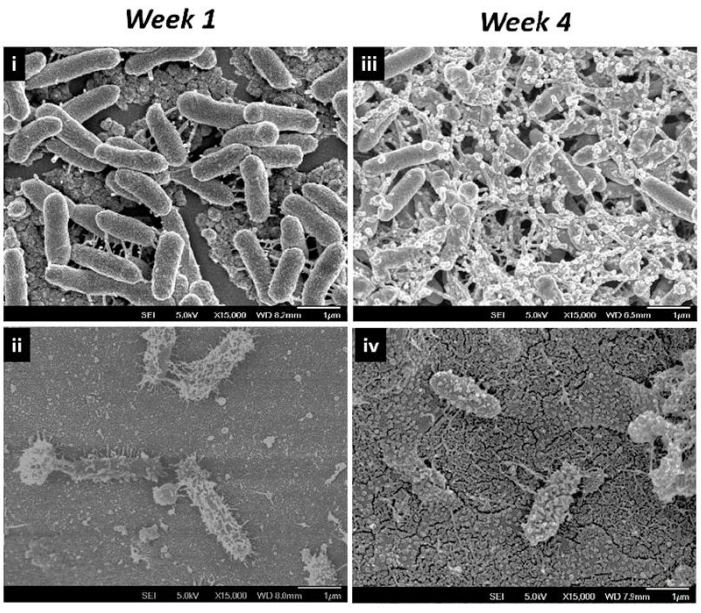

Figure: SEM images of peptide coated, ii and iv, and uncoated Polyurethane, i and iii, incubated with 106 CFU/ml of UPEC after 1 and 4 week.

peptide nanonets enveloping UPEC, emphasizing the importance of structural flexibility in peptide design. Live/Dead staining assays demonstrated that AMP-coated surfaces prevented biofilm formation and reduced bacterial motility, while uncoated surfaces promoted the development of mature biofilms. Additionally, the peptide coating effectively curtailed reactive oxygen species production and sequestered pro-inflammatory cytokines like TNF-α.

These results highlight the promise of AMP-functionalized coatings as a viable long-term strategy to prevent CAUTIs by addressing both bacterial colonization and associated inflammatory responses. Overall, we hope to develop a multimodal peptide-based platform that can address catheter-associated urinary tract infection and its related inflammation.

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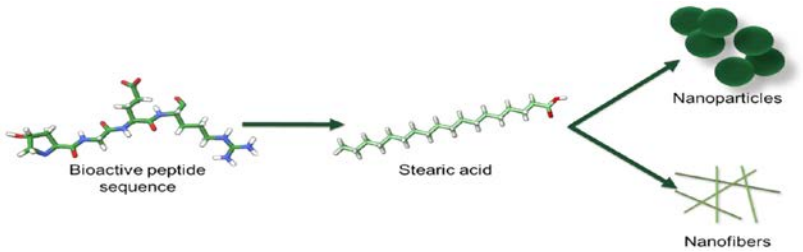
P-066

Bioengineering of Lipopeptides for Therapeutic Applications

Dhriti Santosh Shenoy[1], Michaela Pesenti[1], Kaliroi Peqini[1], Nicolas Simon[2], Stefania Crespi[1], Cecile Echalier[3], Gilles Subra[3], Sara Pellegrino[1]

[1]University of Milan, Milan, Italy
[2]Universitat Politècnica de Catalunya, Bacelona, Spain
[3]University of Montpellier, Montpellier, France

Over the past decade, there has been a significant rise in the use of bio-inspired and naturally derived nanomaterials due to their enhanced efficiency, biocompatibility, and ability to self-assemble into complex, functional structures in response to external stimuli. These materials often exhibit thermodynamically and kinetically driven self-organization, leading to the formation of hierarchically ordered nanostructures with specific properties. This self-assembly process, in response to stimuli such as temperature, pH, or ionic strength, is of great interest for developing materials with tailored functionalities.[1,2]



In this study, we present the synthesis of a small library of lipopeptides, where bioactive peptide sequences are covalently modified by the addition of a stearic acid group at the *N*-terminus.[3] The incorporation of the hydrophobic stearic acid tail is crucial, as it drives the self-assembly of the peptides through hydrophobic interactions, leading to the formation of nanostructures such as micelles or fibrils. These self-assembled lipopeptides demonstrate enhanced stability and bioactivity due to the synergistic effects of the peptide's functional properties and the lipid's self-organization behavior.[4]

This approach provides a versatile platform for designing and engineering nanomaterials with controlled morphology and functionality. The resulting lipopeptide-based nanostructures hold significant potential for a range of applications, including drug delivery, biosensing, and tissue engineering. By tuning the peptide sequence or modifying the lipid component, the properties of these nanostructures can be tailored for specific uses, making them a promising candidate for diverse biomedical and biotechnological applications.[5]

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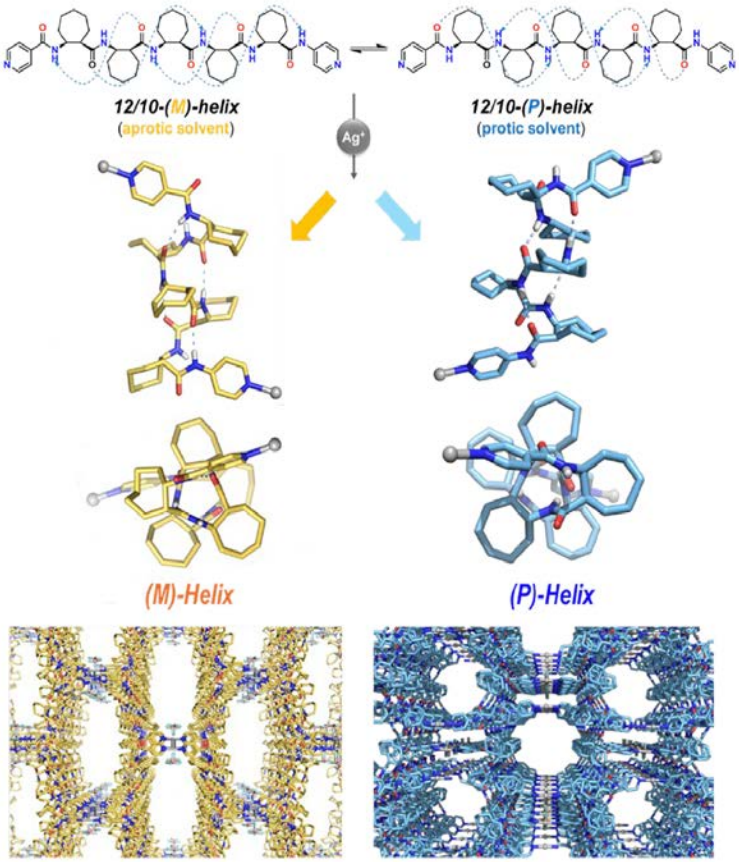
P-067

12/10-Helical Peptidic Foldamer-Based Porous Metal-Helix Frameworks: Screw-Sense Inversion Induces Structural Duality

Ingyu Han, Soo Hyuk Choi

Yonsei University, Seoul, South Korea

Peptide-based materials possess significant advantages in terms of chirality and modularity, as they consist of diverse chiral building blocks, including unnatural amino acids. The secondary structures of peptides, such as helices, sheets, or turns, are another interesting feature of peptides as they show potential for applications in complex systems with unique functionalities. One growing area of interest in peptide research involves complexation of these conformationally rigid helical peptides with metal ions to construct higher-order frameworks.[1] Unlike conventional aromatic ligands used in metal-organic frameworks, MOFs, helical peptide-based ligands enable the construction of systems with greater complexity. Importantly, helical peptides introduce chirality over point chirality, termed handedness, which is difficult to achieve with conventional organic ligands. However, rational design and structural analysis of metal-helix frameworks with helical peptides remain challenging as their helical conformations easily influenced by the external non-covalent interactions and their crystal structures are hard to acquire. Only a limited number of examples have been reported yet.



We previously reported that heterochiral β-Peptides

consist of alternating chirality can adopt both handedness and affected by the solvent condition.[2] Inspired by the conformational duality of 12/10-helical β-Peptides, we envisioned that 12/10-helical peptide could serve as a promising scaffold for an ambidextrous ligand, resulting in two different types of metal-helix frameworks, MHFs, from a single linker.

Herein, we demonstrate two distinct structures of MHFs made from single 12/10-helical pentapeptide foldamers. The inversion of the 12/10-helix leads to different MHF structures with either left- or right-handed helical ligands.

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P-068

Stimuli-Responsive Coiled Coil Fibers via Dynamic Covalent Bonds

Ivan Montes de Oca Estrada, Marcos Daniel García Romero, Elena Pazos Chantrero

University of A Coruña, A Coruña, Spain

The coiled coil is a widely studied supersecondary structure found in many natural proteins, including filaments,[1] which constitutes one of the main protein oligomerization motifs. Given its well-defined sequence-to-structure relationship, ease of programmability, and tunability, this protein motif has garnered significant attention for the design of new peptide-based materials. Extensive research has been conducted in this area over the last two decades, exploiting different assembly strategies to create higher order assemblies of coiled coil building blocks.[2] However, the development of stimuli-responsive materials from coiled coils still remains largely underexplored.[3] Inspired by these systems and taking advantage of the inherent stimuli-responsiveness of dynamic covalent chemistry, we have designed a new family of stimuli-responsive higher order assemblies based on coiled coil structures.

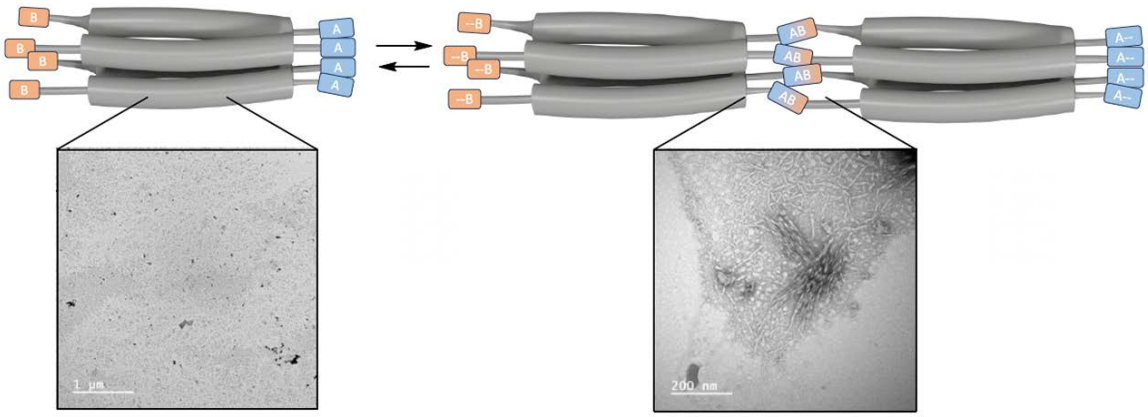

Figure 1. Top: schematic representation of the proposed assembly of coiled coil oligomers into nanostructures. Bottom: TEM images show reversible fiber assembly and disassembly via dynamic covalent chemistry.

We carried out a rational design of a parallel coiled coil domain modified with functional groups suitable to form dynamic covalent bonds. The peptide derivative was synthesized following standard Solid Phase Peptide Synthesis, SPPS, protocols and characterized by HPLC-MS and MALDI. After promoting the assembly of the coiled coil oligomer into higher order assemblies, the resulting structures were characterized by microscopic techniques. Preliminary TEM images revealed fiber-like nanostructures that can be reversibly controlled thanks to their stimuli-responsive behavior.

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P-069

One-Pot Synthesis of Gold Nanoparticles Immobilized on Microbeads Using Gold Ion Reduction Peptide

Koki Yoshida, Shuhei Yoshida, Takaaki Tsuruoka, Kenji Usui

Konan University, Kobe, Japan

Specific properties of gold nanoparticles, GNPs, such as localized surface plasmon resonance, LSPR, and catalytic activities attract attention for various applications such as biosensors and catalysts. Especially, study of immobilized GNPs has been actively performed because immobilization has some merits such as avoiding aggregation of GNPs, and reuse. However, conventional methods of synthesis and immobilization of GNPs require a high temperature conditions and multi-step reactions.[1] Hence, a milder one-step method is required.

Meanwhile, some peptides had been identified that they could reduce gold ions under low environmental impact.[2] A one-step method for synthesis and immobilization of GNPs would be achieved using an immobilized peptide containing an Au ion reduction sequence. We hypothesized that immobilized peptide could provide simultaneous synthesis and immobilization of GNPs. After Fmoc solid peptide synthesis and then removal of protecting groups using microbeads without a cleavable linker, the immobilized peptides could be easily prepared.[3]

At first, we synthesized peptidyl beads, AuBP1-beads, with sequence that has ability to reduce gold ions, AuBP1.[4,5] After gold ion reduction using AuBP1-beads, UV-Vis measurement and SEM-EDX observation confirmed GNPs were generated and immobilized on peptidyl beads. Additionally, all the sample at various $HAuCl_4$ concentrations, 0.5-1000 μM, showed circa 40 nm particles. In ICP-AES measurement, amount of the immobilized GNPs in the sample was increased depending on $HAuCl_4$ concentrations. Moreover, we evaluated total amount of catalytic activities of GNPs in the sample, catalytic amount, using 4-nitrophenol. These results indicated that catalytic amount could be controlled by changing initial $HAuCl_4$ concentrations in this method.

Thus, we achieved the one-step method for synthesis of immobilized GNP using AuBP1-beads. Furthermore, the number of GNPs per bead with similar properties could be adjusted with $HAuCl_4$ concentration in this method.

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P-070

Stable 4-Mercaptoproline Stapled Collagen Mimetic Peptides

Megan Sargent, Amarachi Osuji, Zhengwen Lian, Cassandra Cancemi, Joshua A. Kritzer

Tufts University, Medford, USA

Collagen is an abundant extracellular matrix protein with multiple functions within the body including structural support, direct interactions with receptors, and clustering effects on other proteins. However, studying collagen is challenging because of synthetic limitations of collagen-mimetic peptides, which typically require long peptides, complex synthetic strategies, and/or large stabilizing domains to produce stable triple-helical assemblies. In this project, we apply 4-mercaptoproline stapling to produce high-stability assemblies using short collagen-mimetic peptides. This strategy requires a small, molecular three-amino-acid domain while maintaining the desired proline-rich environment. We computationally designed a homotrimeric assembly with an elegant knotted staple formation, and we demonstrate the high-yield stapling of these assemblies. We also show these stapled triple-helical assemblies are hyperstable with respect to thermal denaturation. The relative ease and modularity of this stapling strategy should provide many advantages over currently applied strategies for assembly and stabilization of synthetic collagen-like triple helices.

P-071

De Novo Design of Porous Peptide Frameworks with Protein Tertiary Structure

Pragati Ganatra, Daniel F. Wang, Savannah Nichols, Viet Thuc Dang, Andy I. Nguyen

University of Illinois Chicago, Chicago, USA

Porous peptide frameworks, PPFs, are crystalline materials featuring well-defined pores or channels with the potential to mimic protein active sites. While PPFs possessing secondary and quaternary structures are widely documented, there are no reports of PPFs with tertiary structures. *De novo* design of a peptide possessing a tertiary structure will enhance our understanding of protein folding and self-assembly, while also allowing for the incorporation of complex enzyme active sites.

In this work, we report the *de novo* design of a peptide that self-assembles into a tertiary structure featuring an α-helix, a loop, and a β-sheet. This structure is achieved by combining an 11-residue α-helix and amyloidogenic sequences 2-6 AAs long. Remarkably, the peptide folds into a tertiary structure with a length of only 15 amino acids. Single crystal X-ray diffraction, SC-XRD, revealed a diversity of tertiary structures that further assemble into a porous lattice replete with functional groups. This strategy can be adapted to include β-sheets of varying lengths and functional group compositions. Our results demonstrate that a well-defined tertiary structure can emerge from combinations of short peptide modules. Additionally, this system exhibits phosphatase activity to catalyze the hydrolysis of ATP to ADP.

P-072

Universal Peptoid Unit Cell Enables Rational Design of High-Precision Nanostructures

Jen Yea Lee

Lawrence Berkeley National Laboratory, Berkeley, USA

Precise control over the dimensionality and molecular packings of nanostructures is central to nanoscience. Self-assembled soft materials from peptides and polymers have expanded morphological diversity, but designing hierarchical nanostructures with atomic-level precision remains challenging due to complex noncovalent interactions and the retrospective characterization of unit cells. Peptoids, as sequence-defined polymers, bridge biological precision with polymeric robustness and exhibit a universal unit cell motif in their self-assembled nanostructures: four peptoid pillars in a 2×2 grid, interacting backbone-to-backbone along the *a*-axis and side chain-to-side chain along the *c*-axis.

Here, we leveraged peptoid unit cell motifs as synthons for supramolecular synthesis. By chemically engineering the primary sequences, we tuned noncovalent interactions within the unit cells and characterized the resulting morphologies and their packing using cryogenic transmission electron microscopy, cryo-TEM, and molecular dynamics, MD, simulations. Chemical point mutations enabled the formation of one-dimensional, 1D, nanofibers with single-unit cell widths, achieving a level of precision that had been challenging. Incorporating perfluoroaromatic side chains further facilitated the assembly of highly ordered, hetero-assembled two-dimensional, 2D, nanosheets with exceptional precision and crystallinity. This approach allowed direct imaging of perfluoro-phenyl interactions within monolayered molecular sheets for the first time.

To further expand the use of these unit cell motifs in nanostructure design, end-group modifications were employed to achieve three-dimensional, 3D, peptoid assemblies. Through combined cryo-TEM imaging and MD simulations, our study offers critical insights into peptoid packing at the nanoscale. Establishing these unit cell motifs as design elements advances the precision and predictability of nanostructure engineering, opening new avenues for nanomaterials research.

P-073

Development of Novel SARS-CoV-2 Papain-Like Protease Inhibitors and their Application as Mpox I7L Protease Inhibitors

Kouki Shinohara[1], Takuya Kobayakawa[1], Yuki Takamatsu[2], Kohei Tsuji[1], Hiroaki Mitsuya[2], Hirokazu Tamamura[1]

[1]Laboratory for Biomaterials and Bioengineering, Institute of Integrated Research, Instituteof Science Tokyo, Tokyo, Japan
[2]National Center for Global Health and Medicine ResearchInstitute, Tokyo, Japan

Papain-like protease, PL^{pro}, is a viral protease essential for SARS-CoV-2 replication in concurrence with a main protease. The active site of SARS-CoV-2 PL^{pro} has 100% homology to that of SARS-CoV PL^{pro}. Previous studies by Mitsuya et al. demonstrated that a SARS-CoV PL^{pro} inhibitor, GRL-0048, exhibits inhibitory activity against SARS-CoV-2 PL^{pro}.

Accordingly, we conducted structure-activity relationship studies using GRL-0048 as a lead compound to develop potent SARS-CoV-2 PL^{pro} inhibitors. We have developed several potent SARS-CoV-2 main protease inhibitors by fluorine substitution, which enhanced inhibitory potency and cell membrane permeability.

In this study, novel derivatives with fluorine substitution were designed and synthesized. As the results of antiviral assays, several compounds showed significantly enhanced potency compared to the parent compound GRL-0048.[1] Next, we focused on the main protease of monkeypox virus, MPXV, I7L protease, $I7L^{pro}$. This protease has high similarities in the active sites, Cys328-His241-Asp248, with that of SARS-CoV-2 PL^{pro}, Cys111-His271-Asp286.

Therefore, we tested our synthesized PL^{pro} inhibitors against $I7L^{pro}$. Intriguingly, we identified several compounds as selective inhibitors against MPXV, which did not show the activity against SARS-CoV-2. Herein, we will present the design and synthesis of the compounds with their anti-viral activity against SARS-CoV-2 and MPXV.

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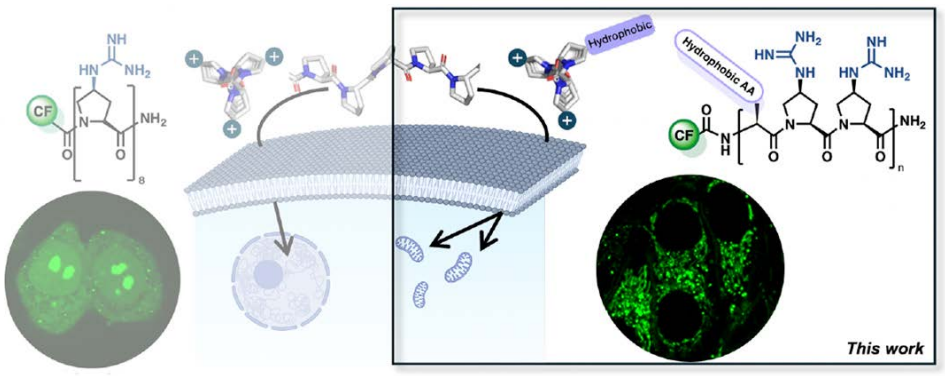
P-074

Amphipathic Proline-Rich Cell Penetrating Peptides for Mitochondria Targeting

Adeline Schmitt, Helma Wennemers

Laboratory of Organic Chemistry, ETH Zürich, Zürich, Switzerland

Cell penetrating peptides, CPPs, cross the lipophilic cellular membrane and serve as delivery vectors to translocate cargo into cells.[1] CPPs can also be useful for target-specific delivery, for example, of bioactive molecules to a specific cellular organelle. Here, targeting mitochondria constitutes an important goal since mitochondria dysfunction is associated with many diseases, including neurodegenerative and auto-immune diseases, diabetes, and cancer. Due to the dense and hydrophobic double membrane, the selective delivery of bioactive compounds to mitochondria is challenging.[2]



Our group developed an oligoproline, Z_8, CPP that exhibits higher cellular uptake than more flexible peptides, for example, octaarginine, and localizes in the cytoplasm and the nucleus.[3] Here, we show that oligoprolines with hydrophobic amino acids installed at every third position allow for mitochondria targeting. Selectivity is achieved by the PPII helical conformation with two cationic faces and one hydrophobic face, enabling the crossing of the mitochondria membranes. The localization of the amphipathic peptides inside cells was evaluated by confocal microscopy and the cellular uptake efficiency by fluorescence-activated cell sorting, FACS.

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P-075

Using Peptides to Study Sliding Clamp-DNA Polymerase Interactions in *Bacillus subtilis* and *Escherichia coli*

Ashley Clemente

Fordham University, Bronx, USA

Bacterial DNA replication is essential for survival and is carried out by the replisome, a molecular machine composed of multiple interacting proteins. Among these, the sliding clamp protein enhances the processivity of DNA polymerases, which is critical for efficient replication. The *E. coli* replisome has served as the primary model for studying sliding clamp-DNA polymerase interactions; however, though the sliding clamp protein is conserved across bacterial species, there are numerous sequence differences in the sliding clamp proteins from different species, including in their DNA polymerase binding sites.

To understand how these differences influence sliding clamp-DNA polymerase interactions, we studied how peptides, derived from different DNA polymerase clamp-binding motifs, interact with the sliding clamp proteins from *E. coli* and *Bacillus subtilis*. Peptide-protein interactions with the sliding clamp proteins from both species were investigated using fluorescence polarization assays with fluorescently labeled clamp-binding motif peptides. Overall, we found that the *E. coli* sliding clamp interacts more strongly with all peptides except the negative controls. These data suggest that *B. subtilis* DNA polymerases do not require a strong interaction with the sliding clamp. These findings revise our current understanding of the role of sliding clamp-DNA polymerase interactions in bacterial DNA replication and may offer new opportunities to develop new peptide-based antibiotics targeting bacterial DNA replication.

P-076

Development of Aryl Diazonium-Containing Peptides for Labeling Endogenous Protein Receptors

Chase Stebbing, Sheryl Sharma, James Checco

University of Nebraska - Lincoln, Lincoln, USA

Many diseases are characterized by the dysregulation of peptide hormones. Often, these peptides do not have known protein receptors, which can limit investigation into designing possible therapeutic treatments. While there are methods to discover signaling proteins and peptides related to disease regulation, there are very limited protocols to uncover the receptors for these signaling molecules.

Previous work by the Checco lab has shown that aryl diazonium AD-containing peptides can label G protein-coupled receptors in overexpressed cellular systems. This current work aims to solve two AD-based labeling drawbacks: the rapid degradation of the AD functional group at room temperatures or warmer, and the mild off-target labeling of cellular surface proteins. We aim to solve these two drawbacks by developing a series of aryl diazonium-amino acid

precursors with para-substituted electron donating groups. We predict the electron rich AD moiety will slow reactivity and degradation due to the increased electron density on the reactive atoms. Consistent with our hypotheses, results show there is a large decrease in degradation rate when electron donating groups are added to the AD at multiple temperatures. Second, to test if AD labeling can be effective at enriching endogenously expressed receptors, we are developing AD-containing insulin analogs to enrich the insulin receptor as a model system. We chose the insulin/insulin receptor system because the insulin receptor is easily detected by known protocols and the receptor exists in all human immortal cell lines.

With the successful completion of these projects, future works may include using AD labeling to identify the receptors for bioactive peptides without known receptors.

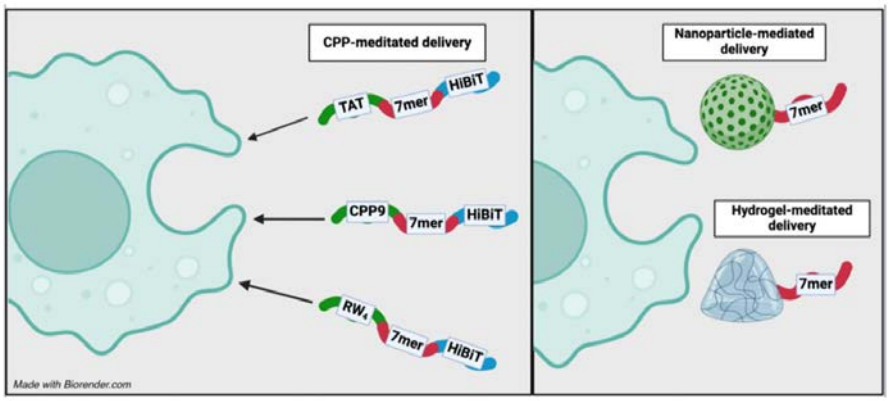
P-077

Enhancing the Cytosolic Delivery of Bioactive Peptides

Kathryn Fincham[1], Yuxue Cao[2], Jie Tang[2], Mark Del Borgo[3], Ray Norton[1]

[1]Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia,
[2]Drug Delivery, Disposition, and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC3052
[3]Department of Pharmacology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia

Peptide drugs offer many advantages over small molecule drugs, including high affinity, target selectivity, and the ability to bind to 'undruggable' targets. However, peptides also present unique challenges, including that they cannot traverse cell membranes.[1] One such class of impermeable peptides is DINNN-containing peptides, which are high-affinity inhibitors of the interaction between the SPSB1, 2 and 4 proteins and inducible nitric oxide synthase, iNOS.[2,3] When delivered to the cytosol of M1 macrophages, DINNN peptides extend the lifetime of iNOS and thus increase NO output,[4] making them potential host-directed antibiotics.



In an attempt to improve the cellular delivery of these peptides, they were conjugated to cell-penetrating peptides CPPs. The Split Luciferase Endosomal Escape Quantitation, SLEEQ assay[5] was used to monitor the cytosolic delivery and endosomal escape of a DINNN peptide conjugated to the CPPs TAT, RW_4, and CPP9.[6] Cytosolic delivery of the cargo peptide was enhanced significantly by each of the CPPs, although endosomal escape remained low. Conjugation to the fluorophore Cy5 was also found to increase cytosolic delivery. Despite these promising results, the peptide conjugates failed to significantly enhance NO production *in vitro*, suggesting that an even greater improvement in delivery is required. In light of this, we are exploring alternative strategies to improve delivery, including β-peptide hydrogels[7] and silica nanoparticles.[8] The results of these studies will be presented.

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P-078

Analyzing the Permeability of Highly Diverse Cyclic Peptides in PAMPA and Caco-2 Assays

Noora Azadvari, Parisa Hosseinzadeh

University of Oregon, USA

Cell permeability remains a major challenge in the development of therapeutic cyclic peptides, limiting both their oral bioavailability and intracellular targeting. Despite extensive research, only a few permeable peptides have been identified, most of which lack therapeutic function. While general rules for cell permeation exist, many aspects remain unexplored.

In this study, we assessed the permeability of 95 structurally diverse peptides using both PAMPA and Caco-2 assays, with some peptides tested under different experimental conditions. Our preliminary findings emphasize the significant influence of experimental variables, which are often overlooked or unreported in the literature. We hope our results promote the standardization of permeability assays, facilitating the discovery of novel permeable peptides.

P-079

Systematic Determination of the Impact of Macrocyclization on Peptide Accumulation into Mycobacteria

Rachita Dash[1], Zichen Liu[1], Irene Lepori[2], Mahendra D. Chordia[1], Karl Ocius[1], Kadie Holsinger[1], Han Zhang[3], Ryan Kenyon[1], Wonpil Im[3], M. Sloan Siegrist[2], Marcos M. Pires[1]

[1]University of Virginia, Charlottesville, USA
[2]University of Massachusetts, Amherst, USA
[3]Lehigh University, Bethlehem, USA

Mycobacterium tuberculosis, the causative agent of tuberculosis, possesses a complex cell envelope with a highly hydrophobic outer membrane, known as the mycomembrane. The mycomembrane serves as a major barrier to antibiotic penetration and contributes to intrinsic drug resistance. Most antibiotics currently used to treat tuberculosis were developed several decades ago and strains resistant to them have emerged, underscoring the need for improved therapeutic strategies. Recently, peptides have emerged as promising drug candidates, offering the synthetic accessibility of small molecules and the selectivity of larger biologics. Peptide-based therapeutics are also being actively developed for mycobacterial infections, highlighted by the discovery of evybactin and cyclomarin A. However, peptides often suffer from poor cellular permeability. In mammalian cells, macrocyclization is a widely used strategy to enhance peptide permeability by promoting intramolecular hydrogen bonding and reducing solvent-exposed surface area. However, given the unique composition of the mycomembrane, empirical evaluation of macrocyclization as a permeation strategy in mycobacteria is essential.

To address this, we **systematically isolated the impact of macrocyclization on peptide accumulation past the mycomembrane**.[1] By leveraging our recently reported PAC-MAN, Peptidoglycan Accessibility Click-Mediated Assessment, platform, we evaluated how structural variations in peptides such as ring size, cyclization chemistry, and topology, impact their accumulation in live cells. Furthermore, we applied these newly understood design principles to the peptide antibiotic Tridecaptin A1, demonstrating improved permeability and efficacy against mycobacteria, in specific contexts. This work provides the first systematic governing rules for the rational redesign of potential antimycobacterial agents.

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P-080

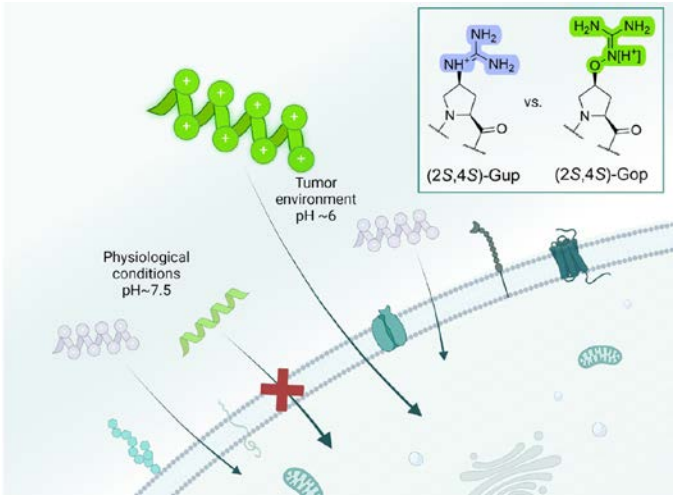
pH-Dependent Cellular Uptake of CPPs – Guanidinium Versus Oxyguanidinium Proline

Rahel Heeb, Laura Massaad, Helma Wennemers

ETH Zurich, Switzerland

Cancer is one of the main causes of death worldwide and, due to its inherent complexity and resistance to conventional therapies, often not treatable. Current therapeutics are often limited by poor cellular uptake or toxicity and reduced efficacy, thus, hindering clinical use. Cell-penetrating peptides, CPPs, have the potential for specific tumor targeting as well as efficient cargo delivery into the tumor cells.[1]

The acidic microenvironment typically found in pathological conditions is an opportunity for the selective and efficient uptake of tumor targeting CPPs. However, CPPs developed for this purpose so far suffer from limitations such as aggregation, endosomal entrapment and uptake efficiency.[2–4]



The Wennemers group has developed cationic oligo guanidinium proline, Gup_8, CPPs, which exhibit higher cellular uptake and low endosomal entrapment compared to previously established peptides, for example, octa arginine.[5] The higher cell penetration is due to the rigidity of the PPII helical structure and the resulting defined spatial charge arrangement. Retaining the concept of the spatial charge arrangement, we developed a new tumor-selective CPP based on oxyguanidinium prolines. The advantage of this novel peptide is a lower p*K*a of the oxyguanidinium functionality allowing for environment dependent protonation and cellular uptake.

Our studies revealed a p*K*a of ~7 for the Ac-(2*S*,4*S*)-Gop-OMe and a preference for the C4-*endo* pucker like the Ac-(2S,4S)-Gup-OMe, and different secondary structures dependent on temperature, solvent and pH. Further, this oxyguanidinium-based CPP has a higher cellular uptake at acidic than neutral pH.[6] These results open exciting prospects for the development of novel tumor-selective delivery agents.

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P-081

A Two-Component Two-Step, TCTS, Transformable Nanoplatform Against Cancers Based on Bioorthogonal Click Chemistry

Ruiqui Huang

Sacramento, Sacramento, USA

Nanomedicine represents a field addressing the issue of conventional anticancer drugs. Nanofibers, the amyloid-like structures of self-assembled peptide β-sheets, have also been widely studied in various biomedical applications. To achieve higher drug targeting and efficacy against EGFR+ α3β1+ cancers, a two-component two-step, TCTS, delivery approach is proposed.

In the first step, a peptide-based transformable nanoparticle, TNPs, was administered, and transformed into nanofibers on binding to the cancer cells. In the second step, when all the nanoparticles in the normal organs were eliminated, the tetrazine-prodrug was administered. Owing to the highly efficient bioorthogonal click reaction, the tetrazine-prodrug was selectively captured by the nanofibers carrying TCO, trans-cyclooctene, at tumor sites to achieve tumor targeting.

In this study, the transformable peptides were successfully synthesized. The peptides were utilized to prepare the TNPs. Cabazitaxel, CBZ, was chosen as the model drug, and chemically modified by tetrazine, Tz. Based on the results, TNPs showed a morphological change from nanoparticles into nanofibers under TEM after incubation with the target protein EGFR and α3β1 see figure A. The confocal microscope results indicated nanofibrillar transformation of TNPs and *in vitro* click chemistry with Tz-Cy3, see figure B, on SKOV-3 cells. The biodistribution studies underscored superior tumor-targeting capability and prolonged tumor retention of the TNPs, see figure C. The TEM images of tumor tissues showed nanofiber structures, demonstrating the transformation occurring *in situ*, see figure C. The following biodistribution of mTz-Cy7 illustrated the occurrence of *in vivo* click reaction, see figure D, reinforcing the remarkable tumor selectivity inherent to the TCTS approach.

In the efficacy study, the TCTS treatment using TNPs and mTz-CBZ showed significantly higher tumor suppression and remarkably reduced toxicity compared to free CBZ, see figure E.

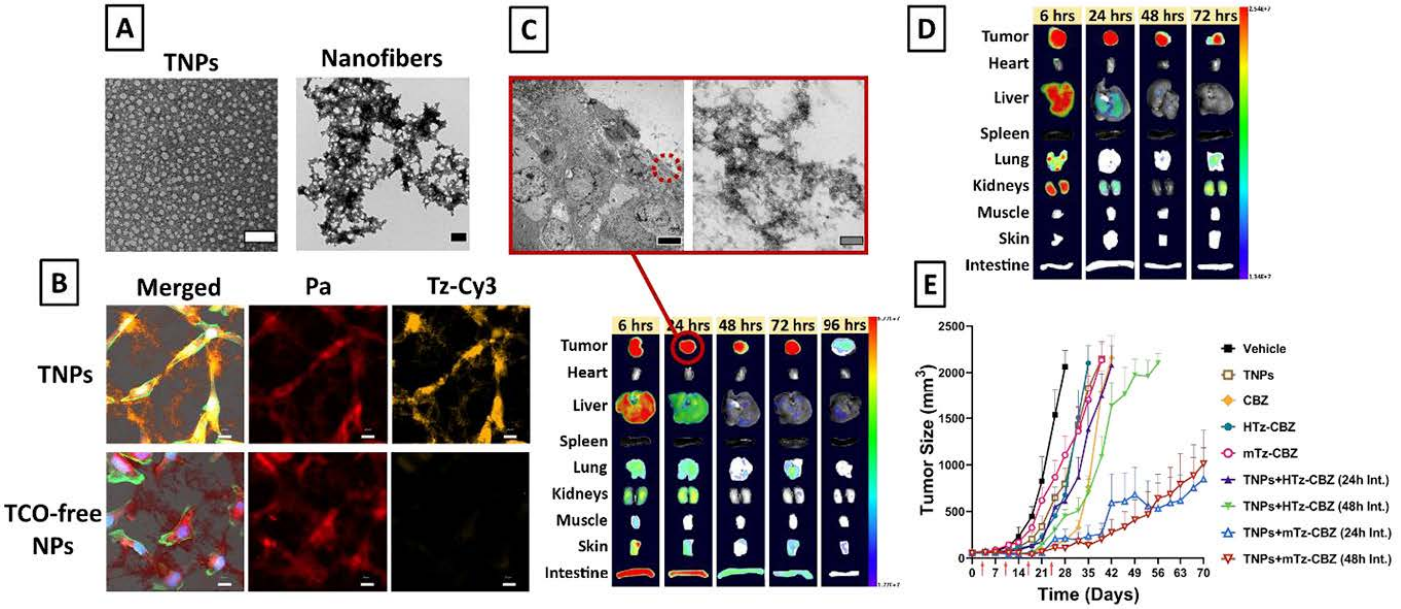

Figure **A** The morphology of TNPs and the nanofibers transformed from TNPs after incubation with EGFR and α3β1 at 37 degrees Celsius. **B, A** The nanofibrillar transformation and in vitro click reaction of Tz-Cy3 with the nanofibers on SKOV-3 (EGFR+ α3β1+) cells. **C** Biodistribution of TNPs in SKOV-3 SQ xenograft model. Nanofibers were detected from the tumor. **D** *In vivo* click chemistry of mTz-Cy7 with TNPs accumulated in tumor. **E** The *in vivo* efficacy study of TCTS drug delivery approach.

P-082

Rational Design of NorA Efflux Pump Inhibitors as Antibacterial Adjuvants

Rupali Singh

New York University, New York

Staphylococcus aureus, S. aureus, are common bacteria that are members of human microbiota. While often harmless, *S. aureus* can cause a range of infections, from minor skin issues to life threatening conditions. Methicillin-resistant *S. aureus,* MRSA, have developed resistance to multiple antibiotics and poses a significant threat to human health due to its high morbidity and mortality. One mechanism for the resistance development involves efflux pumps, for example, NorA, to reduce the intracellular drug concentrations.

Recently, we described design of NorA efflux pump inhibitors, EPI, as antibacterial adjuvants to combat antimicrobial resistance in MRSA, *Nat. Chem. Biol.* **2022**, 18, 706-712. Peptide mimics based on the NorA-specific antibody Fab36 were designed and tested for binding. I will report analogs of NorA binding peptides that block NorA and ablate MRSA growth in combination with Norfloxacin. My current efforts focus on improving the affinity, efficacy and proteolytic stability of the lead analog. Towards this, I am using (i) peptide tethering to identify unnatural fragments and fill unoccupied pockets in NorA, and (ii) Hydrogen bond surrogates to generate cyclic derivatives. By developing EPIs, we hope to revive multiple antibiotics' efficacy and offer a strategy applicable to other bacterial efflux systems. In summary, our peptide-based inhibitors represent a novel class of antimicrobial adjuvants against MRSA and opens new avenues for combinatorial therapies.

P-083

Developing Stapled Peptides that Bind LRP1 and Block Tau Uptake

Amarachi Osuji, Joshua A. Kritzer

Tufts University, Medford, USA

Tauopathies such as Alzheimer's disease are devastating pathologies of the central nervous system characterized by the accumulation of misfolded tau protein in the brain, resulting in neuronal dysfunction. In tauopathies, tau-dependent pathology can be propagated from neuron to neuron through a prion-like mechanism whereby extracellular, misfolded tau is taken up by cells and then induces tau misfolding. The Low-density Lipoprotein Receptor-related Protein 1, LRP1, was recently identified as the neuronal receptor responsible for tau uptake, and depletion of LRP1 slowed spread of tau pathology.

To date, there are no reported therapeutic strategies targeting tauopathies which block the LRP1-tau interaction. In this work, we use peptide ligands of LRP1, many of which were originally developed as blood-brain barrier shuttles, as inhibitors of the LRP1-tau interaction. We measured the abilities of peptide ligands to block the LRP1-tau interaction and are investigating stapling strategies on high-potency inhibitors.

P-084

Synthesis of a Novel PNA Bioreceptor for Rapid Skin Cancer Detection

Eleni Chatzilakou[1], Yubing Hu[1], Othman Al Musaimi[2], Lucia Lombardi[1], Daryl R. Williams[1], Ali K. Yetisen[1]

[1]Department of Chemical Engineering-Imperial College London, London, United Kingdom
[2]Faculty of Medical Sciences - School of Pharmacy, Newcastle University, Newcastle upon Tyne, United Kingdom

Melanoma accounts for 80% of skin cancer deaths, with high metastatic risk. The limitations of biopsies highlight the need for novel biosensors targeting promising protein biomarkers for rapid, minimally invasive diagnosis.[1] Detecting low-concentration proteins in biological fluids requires sensitive and specific methods. This work targets the melanoma biomarker S100B in interstitial fluid, ISF, using a fluorescently labeled peptide-nucleic acid, PNA, beacon as a melanoma-specific bioreceptor.

The molecular design leverages the dimeric nature of the S100B protein, featuring a PNA beacon with two fluorescently labeled peptide arms, each targeting one subunit of the S100B homodimer. The selected fluorophores enable FRET, providing a highly sensitive limit of detection, LOD.[2] The mechanism of action is based on the intramolecular interactions between the complementary PNA bases enabling spatial proximity of the arms, causing donor fluorescence quenching.

The two arms were synthesized separately via solid-phase peptide synthesis, with optimization of protecting groups, coupling reagents, solvents, molecular ratios, mixing order, and coupling time. Special focus was given to the 5-FAM probe attachment, donor of the quenching pair, and hydrazine treatment to ensure efficient coupling and maximized yield with suppression of side reactions.

Both hybrid peptides were cleaved from the resins independently, deprotected, successfully purified, ≥99%, and then inter-molecularly linked via copper-catalyzed azide-alkyne cycloaddition, CuAAC. CuAAC conditions were optimized for our system, adjusting molar ratios, reaction time, and additives to prevent adducts. The final molecule was characterized for sensitivity, response time, tumor microenvironment interference susceptibility, and binding efficiency, achieving a LOD as low as 0.05 nM within 1h.

In conclusion, we developed an innovative point-of-care biosensing tool using an optimized synthetic method offering enhanced diagnostic sensitivity for melanoma skin cancer.

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P-085

Novel Tau Filament Folds in Individuals with Frontotemporal Degeneration Caused by Familial Tau Mutation

Henry Pan, Gregory Merz, Athena Quddus, Anthony Yung, Eric Tse, Rian Kormos, William Seeley, and Daniel Southworth

The University of California, San Francisco, San Francisco, USA

Frontotemporal dementias, FTDs, are a group of neurodegenerative diseases primarily affecting the frontal and temporal lobes. About 30% of cases are familial, linked to mutations in genes such as MAPT. Familial MAPT FTDs may clinically and neuropathologically parallel sporadic tauopathies. Structural studies show that familial FTD-tau mutations can lead to amyloid filaments with distinct folds resembling those in sporadic tauopathies. This suggests that characterization of tau filaments derived from specific MAPT mutations may provide critical insights into the folding pathway of disease-specific structural conformations, potentially revealing key factors that drive tau misfolding.

Using cryo-EM, we determined tau filament structures from frozen postmortem tissue of a patient with a MAPT familial FTD currently classified as an early-onset form of a sporadic tauopathy, and identified two novel tau filament folds distinct from those in the associated sporadic tauopathy and other tauopathies. Histological analysis revealed tau aggregates morphologically similar to the sporadic counterpart but with more severe pathology, including the inferior frontal gyrus. Structural comparisons show that while these fibrils share organizational motifs with the sporadic tauopathy, they exhibit key differences in protofilament packing and β-sheet interface interactions, likely contributing to the unique neuropathological features of the familial FTD.

Our findings revealed that a rare familial MAPT mutation produces tau filaments structurally distinct from those in the associated sporadic tauopathy, despite the patient's histological similarities to the sporadic form. While this mutation was known to cause early-onset FTD with this sporadic tauopathy, whether the resulting tau filaments would match those of sporadic cases remained unclear. The assumption that familial and sporadic tauopathies with similar histopathology share identical filament structures stems from observations of structural uniformity in some cases. However, our findings challenge this notion, emphasizing the need to investigate the structural diversity of tauopathies to better understand their molecular mechanisms and pathological variability.

P-086

Photoprobes for Target Identification of Trefoil Factor Family Peptide 2, TFF2, and its Therapeutic use in Inflammatory Bowel Disease

Stephan Denisov, Markus Muttenhaler

University of Vienna, Vienna, Austria

Inflammatory bowel diseases, IBD, are chronic gastrointestinal conditions characterised by degradation of the mucosal barrier and gut epithelium. IBD include Crohn's disease and ulcerative colitis that affect between 2.4. and 3.1 million people in the USA alone. Considering the growing prevalence and burden on patients' quality of life, developing novel therapeutic strategies is of utmost importance.

Therapeutics that promote epithelium healing without immunosuppression are highly interesting as they prevent undesirable effects such as infections. However, the lack of understanding of the underlying processes of mucosal healing and its molecular agents significantly impedes progress in this direction. The trefoil factor family peptides TFF1, TFF2, and TFF3 are essential players in epithelial protection and repair and are involved in mucosal restitution, cell junction modulation, apoptosis, angiogenesis, and inflammation. Their exact molecular mechanisms remain elusive, with a broad range of receptors and glycoproteins proposed as putative interaction partners yet with missing pharmacology.

Here, we present the synthesis of photo-cross-linking probes for the structurally most complex human TFF – TFF2 – which comprises two trefoil domains supported by 7 disulfide bonds. TFF2 was produced via recombinant expression, its oxidative refolding was optimised, and the obtained native TFF2 was characterised using CD and NMR spectroscopy. Molecular probes containing 4-benzoyl-L-phenylalanine, Bpa, as a photo-reactive moiety were produced via two-fragment expressed protein ligation, refolded, and studied to confirm their structural identity with the native TFF2. Native TFF2 and its probes will be further used in TFF2 target identification by GPCR receptor screening and photo-cross-linking coupled with MS analysis.

P-087

Unraveling Chaperone Selectivity: Peptides as Model Substrates to Investigate Hsp70 Binding Characteristics

Sydney Mager, Lea Doerries, Eugenia Clerico, Lila Gierasch

University of Massachusetts Amherst, Amherst, USA

Protein folding in the cell relies on molecular chaperones to avoid misfolded states. The 70-kilodalton heat shock protein, Hsp70, chaperones are ubiquitous and highly conserved in both prokaryotes and eukaryotes, maintaining protein homeostasis in the cell by performing several housekeeping functions such as reducing protein aggregates, unfolding misfolded or denatured proteins, and protecting nascently translated proteins. They also protect cells against stress.

As such, Hsp70s serve a critical role in cell survival. Hsp70 function relies heavily on the chaperone's ability to recognize and bind misfolded proteins. The most highly studied Hsp70 is the *E. coli* DnaK, a constitutively expressed

cytosolic Hsp70. The residues lining the Hsp70 substrate binding domain, SBD, have been shown to have sequence preferences in substrate binding but not stringent ones. They have been observed to bind some hydrophobic amino acid residues, but not all. These binding patterns have been characterized as "selectively promiscuous".

This project seeks to identify and compare the binding characteristics of two human cytoplasmic Hsp70s, including Hsc70, constitutively expressed, and HspA1, stressinduced, and drawing comparisons with the previously studied *E. coli* DnaK. While the two human Hsp70s share 82% sequence identity, differences are found in the residues that line the central binding pocket of the SBD. Because of the differences in protein sequence in the central binding pocket, we expect there to be differences in substrate binding selectivity, which is supported by preliminary peptide arrays, and potentially their responses to drug candidates. This project aims to elucidate the differences in substrate interactions between these chaperones using peptide models of substrates and fluorescence anisotropy-based binding assays to compare substrate affinities, methyl-NMR to identify substrates binding modes, and disulfide cross-linking to look at binding orientations. Differences in binding preference may have fundamental implications for the evolutionarily selected substrate subproteomes handled by the different Hsp70 chaperones.

P-088

Disrupting the WASF3 Regulatory Complex in Pancreatic Cancer Using Stapled Peptides

Taylor Dill, Eileen Kennedy

University of Georgia at Athens, USA

Pancreatic cancer makes up 3% of diagnosed cases in the U.S. but it is responsible for 7% of all cancer related deaths. Pancreatic cancer is aggressive and is associated with rapid growth, invasion, and poor prognosis. With few effective treatments and low survival rates, alternative therapeutic approaches are needed for effective treatment.

Upregulation of the WASF3 Regulatory Complex has been identified in pancreatic cancer and associated with poor prognosis. WASF3 is a member of the Wiskott Aldrich Syndrome Family Proteins whose activation occurs through the WASF3 Regulatory Complex, which exists in a heteromeric complex alongside ABI, NKCAP, CYFIP, and BRK. The active complex initiates actin polymerization and the formation of filament networks aiding in cellular motility and cellular matrix dynamics. Upregulation of WASF3 in pancreatic cancer promotes invasion and metastasis.

We have designed hydrocarbon stapled peptides that disrupt the formation and activation of the WASF3 Regulatory Complex and demonstrate that these peptide-based compounds inhibit the invasion and migration phenotypes. These peptides will be further explored for their efficacy in targeting pancreatic cancer metastasis by disrupting the formation and activation of the WASF3 Regulatory Complex. This work may serve as a foundation to broaden the window for pancreatic cancer treatment by downregulating the metastatic burden for a larger time period.

P-089

Oligonucleotide Thioesters: Synthesis, Characterization, and Native State Ligation to Peptides and Proteins

Albert Kakkis, Youlin Guo, and Philip E. Dawson

Scripps Research Institute, La Jolla, CA, USA

Oligonucleotides, including silencing RNA, siRNA, and anti-sense oligos, ASOs, represent an emergent class of therapeutics, yet in their native forms they are poor drug candidates owing to low cell-penetrability and circulation half-life.[1] Recently, conjugation of oligonucleotides to lipids,[2] sugars,[3] and antibodies,[4] has improved their therapeutic potency. Given these recent successes, it is vitally important to expand the repertoire of bioconjugation chemistries that can be harnessed to render oligonucleotide conjugates in high yield and with high chemo-/regio-selectivity. Native chemical ligation, NCL, is a high-yielding, chemo-selective, regio-selective, and scarless conjugation chemistry in which

N-terminal cysteines react with latent thioesters to form an amide bond.[5] While NCL has seen extensive application in protein synthesis, there have been limited efforts to apply it to oligonucleotide modification.[6]

Herein, we describe the synthesis and characterization of molecules featuring siRNA/ASO and thioester surrogate moieties. We demonstrate the ligation of these oligo-thioesters to peptides and proteins in high conversion and, notably, in the absence of denaturants, that is, "native-state," which enables ligations that preserve the secondary/tertiary structures of the oligonucleotide electrophile and protein nucleophile. Future work is oriented toward the ligation of oligos to larger biomacromolecules and the introduction of multiple distinct payloads within an antibody-oligonucleotide conjugate.

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P-090

Novel Peptide Nucleic Acid Conjugates with Hydrocarbon-Stapled Peptides and Aminoglycoside Antibiotics to Target Gram-Negative Bacteria

Izabela Siekierska, Michał Burmistrz, and Joanna Trylska

Centre of New Technologies, University of Warsaw, Warsaw, Poland

Antimicrobial resistance is a major global health threat, challenging the effectiveness of modern medicine. The slow development of new antibiotics and rapidly rising levels of resistance force researchers to look for alternative approaches. Among them, peptides emerge as promising agents.[1] Their ability to disrupt bacterial membranes causes cell death directly or facilitates the transport of other antibacterial components to the cytoplasm. The latter is particularly useful when applied in conjugates with antisense agents,[2] such as peptide nucleic acids, PNAs - nucleic acid analogs composed of N-substituted 2-aminoethylglycine units connected via amide bonds.[3]

We focused on a naturally occurring peptide anoplin and its hydrocarbon-stapled analog to test their ability to transport PNA, targeting the essential bacterial gene *acpP* into gram-negative bacteria.[4]

In another approach, based on the similarity between antimicrobial peptides and aminoglycosides, AGs, in terms of positive charge, we investigated whether AGs may help internalize antisense PNA oligomers into bacteria. Our results showed that AG-PNA conjugates are active against aminoglycoside-resistant strains. Moreover, we confirmed that the activity of conjugates can be attributed to the antisense mode of action of the PNA oligomer, confirming its AG-facilitated entry into the bacterial cytoplasm.

This work was supported by the National Science Centre, Poland, 2023/49/N/ST5/01824, awarded to IS, and 2020/37/B/NZ1/02904, awarded to JT.

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P-091

Discovery of G-Quadruplex-Binding Peptides via Structure-Based and Sequence Alignment Approaches

Nicola Grasso, Francesco Merlino, Paolo Grieco, Bruno Pagano, Jussara Amato

University of Naples Federico II, Naples, Italy

Noncanonical nucleic acid structures, particularly G-quadruplexes, G4s, have emerged as attractive targets in anti-cancer therapy due to their structural uniqueness and involvement in key regulatory processes.[1] Their formation is modulated by G4-binding proteins, G4BPs, whose dysregulation has been associated with several diseases.[2] Despite the increasing number of G4-targeting ligands, achieving high selectivity and specificity remains challenging. In this context, peptides represent a promising class of ligands, offering structural adaptability and specificity.

Here, we describe two complementary strategies for the discovery of G4-binding peptides. The first is a structure-based approach involving a G4-binding domain of the Rap1 protein, whose crystallographic complex with G4 DNA enabled the design of a selective peptide ligand.[3] Subsequent alanine scanning and conjugation with cell-penetrating peptides, CPPs, led to derivatives exhibiting potent G4-mediated cytotoxicity across multiple cancer cell lines.
In parallel, to address the limited structural data available for most G4-protein complexes, we implemented a strategy based on the sequence alignment of known G4BPs. Through this approach, we identified a 20-mer RGG-rich peptide motif, named NIQI.[4]

Results of biophysical characterization via circular dichroism, CD, microscale thermophoresis, MST, isothermal titration calorimetry, ITC, and nuclear magnetic resonance, NMR, confirmed NIQI's ability to bind several G4 topologies with high affinity. Alanine scanning further highlighted key residues involved in G4 recognition, providing a rational basis for ligand optimization.

Collectively, these strategies underscore the potential of integrating structural biology with bioinformatics to rationally design peptide ligands, paving the way for the development of next-generation G4-targeting therapeutics in oncology and beyond.

[1]Spiegel, J.; Adhikari, S.; Balasubramanian, *S. Trends Chem*. **2020**, 2, 123–136.
[2]Rhodes, D.; Lipps, HJ. *Nucleic Acids Res*. **2015**, 15;43(18):8627-37.
[3]Merlino, F.; Marzano, S.; Zizza, P.; D'Aria, F.; Grasso, N.; Carachino, A.; Iachettini S.; Biroccio, A.; Di Fonzo, S.; Grieco, P.; Randazzo, A.; Amato, J.; Pagano, B. *Nucleic Acids Research*. **2024**, 6748–6762.
[4]Grasso, N.; Graziano, R.; Marzano, S.; D'Aria, F.; Merlino, F.; Grieco, P.; Randazzo, A.; Pagano, B.; Amato, J. *Int J Biol Macromol*. **2023**, 253, 126749.

P-092

A Peptidomimetic Scaffold for Base-Specific Recognition of Duplex RNA

Oliver Swart, Zhi Yuan, Chloe Sung, and Paramjit Arora

New York University, New York City, USA

Targeting specific sequences of RNA has shown enormous potential both in illuminating cell behavior and treating disease but remains a challenging feat due to the large variety and dynamic nature of RNA structures. The simplest and most common RNA structure is the A-form duplex, yet there is currently a scarcity of ligand classes that can reliably target this structure in a sequence specific manner. Drawing inspiration from a unique RNA binding protein, tomato aspermy virus 2b, TAV2b, a peptidomimetic ligand was developed which enabled sequence-specific base readout in RNA major grooves. This ligand displayed selective binding to helical RNAs with repeated CNG sequences, including the CUG-repeat implicated in myotonic dystrophy type 1 and the CAG-repeat sequence of Huntington's disease.

Further development of this ligand has resulted in improved binding affinities and ability to bind non-repeat sequences. This work demonstrates a novel bulge-independent targeting strategy for repeat expansion diseases and more generally provides a much-needed expansion of the RNA-targeting toolbox

Kwok JG, Yuan Z, Arora PS. *Angew Chem Int Ed Engl*. **2023**.

P-093

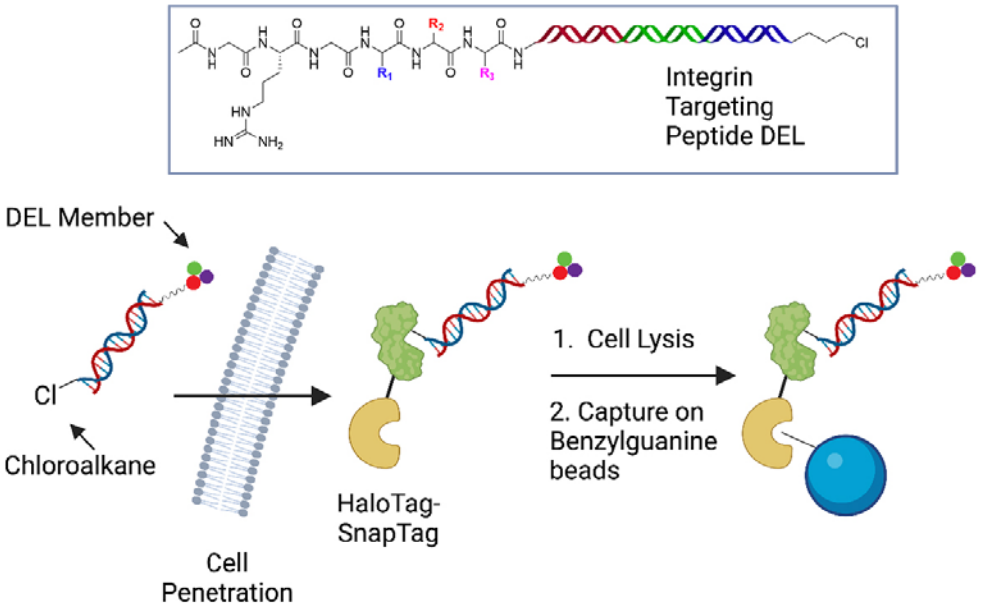
Development of Cellular Assays of DNA-Encoded Chemical Libraries to Identify Peptide Shuttle Ligands for Targeted siRNA Delivery

Siavash Shahbazi Nia, Casey J. Krusemark

Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy,Purdue University and Purdue University Center for Cancer Research, 575 Stadium Mall Drive, West Lafayette, IN, USA

Small interfering RNAs, siRNAs, and antisense oligonucleotides, ASOs, have emerged as powerful therapeutic tools. A key challenge in RNA-based therapy is efficient delivery into the cellular cytoplasm. Currently, there are several FDA-approved RNA therapeutics that rely on uptake via binding and receptor-mediated endocytosis of the TriGalNAc-Asialoglycoprotein receptor 1, ASGR1. This approach, however, is largely restricted to delivery into the liver where ASGR1 is highly expressed.

To overcome this limitation, we have developed selection assays for DNA encoded chemical libraries to enable discovery of new targeting ligands. Similarly composed of double-stranded nucleotides, the DNA barcodes of a DEL member, ~70 bps, hope to serve as a surrogate of an siRNA, ~25 bps, such that discovered ligands can be used directly.



Our approach involves two separate assays: **1|** selecting for molecules that enter cells by simple washing and DNAse treatment of cells post DEL incubation and **2|** selecting for molecules capable of delivering the DNA into the cytoplasm by capture of a chloroalkane-containing DEL with a cytosolically-expressed HaloTag-SNAPtag fusion. As a model system, we have used the TriGalNAc-ASGR1 system in HepG2 cells to optimize the conditions for delivery DNA conjugates. We present progress targeting the α5β1 integrin protein using DNA conjugates of RGD peptides of varying affinity, GRGDNP, 30 nM IC_{50} and a cyclic RDG peptide, ATN-161, 4 nM IC_{50}.

We present progress in design and synthesis of a 13,824 membered peptide DEL to target RGD peptide-binding integrins.

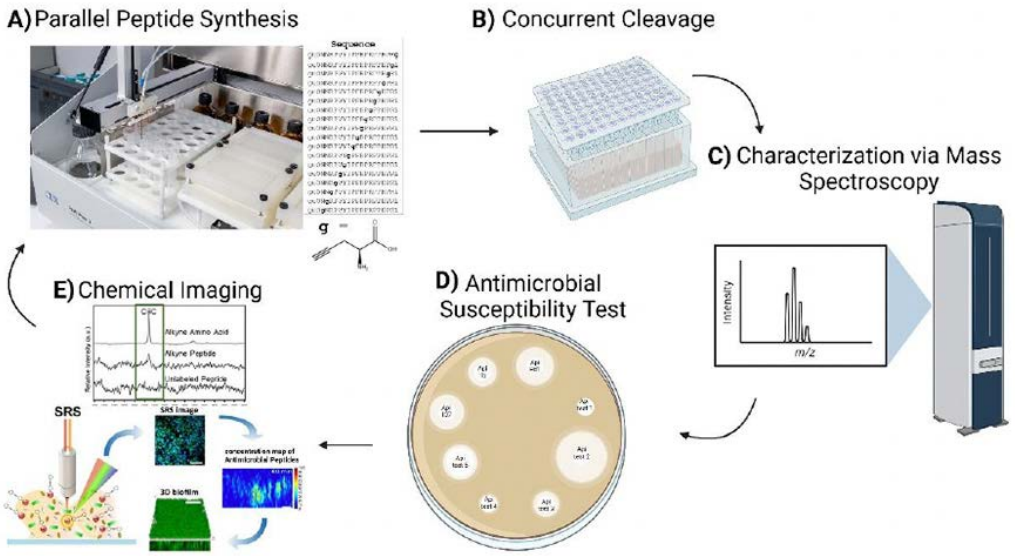
P-094

Harnessing Chemical Imaging to Advance Antimicrobial PeptideResearch

A'Lester Allen, Trevor Dean, and Terry Moore

University of Illinois Chicago, Chicago, USA

Peptide-based therapeutics offer a powerful strategy for tackling persistent infections, yet their clinical potential is often hindered by imaging challenges. Conventional fluorescent labels, though widely used, introduce steric bulk that can disrupt native peptide behavior. Here, we demonstrate a minimally invasive alternative: vibrational tags detectable via Stimulated Raman Scattering microscopy. This cutting-edge approach preserves peptide functionality while enabling real-time visualization of their biological effects in living cells.



As a proof of concept, we have strategically incorporated Ångström-sized alkyne imaging probes into the natural product Apidaecin during solid-phase peptide synthesis. Apidaecin, a type II proline-rich antimicrobial peptide, PrAMP, relies on specific bacterial transporters for uptake and exerts its activity by disrupting ribosomal function. We will benchmark the antimicrobial efficacy of our tagged Apidaecin in well-characterized *E. coli* strains with known peptide transport pathways, providing critical insights into transporter-mediated uptake. We also assess its activity in cyanobacteria—an underexplored bacterial group—to determine whether similar peptide-like transporters exist in these species and to visualize Apidaecin's penetration through their protective sheath and cell wall.

This work has broad-reaching implications. In addition to optimizing Stimulated Raman Scattering imaging for tracking antimicrobial peptides, our findings will illuminate the taxonomic diversity of peptide transport mechanisms in cyanobacteria. More importantly, these advances will lay the groundwork for clinically relevant applications, such as imaging-driven strategies to combat biofilm-associated *S. aureus* infections. By refining both peptide-based drug development and real-time visualization techniques, this research paves the way for next-generation therapeutics that can outmaneuver antibiotic resistance and improve patient outcomes.

[1]K. Skowron, et al. *J. Med. Chem.* **2023** 66, 17, 11831–11842
[2]C. Baliga, et al. *PNAS*. **2021** 118 (10). e2026465118
[3]Centers for Disease Control. *Antibiotic Resistance Threats in the United States* **2019**
[4]T. Florin, et al. *Nature Structural and Molecular Biology*. **2017** 24 (9). 752-757.
[5]L. Wei, et al. *Nat. Methods*. **2014** 11 (4), 410-412t

P-095

Peptide Backbone Editing via Post-Translational C–C Bond Formation

Carly Schissel, Matthew Francis, and Alanna Schepartz

UC Berkeley, Berkeley, USA

Despite tremendous efforts to engineer translational machinery, replacing the encoded peptide backbone with new-to-Nature structures remains a significant and largely unmet challenge. C, H, O, and N are the elements of life, and yet ribosomes are only capable of forming C–N bonds as amides, C–O bonds as esters, and C–S bonds as thioesters.

Therefore, post-translational backbone editing reactions are required to install unnatural backbone linkages in peptides made by chemical or ribosomal synthesis. We discovered that peptides containing a dehydrolactic acid motif rapidly isomerize to generate a backbone-embedded α,ɣ-diketoamide via a spontaneous formal O to C acyl shift. The dehydrolactic acid motif can be introduced into peptides ribosomally or via solid-phase synthesis using α-hydroxy phenylselenocysteine followed by oxidation. Subsequent incubation at physiological pH produces an α,ɣ-diketoamide that can be diversified using a variety of nucleophiles, including hydrazines and hydroxylamines to form pyrazoles and oximes, respectively. All of these groups remain embedded directly within the polypeptide backbone. The genetically encoded, new-to-nature biopolymers produced should accelerate the discovery of genetically encoded molecules whose properties better resemble those of bioactive natural products.

P-096

Rapid Evaluation of Diverse Chemical Transformations on Bacteriophage-Displayed Peptides

Chuanhao Peng, Ratmir Derda

University of Alberta, Edmonton, Canada

Chemically modified genetically encoded peptide libraries encompass broader chemical space that enables rapid discovery of proteolytically resistant and potent drug leads. However, evaluating chemical modifications on phage-displayed peptide remains challenging due to their ultra-low concentrations. Despite extensive efforts to assess these modifications—such as using ESI-MS for identifying modifications of phage displayed peptides or using MALDI-TOF-MS to detect chemical transformations on p8-displayed peptides—current methods are often time-consuming or impractical for certain applications, hindering the development of new modification strategies for generating chemically modified peptide libraries in drug discovery.

Herein, we propose a strategy that enables rapid display of peptides regards of sequence and size on phage and offer convenient kinetic evaluation of chemical transformation on phage-displayed peptide. In this work, DBCO was installed on p8 proteins of M13 phage, followed by "click" chemistry attachment of chemically synthesized azido peptides. Subsequently, the kinetics of diverse chemical transformations on phage-displayed peptide were evaluated using MALDI-TOF-MS, highlighting the extreme convenience and rapidity of this method. All modification, detection, and purification, if needed, steps can be performed in parallel, with the potential for fully automation, significantly advancing the development of new chemical modification strategies of peptide libraries.

P-097

Combining AlphaFold and mRNA Display Technologies - *De Novo* Discovery and Maturation of Functional CD206 Peptide Binders

Esther Meron[1], Mads Nygaard[1], Nina Buch-Månson[1], Dennis Worm[1], Victor Møller[1], Kasper Einarson[1], Morten Lundh[1], Joseph Rogers[2], and Jens Nielsen[1]

[1]Gubra, Hørsholm, Denmark
[2]Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark

Recently, *de novo* peptide discovery technologies have emerged as powerful tools to expand the druggable target space. Here we show the combined use of AlphaFold and mRNA display for high-throughput discovery and sequence optimisation of a *de novo* peptide binder of CD206, to explore how these technologies could complement each other.

The CD206 receptor is found on M2-like macrophages and is a potential target for treatment of pulmonary fibrosis. We used computational de novo design to generate CD206 peptide binders in silico. Sequences were generated using a peptide hallucination protocol using AlphaFold2 and were modelled to bind a previously reported active binding site on the receptor extracellular domain. Based on ipTM and pLDDT scores, we selected 180 binders for synthesis and functional characterisation. The peptides showed potencies down to 2.5 µM. In order to further improve the potency of one de novo designed peptide we applied a focused mRNA display selection to identify variants with improved binding properties.

A deep-mutational scan was performed using mRNA display. An mRNA-tagged peptide library was created from a custom DNA library encoding for all single and double mutations of the hit peptide sequence. The library was panned against recombinant CD206 extracellular domain. By next-generational sequencing of the mRNA tag, we measured the binding of peptide mutants to determine the importance at each position for binding as well as effects of multiple mutations. Ongoing experiments are expected to shed light on how the use of AlphaFold and hallucination protocols can create novel site-selective peptide hits, and how this can be combined with mRNA display protocols for high-throughput hit maturation.

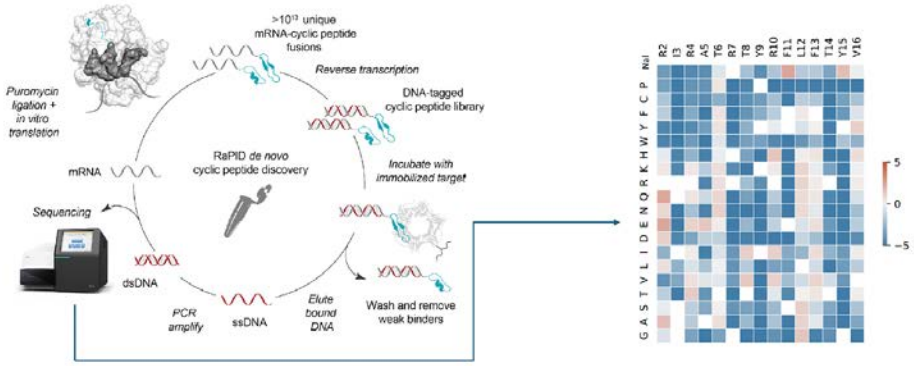
P-098

Enhancing and Characterizing the Binding of Novel RaPID-Derived Cyclic Peptides Using Deep Mutational Scanning

Jing Zhao, Fabian Hink, Signe Simonsen, Birthe Kragelund, and Joseph Rogers

University of Copenhagen, Copenhagen, Denmark

The Random Nonstandard Peptide Integrated Discovery, RaPID, system is a powerful screening approach for discovering cyclic peptides de novo that bind to target proteins. RaPID efficiently screens trillions of DNA-tagged cyclic peptides, rapidly identifying the highest-affinity binders by utilizing randomized sequence mRNA and genetic code reprogramming.



To optimize macrocyclic RaPID hits, a combination of chemical modifications and biophysical measurements is commonly employed. However, this approach relies on extensive chemical synthesis and requires one-at-a-time synthesis and biophysical characterization, making it time-intensive. Computational modeling can aid this process by predicting peptide-protein interactions, but it typically requires highresolution 3D structures of the target protein to generate accurate models.

In this project, we developed and implemented RaPID-based deep mutational scanning, enabling the simultaneous analysis of thousands of peptide variants to identify essential binding residues and guide peptide optimization. By employing saturation mutagenesis on multiple hit cyclic peptides in one-pot experiments, we successfully applied this method across diverse systems, including bacterial DNA sliding clamps, RNA-binding proteins, and protein-protein interactions.

P-099

Development of Immunoproteasome Substrate Labeling Assays - iSLAy

Juan Dantis

University of California, Irvine, Irvine, USA

The immunoproteasome, iCP, is an isoform of the proteasome that is expressed constitutively in immune cells and can be induced upon inflammatory insults in others. The iCP differs from the standard proteasome, sCP, in its catalytically active subunits, LMP2, β1i, MECL-1, β2i, and LMP7, β5i, attenuating its cleavage preferences, producing peptides that are more amenable for MHC-I binding, rendering it vital to antigen presentation. Compared to the sCP, little is known of the iCP's interactors, including its substrate profile. At present, no proteomic platform is selective to profiling the iCP.

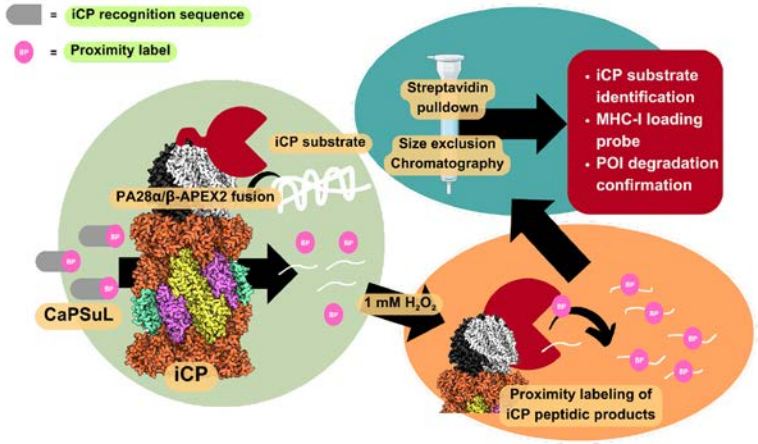


Figure 1. Overview of iSLAy technology. This new platform that is activity and proximity-based will be able to tag and enrich iCP protein substrates, allowing for their identification.

Herein, we describe a novel technique we have named Immunoproteasome Substrate Labeling Assays, iSLAy. We expand the capabilities of a mature proximity labeling strategy, APEX2-MS, a platform demonstrated to be adept at profiling transient protein interactions such as between enzymes and their substrates.[1] With our discovery of an iCP-specific peptide recognition sequence, ATMW, we introduce the concept of Caged Proximity Substrate Labels, CaPSuLs, comprised of a proximity label that is liberated only upon the iCP-mediated hydrolysis of the sequence. This allows for subsequent activation of the label by APEX2 fused to iCP regulator, PA28, and labeling only the proteome proximal to the iCP. With the development of this technology, we hope to isolate iCP substrates and glean insights on the properties of the iCP and its role on the cellular phenotype.

[1]Lam, S. S.; Martell, J. D.; Kamer, K. J.; Deerinck, T. J.; Ellisman, M. H.; Mootha, V. K.; Ting, A. Y. Directed Evolution of APEX2 for Electron Microscopy and Proximity Labeling. *Nat Methods* **2015**, 12 (1), 51–54. https://doi.org/10.1038/nmeth.3179.

P-100

A Peptide-Based Platform for Evaluation of Transpeptidase Activity and Screening for Inhibitors of Mycobacterium-derived L,D-Transpeptidases

Karl Ocius

University of Virginia, Charlottesville, USA

Mycobacteria poses an important threat to public health across the globe. The most common mycobacteria infection, Tuberculosis, caused by *Mycobacterium tuberculosis*, claimed the life of more than 1 billion people over the past 200 years with a quarter of the world's current population infected by this bacterium. Though astounding, TB is not the only mycobacterium that causes public health concern. *Mycobacterium abscessus*, and *Mycobacterium avium* have also garnered attention as they cause non-tuberculosis lung infections. The development of resistance to current antibacterial agents supports the need for development of new antibacterial agents.

As such, we decided to target the major component of all bacterial cell walls, the peptidoglycan, PG. The PG confers physical and mechanical stability to the cell wall making it an ideal target. The PG is a biopolymer with a rigid backbone consisting of glycan strands of repeating disaccharide units of N-acetylglucosamine, GlcNAc, and N-acetylmuramic acid, MurNAc, held together by ß (1→4) glycosidic bonds. The MurNAc moiety of each PG unit harbors a pentameric stem peptide which is crosslinked to other stem peptides on adjacent PG units by transpeptidase enzymes. These enzymes are the target of the largest class of antibiotics developed, beta lactams.

Herein, we use synthetic peptide mimics of the PG, one of which is stabilized onto a solid support to develop a flow-based high-throughput fluorescent platform for the evaluation of crosslinking activity and for screening of inhibitors of L,D transpeptidases, the major class of PG transpeptidase of mycobacteria.

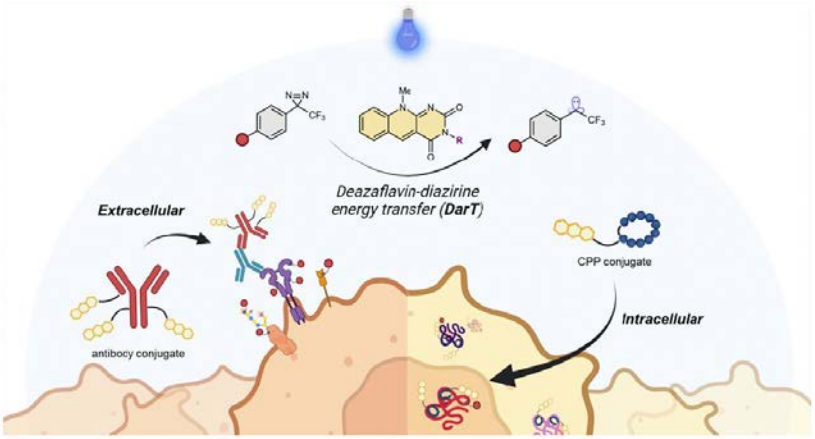
P-101

Mapping Peptide Interactomes Using an Organic Energy Transfer Photocatalyst

Leander Crocker[1], Judith Müchler[2], Jan Vincent Arafiles[2], Max Ruwolt[2], Kristin Kemnitz-Hassanin[2], Kilian Roßmann[2], Christian Stieger[2], Fan Liu[2], Christian Hackenberger[2]

[1]Berlin, Berlin, Germany
[2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin,Germany

Photocatalytic proximity labelling has recently emerged as a powerful tool to resolve a wide variety of biomolecular and cellular interactions.[1] While the use of high-resolution probe species, such as diazirines, enables cell-surface protein labelling with nanometre precision by generating highly reactive intermediates, intracellular applications are limited either by the intrinsic toxicity of frequently employed photocatalysts or lower resolution when long-lived reactive intermediates are used.



In this work, we describe the discovery and application of an organic flavin cofactor derivative, deazaflavin, capable of diazirine activation to form carbenes through triplet energy transfer and offers unparalleled biocompatibility. We demonstrate deazaflavin-diazirine energy transfer labelling, DarT-labelling, allows for targeted extracellular mapping using antibody conjugates and also intracellular interactome mapping of cell-penetrating peptides, CPPs. We successfully mapped the localisation of two popular polyarginine CPPs and identified potential key membrane interactors. Furthermore, we show the applicability of DarT-labelling over extended time by mapping the intracellular trafficking of a stable cyclic derivative to reveal its eventual exocytosis from the cell. We envision DarT-labelling has the unmet potential to enable detailed profiling of intracellular dynamics across diverse biological systems with unprecedented spatiotemporal control.

Knutson et al. *Cell Chemical Biology*, **2024**, 31, 1145 – 1161

P-102

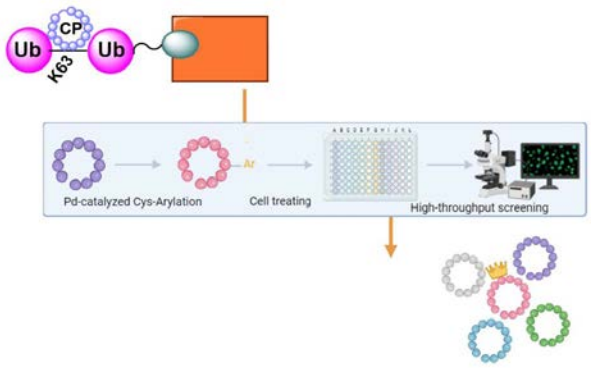
Pd-Mediated Arylation for the Discovery of Cyclic Peptide Binders for Lys63-Linked Ub Cthain

Mahdi Hasan, Nagaraj Vodnala, Yuri Glagovsky, and Ashraf Brik

Technion-Israel Institute of Technology, Haifa, Israel

Cyclic peptides have gained significant attention as modulators of protein-protein interactions, particularly in ubiquitin, Ub, signalling pathways, offering therapeutic potential in various diseases. Recent advancements from our lab included the the use of the Random Non-standard Peptides Integrated Discovery, RaPID, platform and protein syntehsis that have enabled the discovery of cyclic peptides that selectively bind to Lys48 and Lys63-linked ubiquitin chains.[1,2] The latter cyclic peptides were found to disrupt DNA damage repair, DDR, pathways, induce apoptosis, and sensitize cancer cells to therapies like ionizing radiation.

However, challenges such as poor solubility, limited cell permeability, challenging synthesis and limited scalability, hindered its further development. This is exemplified by CP7, a cyclic peptide featuring a thioacetal linker, has demonstrated exceptional efficacy as an inhibitor of non-homologous end-joining, NHEJ, repair, which shows exceptional biological activity.[3] However, CP7's challenging synthesis due to the thioacetal linkage, limited its translational potential, necessitating the exploration of alternative strategies.



To address these challenges, we have developed a novel strategy, based on Pd-catalyst mediated Cys-arylation of 14 modifications across 10 different cysteine positions in CP4, a cyclic peptide scaffold bearing the chlorobenzyl linker which can be made in strighforward manner. This systematic approach was further developed to allow *in situ* screening of the library on live cell allowing the rapid discovery of a new lead compound. The new cyclic peptide was tested in different biolgical setting where Lys63 plays a key role in these patheways.

[1]Nawatha, Mickal, et al. "De novo macrocyclic peptides that specifically modulate Lys48-linked ubiquitin chains." *Nature Chemistry,* **2019** 11.7 (2019): 644-652.
[2]Vamisetti, Ganga B., et al. "Selective macrocyclic peptide modulators of Lys63-linked ubiquitin chains disrupt DNA damage repair." *Nature Communications,* **2022** 13.1 6174.
[3]Saha, Abhishek, et al. "Exocyclic and Linker Editing of Lys63-linked Ubiquitin Chains Modulators Specifically Inhibits Non-homologous End-joining Repair." *Angewandte Chemie International Edition* **2024** 63.44 (2024): e202409012.

P-103

De Novo Design of Stable Transmembrane Protein Complexes with Apolar Specificity Encoded by Data-Mined Sterically Restricted Interfaces

Minghao Zhang, Marco Mravic, Charlie Anderson

The Scripps Research Institute, San Diego, USA

Different chemical principles govern protein-protein interactions in the cell membrane than in water. The hydrophobic effect does not play a major role in protein folding within lipid bilayers since most amino acids and interactions involved are apolar. Our working theory is that apolar interactions between transmembrane, TM, alpha-helices are not non-specific or "greasy." Rather, to be energetically favorable and more stabilizing than the surrounding lipid environment, van der Waals sidechain packing must be rigid and sterically specific – and likewise can be encoded by particular sequence motifs.

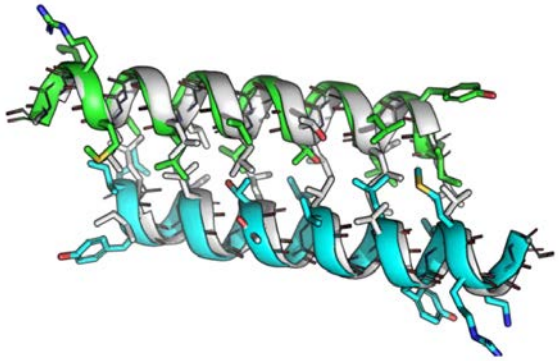


Fig1. Aligned X-ray solved model with in silico model

In contrast to well-known small residue motifs for tight TM domain interactions, wider apolar helix-helix interfaces have no currently recognized patterns. We used *de novo* protein design to address whether highly stable synthetic membrane protein complexes of an intended topology could be engineered exclusively using fully apolar synthetic sequences – testing how complex networks of sterically restricted sidechains encode stable and specific TM domain interfaces. We designed 10 synthetic single-pass TM proteins to self-associate as left-handed antiparallel dimers, ~10 Å interface, and tested the folding/oligomerization of purified proteins via size-exclusion chromatography, cross-linking, Nanobit, and X-ray crystallography.

Of the 10 *de novo* TM proteins, 7 had specific folding, homodimerization, as intended, with some complexes thermostable to above 70 C and resistant to SDS denaturation on SDS-PAGE. Moreover, X-ray crystallography data were collected for 3 of these designs to get atomic details of core apolar sidechain packing patterns and one of the solved structures confirms our *in silico* model. These results emphasize the biophysical basis for apolar interactions in lipid environment, expanding capacity for designs of more diverse helix-helix interactions and synthetic folded architectures in the membrane.

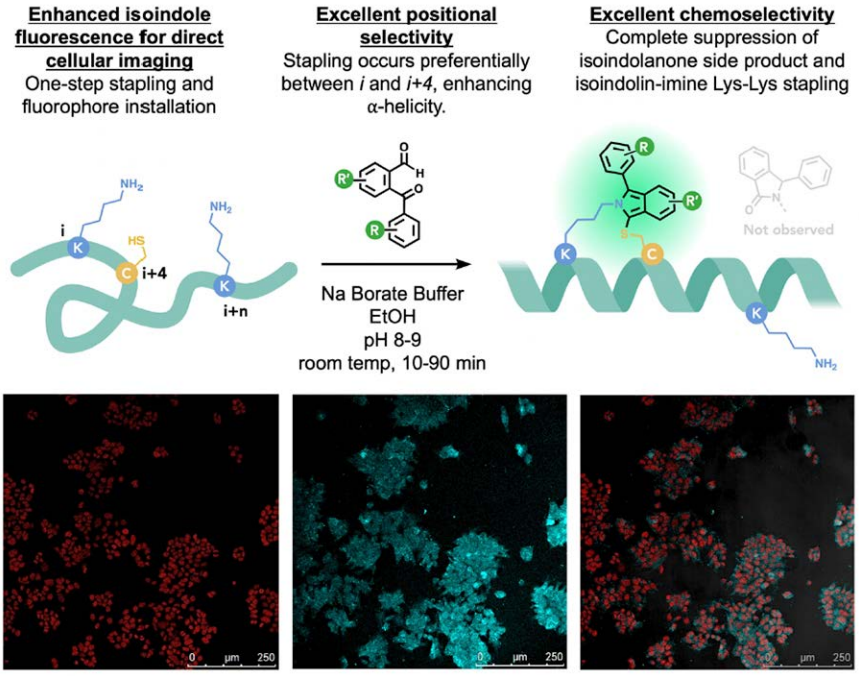
P-104

Chemoselective, Regioselective, and Positionally Selective Fluorogenic Stapling of Unprotected Peptides for Cellular Uptake and Direct Cell Imaging

Naysilla Dayanara, Juliette Froelich, Pascale Roome, and David Perrin

University of British Columbia, Vancouver, Canada

Peptide stapling reactions represent powerful methods for structuring linear peptides into macrocyclic scaffolds in the pursuit of targeting protein-protein interactions, PPIs.[1] In light of a growing need for regio- and positionally selective



stapling methods, particularly those showing late-stage applications on native unprotected peptides, we present a rapid and mild three-component stapling reaction utilizing a class of molecular linchpins based on 2-arylketobenzaldehydes, ArKBCHOs.[2] This approach yields a stable and inherently fluorescent isoindole staple with appreciable quantum yields. Moreover, this methodology has demonstrated improved chemoselectivity by minimizing the formation of isoindolinone and isoindolin-imine byproducts,[3] therefore allowing us to achieve excellent stapling positional selectivity at the helical *i* and *i*+4 positions in the presence of competing nucleophiles on unprotected peptides. In our efforts to further validate this chemistry, we have successfully demonstrated the *in vitro* cytotoxicity of an isoindolestapled BIMBH3 analog, IC_{50}=5.10 ± 1.27 μM, equipotent to an olefin-stapled congener.[4] By leveraging the intrinsic fluorescence of the isoindole, we were able to also directly evaluate peptide cellular uptake by virtue of the staple itself, thus bridging therapeutic potential with cytological probe development.

[1]M. Pelay-Gimeno, A. Glas, O. Koch, T. N. Grossmann, *Angew Chem Int Ed Engl* **2015**, 54, 8896-8927.
[2]N. L. Dayanara, J. Froelich, P. Roome, D. M. Perrin, *Chem Sci* **2025**, 16, 584-595.
[3]B. Li, L. Wang, X. Chen, X. Chu, H. Tang, J. Zhang, G. He, L. Li, G. Chen, *Nat Commun* **2022**, 13, 311.
[4]A. L. Edwards, F. Wachter, M. Lammert, A. J. Huhn, J. Luccarelli, G. H. Bird, L. D. Walensky, *ACS Chem Biol* **2015**, 10, 2149-2157.

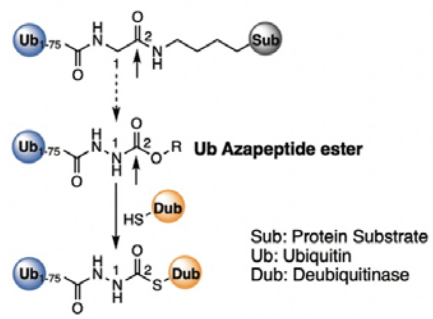
P-105

Ubiquitin Azapeptide Ester as Next-Generation Activity-Based Probes for Cysteine Enzymes in the Ubiquitin Signal Pathway

Saibal Chanda[1], Sandeep Atla[1], Xinlei Sheng[2], Yingming Zhao[2], Wenshe Ray Liu[1]

[1]Texas A&M University, College Station, USA
[2]University of Chicago, Chicago, USA

Ubiquitination is a pivotal cellular process that controls protein homeostasis and regulates numerous biological functions. Its pathway operates through a cascade of enzyme reactions involving ubiquitin-activating, E1, ubiquitin-conjugating, E2, and ubiquitin-ligating, E3, enzymes and deubiquitinases, DUBs, many of which are cysteine enzymes. Activity-based ubiquitin probes were previously developed for profiling these enzymes. However, most conventional probes do not mimic natural enzyme–substrate interactions and involve chemical mechanisms different from enzyme catalysis. Their uses potentially affect the comprehensiveness of enzyme profiling results. The current study introduces a novel class of activity-based ubiquitin probes, ubiquitin azapeptide esters, designed to overcome these limitations.



These probes incorporate an azaglycine ester at the ubiquitin C-terminus. They structurally mimic a ubiquitinated protein substrate and react with a cysteine enzyme via a mechanism like the enzyme catalysis. It was demonstrated that ubiquitin azapeptide esters are reactive toward a large variety of DUBs and several tested E1, E2, and E3 enzymes as well. Compared to a conventional probe, ubiquitin propargylamine, ubiquitin azapeptide esters generally provide superior labeling and profiling of active cysteine enzymes in the ubiquitination/deubiquitination cascade in both HEK293T cells and mouse tissue lysates. Activity-based protein profiling using these probes in mouse tissue lysates also revealed distinct patterns of labeled enzymes, confirming their potential in understanding the unique roles of these enzymes in different tissues.

P-106

Evaluating Staple Position and Linker Length on Coiled-Coil Stability via Experiment and Molecular Dynamics

Samantha Hatfield

Brigham Young University, Provo, USA

Stapling or macrocyclization is a widely used strategy to enhance the conformational stability, proteolytic resistance, and target-binding affinity of peptide and protein drugs. Stapling is generally thought to enhance peptide/protein conformation stability and target-binding affinity by covalently preorganizing the peptide/protein into a conformation that mimics the folded/bound state, thereby prepaying some of the energetic cost of folding, through both entropic and enthalpic effects.

Despite significant advancements, a lack of clear structure- or sequence-based guidelines for selecting staple sites, linker lengths, and reaction chemistries presents a major challenge. While empirical methods can identify optimal stapling sites or linker lengths, these approaches are time-consuming and resource intensive. Predictive tools, on the other hand, have the potential to streamline drug development by identifying stapling parameters that improve stability and proteolytic resistance more efficiently.

Our lab is uniquely positioned to address this gap by systematically studying stapling in a model system, with a particular focus on linker length—an underexplored parameter in the field. Most studies have relied on a narrow range of linker lengths, often settling on an arbitrary choice. Using a combination of experimental techniques and molecular dynamics simulations, we aim to provide a biophysical assessment of how staple site and linker length impact conformational stability. This work seeks to establish a set of design rules for peptide stapling, enabling more efficient and rational development of stabilized peptide therapeutics.

P-107

A Dual Assembly Pathway of Liraglutide Uncovered by Advanced Integrative Native Mass Spectrometry

Syuan-Ting Kuo, Zhenyu Xi, Xin Yan, and David Russell

Texas A&M, College Station, USA

Liraglutide, a glucagon-like peptide-1, GLP-1, receptor agonist, is a therapeutic peptide conjugated with palmitic acid, promoting self-association to enable slow release and prevention of renal clearance. Despite advancements in biophysical characterization methods,[1, 2] the comprehensive elucidation of its oligomeric states remains elusive. Traditional techniques, such as analytical ultracentrifugation and light scattering, reported ensemble-average responses and have difficulties resolving oligomeric states and the identities of oligomers with high molecular weights.

Here, we present a novel integration of native mass spectrometry, nMS, that has provided new insights into the roles of hydrophobic and hydrophilic interactions that drive liraglutide self-assembly. While the self-assembly process mediated by conjugated 16-carbon fatty acid chain has been widely observed, the transition from soluble oligomers to pre-fibrillar high-molecular-weight intermediates—a critical step in forming insoluble aggregates detrimental to biomedical efficacy and production—remains poorly understood.

Our study leverages advanced nMS techniques, including electron-capture dissociation, ECD, and Direct Mass TechnologyTM, DMT, that is capable of individual ion detection, to reveal these elusive intermediates. ECD analysis uncovered a restricted C-terminus in oligomeric liraglutide compared to its monomeric form, suggesting structural stabilization during 14-mer formation. Additionally, high-resolution DMT identified oligomers ranging from n=25 to n=62, resolving their identity in a highly heterogeneous system by directly determining ion charges.[3] Molecular dynamics simulations further demonstrated that the 14-mer associates via hydrophilic interactions to form high-molecular-weight oligomers. We envision these findings offer a comprehensive framework for analyzing peptide therapeutics and advancing their future discovery and development.

[1]Wang, Y.; Lomakin, A.; Kanai, S.; Alex, R.; Benedek, G. B. Transformation of oligomers of lipidated peptide induced by change in pH. *Mol Pharm* **2015**, 12 (2), 411-419. DOI: 10.1021/mp500519s From NLM Medline.
[2]Frederiksen, T. M.; Sonderby, P.; Ryberg, L. A.; Harris, P.; Bukrinski, J. T.; Scharff-Poulsen, A. M.; Elf-Lind, M. N.; Peters, G. H. Oligomerization of a Glucagon-like Peptide 1 Analog: Bridging Experiment and Simulations. *Biophys J* **2015**, 109 (6), 1202-1213. DOI: 10.1016/j.bpj.2015.07.051 From NLM Medline.
[3]Kafader, J. O.; Melani, R. D.; Durbin, K. R.; Ikwuagwu, B.; Early, B. P.; Fellers, R. T.; Beu, S. C.; Zabrouskov, V.; Makarov, A. A.; Maze, J. T.; et al. Multiplexed mass spectrometry of individual ions improves measurement of proteoforms and their complexes. *Nat Methods* **2020**, 17 (4), 391-394. DOI: 10.1038/s41592-020-0764-5 From NLM Medline.
[4]Syuan-Ting Kuo, Zhenyu Xi, Xiao Cong, Xin Yan, and David H. Russell. Dissecting Liraglutide Oligomerization by Native Mass Spectrometry Coupled with Molecular Dynamic Simulation. Manuscript in preparation

P-108

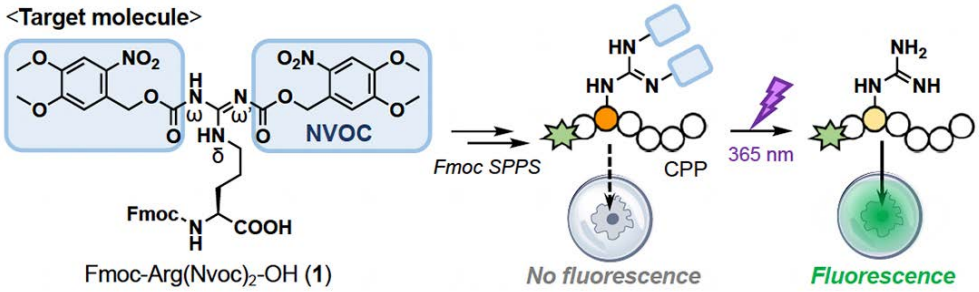
Development of Fmoc SPPS-Compatible Caged Arginine Monomer that Enables Photoactivation of Bioactive Peptide and Protein Synthesis

Yuya Nakajima[1], Yoshiki Konda[2], Ryosuke Sakamoto[2], Akimitsu Okamoto[2], HiroshiMurakami[1], and Gosuke Hayashi[1]

[1]Department of Biomolecular Engineering, Graduate School of Engineering, NagoyaUniversity, Nagoya, Japan
[2]Department of Chemistry and Biotechnology, Graduate Schoolof Engineering, The University of Tokyo, Tokyo, Japan

Photocaged peptides and proteins that can restore their intrinsic activity upon light irradiation have been utilized to regulate various cellular processes in a spatiotemporal manner. To aim the efficient chemical synthesis of caged peptides by Fmoc solid-phase peptide synthesis, SPPS, Fmoc-protected caged amino acid derivatives have been developed for several amino acids, such as cysteine, serine, and tyrosine.

In this research, we newly developed a caged arginine monomer compatible with Fmoc SPPS. The Fmoc-protected arginine derivative contains photolabile nitroveratryloxycarbonyl, Nvoc, moieties both at ω- and ω'-amino groups of the side chain guanidino group, **1**. Coupling of **1** quantitatively proceeded with base-free coupling condition with DIC/HOAt, followed by the optimal Fmoc deprotection condition, 2% DBU in DMF. We successfully introduced **1** not only into several bioactive peptides such as cell penetrating peptide (CPP) and cell-adhesive peptide but also a chemically synthesized protein, indicating the compatibility of monomer **1** with Fmoc-SPPS and native chemical ligation. Removal of Nvoc groups on **1** was confirmed to complete within 30 min by UV irradiation in PBS buffer.



Furthermore, Nvoc arginine-containing penetratin, a cell penetrating peptide, was used for light-induced cellular uptake experiments. Given that arginine is a key amino acid to interact with nucleic acids and proteins, the caged arginine monomer provided here would expand opto-chemical toolbox for biomolecular research and spatiotemporally controllable drug delivery agents.

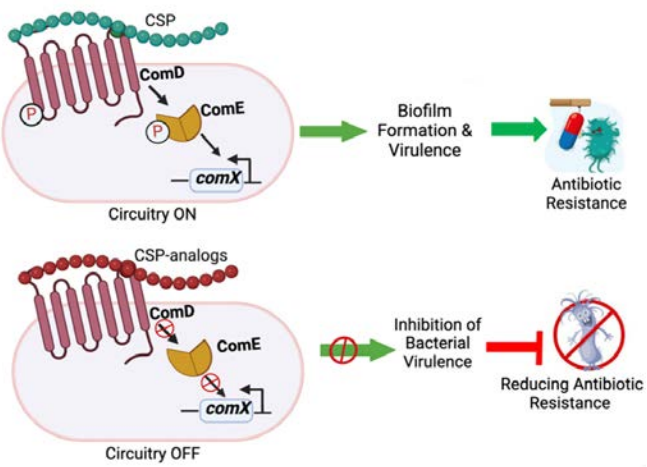
P-109

Developing Competence Stimulating Peptidet CSP-Based Quorum Sensing Modulators in *Streptococcus sanguinis*

Anju Basnet, Yftah Tal-Gan

University of Nevada at Reno, Reno, USA

Streptococcus sanguinis is a prevalent commensal microbiome that colonizes the tooth's surface and contributes to a caries-free environment. However, it can cause infective endocarditis when introduced into the bloodstream. Unlike other streptococci that possess a five-component ComABCDE competence regulon quorum sensing, QS, circuitry, *S. sanguinis* competence is mediated through a unique ComCDE QS circuitry regulated by the competence stimulating peptide, CSP.[1] Bacteria utilize QS to coordinate many group behavior phenotypes, such as acquiring antibiotic-resistance genes and initiating virulence and pathogenesis.[2]



The rapid evolution of antibiotic resistance poses a constant threat to global health. Targeting bacterial QS pathways could be a promising approach to attenuate bacterial infectivity without introducing selective pressure for resistance development. In *S. sanguinis*, induction of the competence regulon is centered on the binding of CSP to its cognate receptor, ComD. However, the molecular mechanisms behind CSP:ComD receptor binding, QS circuitry activation, and pathogenesis associated with *S. sanguinis* are not fully understood. In this study, we systematically replaced amino acid residues within the *S. sanguinis* CSP pheromone and assessed the ability of these mutated analogs to modulate the competence regulon. We were able to identify structural features that are important to ComD binding and QS activation.

P-110

Natural Product-Inspired Cyclic Peptides Active Against Free-Living Amoeba

Gabriela Coy, Chenyang Lu, Sam Nelson,Christopher Neumann, Christopher Rice, ElizabethParkinson

Purdue University, West Lafayette, USA

Natural products are an important source of drug discovery, with bacterial natural products having particularly significant therapeutic activities. Traditional methods to find novel natural products involve arduous purification, characterization, and optimization, frequently leading to the re-discovery of already known molecules. To overcome this, we focus on biosynthetic gene clusters, BGCs, the genes that encode for the enzymes that synthesize natural products, containing non-ribosomal peptide synthetases, NRPS. Bioinformatics analyses of the NRPS domains facilitate the prediction of nonribosomal peptide, NRP, structures. The Synthetic Natural Product Inspired Cyclic Peptides, SNaPP, method consists of this bioinformatic prediction followed by chemical synthesis of the predicted peptides using solid-phase peptide synthesis, SPPS. This method generates a library that can be assessed for bioactivities.

Herein, we describe the identification of antiamoebic peptides from the SNaPP library. *Balamuthia mandrillaris, B. mandrillaris,* is a pathogenic, opportunistic free-living amoeba. It mainly affects the central nervous system, leading to lethal granulomatous amoebic encephalitis. In the United States, it is reported that 9 out of 10 people infected die. While no FDA-approved drugs exist, recommended treatments include a cocktail of six drugs. Unfortunately, these molecules are not potent with IC50 values from 18.35 μM for pentamidine to > 163.25 μM for fluconazole. Therefore, there is a critical need to develop new therapeutics to treat *B. mandrillaris* infections.

Here, we present natural product-inspired cyclic peptides with an IC_{50} against *B. mandrillaris* of 3.9 μM and no cytotoxic activity to the A549 lung carcinoma cells, IC_{50}>100 μM, showing a promising therapeutic for treating amoeba infections.

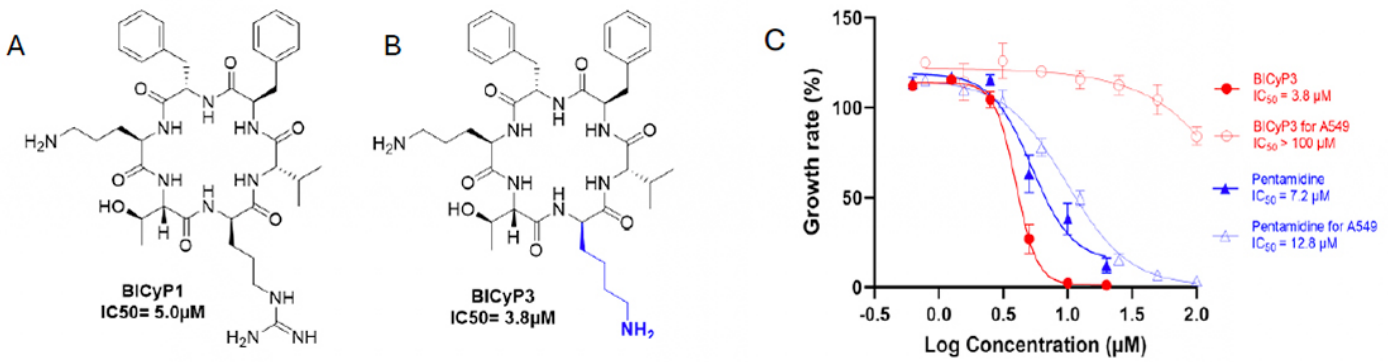


Figure 1: A| Synthetic Natural Product Inspired Cyclic Peptide, BICyP1, active against *B. mandrillaris*. IC_{50} = 5.0 μM. **B|** Optimized derivative most active against *B. mandrillaris,* BICyP3, IC_{50} = 3.8 μM. **C|** Dose-response curves for BICyP3, red, and pentamidine, blue, against *B. mandrillaris* and Cytotoxicity to A549 cells.

P-111

Discovery of D-Peptide Inhibitors of Henipavirus Entry

Matthew Lloyd, and Michael Kay

University of Utah, Salt Lake City, USA

Nipah and Hendra viruses have recently emerged as highly lethal, zoonotic pathogens belonging to the genus henipavirus. Pathogenic henipaviruses have a 38-75% fatality rate, with no approved therapeutic interventions. Henipaviruses follow a conserved mechanism in which the HR1 and HR2 regions of the F fusion protein interact to form a six-helix bundle that brings the viral and cellular membrane together for viral fusion. Hendra and Nipah have 100% sequence identity in the targeted HR1 region, and thus, HR1 is an ideal target to prevent the fusion of both viruses.

The Kay lab specializes in discovering and developing D-peptide drugs that target viral entry. Natural L-peptide inhibitors suffer from fast degradation via proteolysis and are highly immunogenic, and therefore require high/frequent dosing. In contrast, enantiomeric, mirror-image, D-peptides avoid degradation by proteases and have greatly reduced immunogenicity, enabling longer drug half-life and lower costs.

We have synthesized and characterized a mirrorimage target to be used in mirror-image phage display. After screening our three naïve phage libraries, we have identified a midnanomolar binder with high specificity. Through the use of our scaffolding technology, we have improved the binding to picomolar affinity. We are currently using X-ray crystallography to inform structure-guided 2nd generation phage libraries to further optimize affinity. We hypothesize that this D-peptide inhibitor will prevent Nipah/Hendra entry into host cells, acting as an effective antiviral treatment while establishing a new drug discovery pipeline for emerging henipaviruses.

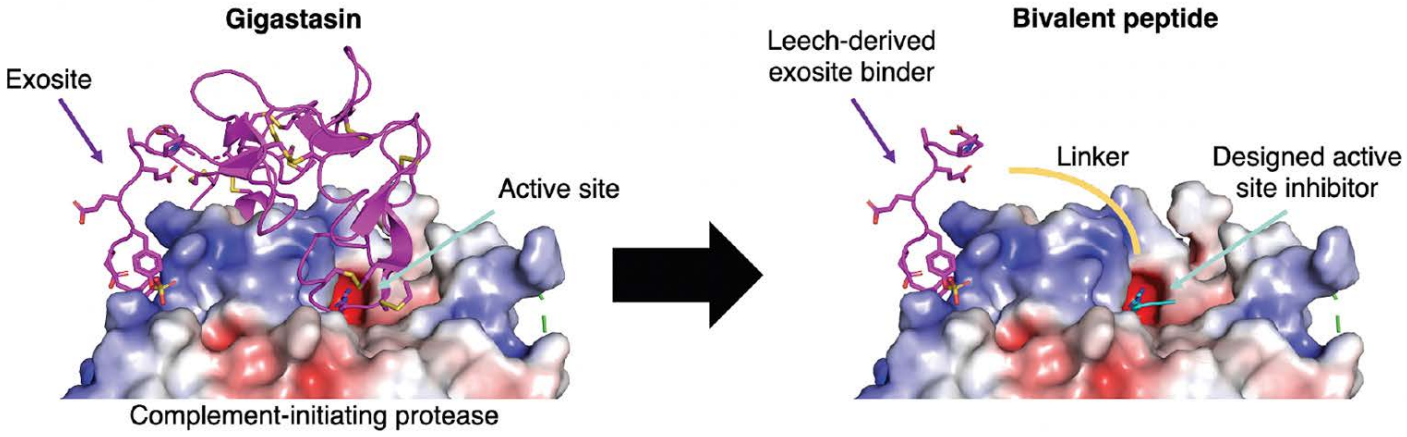
P-112

Leech-Inspired Bivalent Peptides for Multi-Target Modulation of Host-Defense Responses

Alexander J. Lander, Aleksandra Blagojevic, Stephanie A. Vogt, Colm Power-Kennelly, Jerome Eberhardt, Florian D. Meyer, Peter Rüthemann, Oliver Schwardt, and Daniel Ricklin

University of Basel, Basel, Switzerland

The complement and coagulation systems are blood-based proteolytic cascades that are essential for innate immunity and maintaining hemostasis. Though tightly controlled by the action of various serine proteases, dysregulation of both systems can occur simultaneously and lead to thromboinflammation - creating a need for new therapeutic strategies.[1] Leeches have evolved to secrete proteins that inhibit complement and/or coagulation, but issues regarding production, PK, and immunogenicity may limit their direct application as therapeutics. Inspired by nature, we have utilized structural insights of gigastasin, a protein from the giant Amazon leech, in complex with a protease target as a template to design bivalent peptides capable of mimicking its mechanism of action.[2]



We first designed peptides capable of blocking the protease active site in a substrate-like binding mode. These peptides were then conjugated to a sulfated-peptide derived from gigastasin that blocks a functional exosite on the proteases. The resulting bivalent peptides could inhibit complement activation at low micromolar concentrations in human serum via both the classical, antibody-mediated, and lectin, glycaninduced, pathways, by blocking homologous serine proteases. The peptides will be subject to selectivity profiling and structure guided design against various related proteases within complement and coagulation systems. Taking advantage of the exquisite structural control of peptides, we aim to develop a family of bivalent peptide inhibitors with tailored activity and selectivity profiles against host-defense proteases for broad impairment of complement and coagulation systems. Collectively, this work illustrates the potential of designing peptides inspired by naturally-evolved proteins – yielding functionality difficult to achieve using *de novo* discovery efforts.

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[2]S. Pang, et. al., *J Immunol*, **2017**, 199(11), 3883-3891.

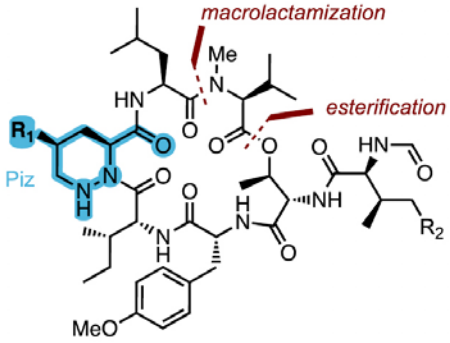
P-113

Total Synthesis of Marformycins A and D

Avraz Anwar[1], Yassin Elbatrawi[1], David Degen[2], Richard Ebright[2], and Juan Del Valle1

[1]University of Notre Dame, Notre Dame, USA
[2]Rutgers University, Piscataway, USA

The search for antibiotics with novel mechanisms of action, MoA, is crucial for combating the growing threat of antimicrobial resistance. The marformycins, Mrf, are a class of piperazic acid, Piz-containing depsipeptide natural products that exhibit selective activity against *Cutibacterium* and *Micrococcus luteus* at 0.1 µg/mL concentrations. Their remarkable selectivity suggests that their activity may be attributed to a unique MoA.



Marformycin A (R_1,R_2 = H)
Marformycin D (R_1 = OH R_2 = Me)

- *MIC* = 0.10 µg/mL for cutibacterium
- *No hemolytic activity*
- No mammalian cell toxicity
- > 10 analogs synthesized
- Mechanism unknown

We report the first total synthesis of Mrf A and D using a solid-phase approach, and describe a rapid and scalable solution-phase synthesis of the ɣ-hydroxy-Piz precursor from hydroxyproline. Interestingly, we observed complete C-terminal epimerization at the Leu α-carbon of linear Mrf A upon macrocyclization using HATU/DIEA in DMF. No epimerization was detected when using T3P/DIEA in DCM. X-ray crystallography of Leu-*epi*-Mrf A showed that it does not adopt the same antiparallel β-sheet conformation as Mrf A, which may explain its lack of antimicrobial activity. Synthetic Mrf A and Mrf D exhibited no hemolytic activity, mammalian cell cytotoxicity, or hepatotoxicity, making them promising antimicrobial candidates. A streamlined solid-phase approach enabled us to carry out sequence-and conformation–activity relationship studies. We also present ongoing efforts to elucidate the MoA of these potent depsipeptide antibiotics.

[1]Anwar, A.F.; Elbatrawi. Y.M.; Degen, D.; Ebright, R.H.; Del Valle, J.R. "Total Synthesis of Marformycin A and D" *Org. Lett.* **2024**, 24, 46, 10056–10060

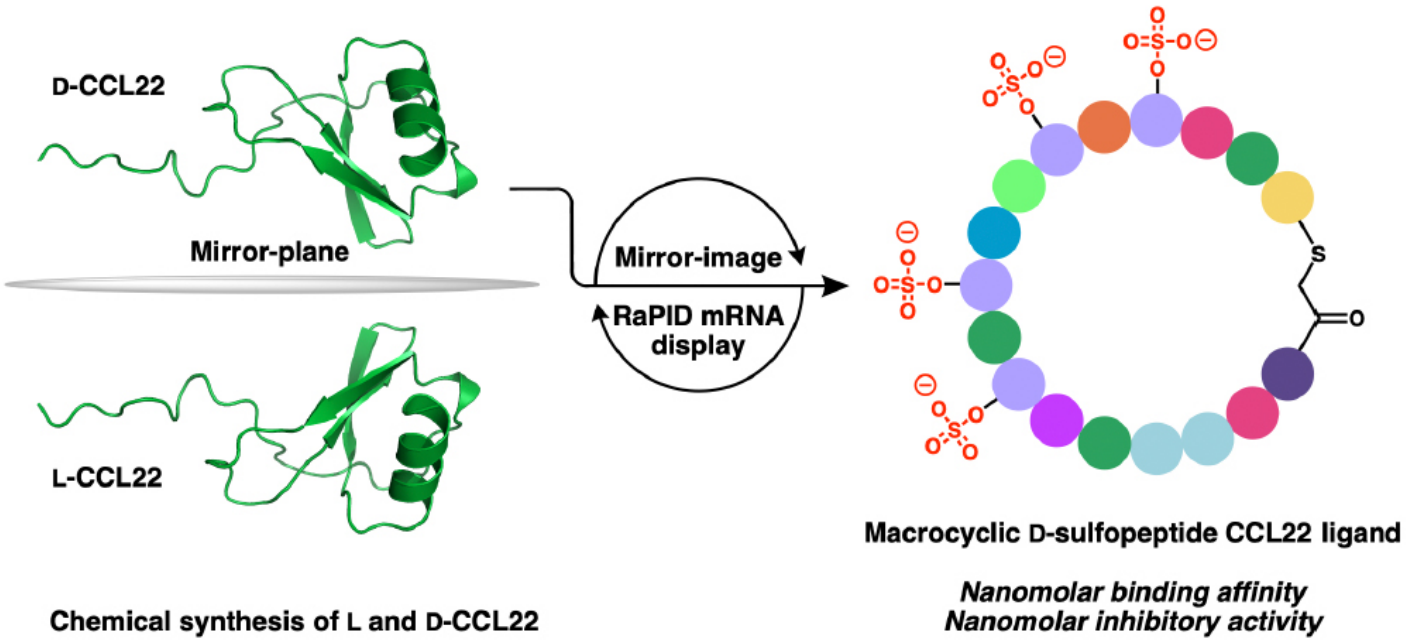
P-114

Discovery of Selective Cyclic D-Sulfopeptide Ligands of the Chemokine CCL22 via Mirror-Image mRNA Display with Genetic Reprogramming

Belinda Zhang[1], Katriona Harrison[1], Yichen Zhong[1], Joshua Maxwell[1], Daniel Ford[1], LiamCalvey[1], Sean So[2], Francis Peterson[3], Brian Volkman[3], Martin Stone[2], Ram Bhusal[2], Sameer Kulkarni[1], and Richard Payne[1]

[1]University of Sydney, Sydney, Australia
[2]Monash University, Clayton, Australia
[3]MedicalCollege of Wisconsin, Milwaukee, USA

Inflammation is driven by chemokines – small proteins involved in recruiting leukocytes through interactions with specific cell-surface receptors.[1] CCL22 is a chemokine that plays a crucial role in inflammatory diseases such as asthma and atopic dermatitis, thus inhibition of CCL22 presents an emerging therapeutic strategy.[2]



In this work, we discovered cyclic D-sulfopeptide inhibitors of CCL22 using mirror-image mRNA display with genetic reprogramming. We first synthesised mirror-image DCCL22, which was subsequently screened against a cyclic peptide library composed of all L-amino acids, including reprogrammed L-sulfotyrosine to mimic this post-translational modification present on native chemokine receptors. The most enriched peptides were prepared as mirror-image D-ligands and assessed for binding against native L-CCL22. We found that the most potent ligand, a plasma-stable D-cyclic peptide with four D-sulfotyrosine residues, showed nanomolar affinity for CCL22, nanomolar inhibition of CCL22 signaling via CCR4, and remarkable selectivity over other chemokines. Thus, this work highlights the potential of mirror-image mRNA display as a technology for discovering proteolytically stable D-peptide inhibitors of protein-protein interactions relevant across a range of therapeutic indications.[3]

[1]Hughes, C. E.; Nibbs, R. J. B. A Guide to Chemokines and Their Receptors. *FEBS J.* **2018**, 285 (16), 2944-2971.
[2]Kufareva, I.; Salanga, C. L.; Handel, T. M. Chemokine and Chemokine Receptor Structure and Interactions: Implications for Therapeutic Strategies. *Immunol. Cell Biol.* **2015**, 93 (4), 372-383.
[3]Zhang, B. B.; Harrison, K.; Zhong, Y.; Maxwell, J. W. C.; Ford, D. J.; Calvey, L. P.; So, S. S.; Peterson, F. C.; Volkman, B. F.; Stone, M. J.; Bhusal, R. P.; Kulkarni, S. S.; Payne, R. J. Discovery of Selective Cyclic D-Sulfopeptide Ligands of the Chemokine CCL22 via Mirror-Image mRNA Display with Genetic Reprogramming. *J. Am. Chem. Soc.* **2024**, 146 (50), 34253-34259.

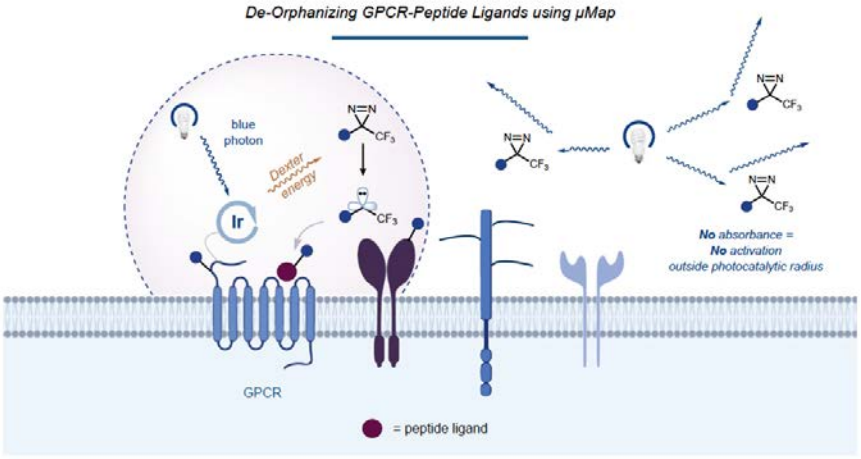
P-115

From Targets to Therapies: Redefining Peptide Discovery with Photocatalytic µMap Proximity Labeling

Danielle Morgan, Steve Knutson, Chenmengxiao, Roderick, Pan, and David MacMillan

Princeton University, Princeton, USA

The therapeutic landscape is rapidly being reshaped by peptides, a class of biomolecules that uniquely bridge the strengths of biologics and small molecules. Their ability to combine high binding affinity and selectivity with favorable pharmacokinetic properties has been powerfully demonstrated by the clinical success of agents such as Semaglutide. Despite their promise, many aspects of peptide–target interactions within complex biological systems remain poorly understood, presenting both a challenge and an opportunity for innovation.



In the MacMillan group, we are leveraging advanced technologies using photocatalysis to address this gap, with a particular emphasis on the µMap proximity labeling platform. By enabling precise mapping of biomolecular interactions in live cellular contexts, µMap is unlocking new strategies for rational peptide therapeutic design. Our research portfolio spans several high-impact areas, including the discovery of ligands for orphan G protein–coupled receptors, GPCRs, the development of brain-penetrant shuttle peptides to enhance central nervous system drug delivery, and the expansion of photocatalytic methodologies for high-throughput screening applications.

P-116

Targeting Transient Receptor Potential Channels with Teretoxins for the Treatment of Liver Cancer

Diana A. Martinez-Baquero[1], Favour Achimba[2], and Mandë Holford[1]

[1]Hunter College of CUNY, New York, USA.
[2]Gradcenter of CUNY, New York, USA

Channelopathies are emerging as an innovative approach to cancer treatment, and peptides uniquely combine the high specificity of protein structures with advantageous drug-like properties. The challenges in diagnosing liver cancer and the limited availability of safe treatments highlight the need for targeted therapies. In Prof. Mandë Holford's lab, we explore the largely untapped diversity of marine animal venoms[1], particularly those of *Terebridae* auger snails — predatory animals that produce a rich repertoire of peptides that naturally target ion channels. Using venomics, we characterize toxins and discover new bioactive peptides with a focus on cancer and pain.

We have demonstrated that Tv1, a 21-mer peptide isolated from *Terebra variegata*[2], selectively inhibits TRP channels in hepatocellular carcinoma cell lines and reduces tumor growth in an allograft mouse model[3]. Here, we present

our latest advancements in elucidating the mechanism of action of Tv1. The efficacy of the peptide and its cysteine-framework analogs against a panel of hepatocellular carcinoma cell lines is assessed using immunofluorescence calcium imaging, microscopic co-localization, and siRNA silencing of TRP channels to validate the target. Additionally, the proteolytic resistance of these highly constrained, disulfide-rich peptides will be challenged in stability studies to assess their potential as drug candidates.

We present the Holford lab's research pipeline, encompassing venom characterization, peptide production, optimization, and biological evaluation.

[1]Holford, M., Daly, M., King, G. F. & Norton, R. S. Venoms to the rescue: Insights into the evolutionary biology of venoms are leading to therapeutic advances. *Science,* **2018,** 361: 842–843
[2]Anand, P. et al. Sample Limited Characterization of a Novel Disulfide-Rich Venom Peptide Toxin from Terebrid Marine Snail Terebra variegata. *PLoS One,* **2014** 9, e94122.
[3]Anand, P. et al. Selective Inhibition of Liver Cancer Cells Using Venom Peptide. *Marine Drugs*, **2019** 17: 587

P-117

Controlling the Nrf2/MafG Coiled Coil with Rationally Designed Peptide Inhibitors

Ellie Hyde, Emily Hobson, Maria O'Connell, Mark Searcey, and Andrew Beekman

University of East Anglia, Norwich, United Kingdom

Nrf2 is a leucine zipper transcription factor that activates cytoprotective gene expression in response to reactive oxygen species and cellular toxins.[1] Cancers can overexpress Nrf2 generating resistance of chemotherapy.[2] Here we describe the use of peptides to prevent Nrf2 from initiating gene expression in cancers.

Nrf2 binds to DNA by forming a heterodimer with the MafG transcription factor. This protein-protein interaction, PPI, is known as a coiled coil, where alpha helical sections of the proteins from a supercoil. Coiled coils follow a heptadic repeat of hydrophobic and ionic residues that stabilise inter/intra-strand interactions.[3] In the absence of a crystal structure, we generated an AlphaFold homology model,[4] to identify key amino acids of the Nrf2-MafG coiled coil.

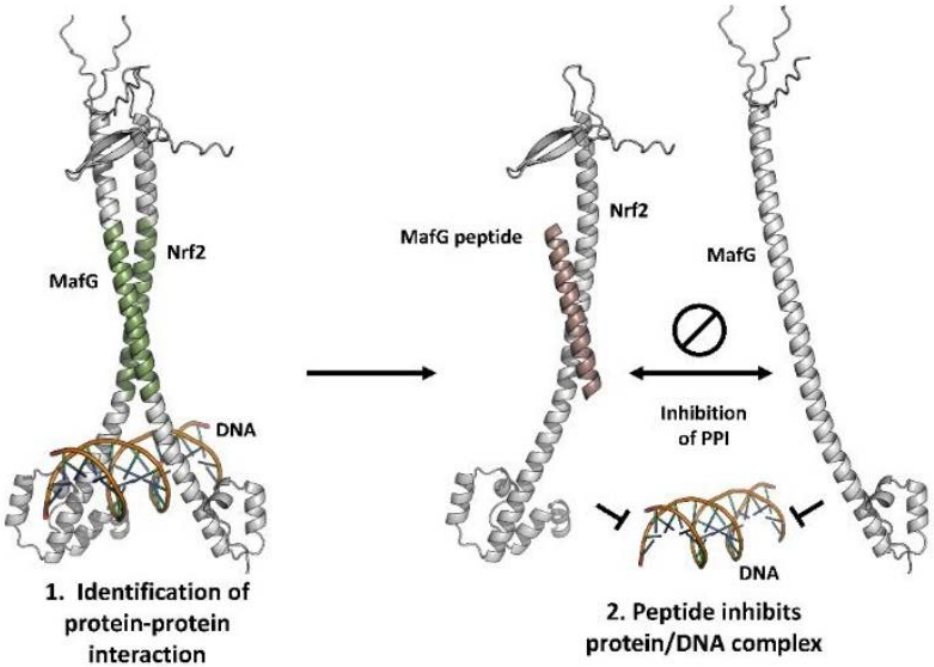


Figure 1: AlphaFold Homology Model of Nrf2/MafG. The site of the protein-protein interaction, green, the section of MafG that peptides synthesised mimic, pink.

Our approach has shown that peptides mimicking the leucine zipper of MafG can bind to Nrf2 and inhibit the PPI required for cytoprotective gene transcription. This presentation will show the design, synthesis and evaluation of peptides derived from MafG. We demonstrate that MafG peptides are α-helical, can bind Nrf2 with high affinity, and can prevent Nrf2/MafG/DNA ternary complex formation. The impacts on resistant and sensitive cancer cell lines will be discussed.

The MafG mimetic peptide inhibitor is now being used for peptide-directed ligand design to identify peptide-small molecule hybrids to vary inhibition, stability, cell permeability and compound solubility. A novel approach demonstrating an exciting new direction for peptide-based drug discovery.[5]

By researching inhibitors of the critical Nrf2 regulatory pathway, we reveal insights into the behaviour of resistant and sensitive cancer cell lines, offering fresh perspectives on potential therapeutic strategies for chemotherapy-resistant cancers.

[1]Farooqui, Z.; Mohammad, R. S.; Lokhandwala, M. F.; Banday, A. A. Nrf2 Inhibition Induces Oxidative Stress, Renal Inflammation and Hypertension in Mice. *Clin Exp Hypertens* **2021**, 43 (2), 175–180.
[2]Wang, X. J.; Sun, Z.; Villeneuve, N. F.; Zhang, S. Zhao, F.; Li, Y.; Chen, W.; Yi, Z.; Zheng, W.; Wondrak, G. T.; Wong, P.K.; and Zhang, D.D. Nrf2 Enhances Resistance of Cancer Cells to Chemotherapeutic Drugs, the Dark Side of Nrf2. *Carcinogenesis* **2008**, 29 (6), 1235–1243.
[3]Baxevanis, A. D.; Vinson, C. R. Interactions of Coiled Coils in Transcription Factors: Where Is the Specificity? *Current Opinion in Genetics & Development* **1993**, 3 (2), 278–285.
[4]Bryant, P.; Pozzati, G.; Elofsson, A. Improved Prediction of Protein-Protein Interactions Using AlphaFold2. *Nature Communications* **2022** 13:1 2022, 13 (1), 1–11.
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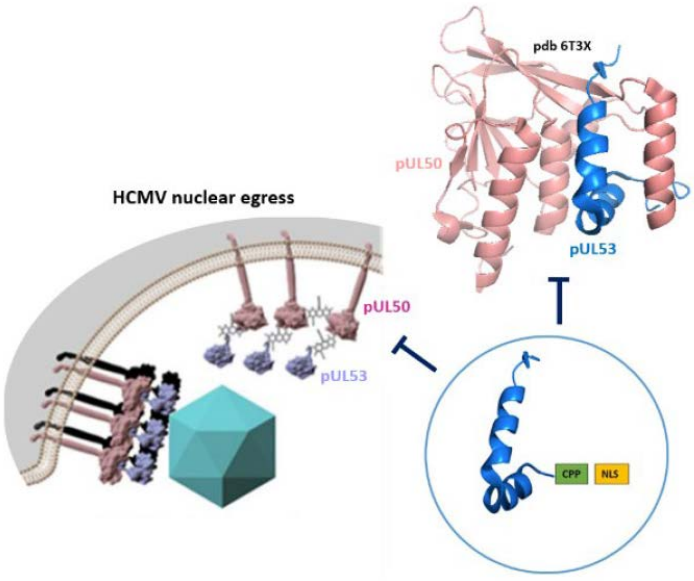
P-118

Peptide Inhibitors of the Human Cytomegalovirus Core Nuclear Egress Complex

Ferdinand Braun, Sewar Alkhashrom, Johannes Schweininger, Jintawee Kicuntod, ManfredMarshall, Yves Muller, and Jutta Eichler

Friedrich-Alexander-University Erlangen, Erlangen, Germany

During replication of human cytomegalovirus, HCMV, nuclear egress enables migration of newly formed viral capsids from the nucleus into the cytoplasm. Inhibition of the HCMV core nuclear egress complex, core NEC, composed of viral proteins pUL50 and pUL53, has therefore been proposed as a potential new antiviral target.[1] Based on the crystal structure of the pUL50-pUL53 complex,[2] we have designed a peptide presenting the N-terminal α-helical hook-like segment of pUL53, through which it contacts pUL50.[3]



This hook peptide was shown to bind to pUL50 and inhibit the pUL50 – pUL53 interaction *in vitro*, as well as interfere with HCMV infection of cells,4 substantiating the HCMV core NEC as a potential antiviral target. As the target of the peptide, that is, the pUL50 – pUL53 interaction, is localized at the inner nuclear membrane of the infected cell, the peptide had to be equipped with translocation moieties that facilitate peptide uptake into the cell and the nucleus, respectively.

We have now significantly improved the affinity of the hook peptide through a convergent approach involving directed evolution, as well as structure-based design. This process resulted in a 40% shorter peptide having a 10-fold higher affinity and inhibitory activity, respectively, highlighting the potential of peptide-based inhibition of the HCMV core NEC specifically, as well as protein-protein interactions in general.

1Marschall, M., Muller, Y.A., Diewald, B., Sticht, H., Milbradt, *J. Rev.Med.Virol.*, **2017**, 27, e1934.
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[3] Muller, Y.A., Häge, S., Alkhashrom, S. et al. *J.Biol.Chem.* **2020**, 295, 3189–3201.
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P-119

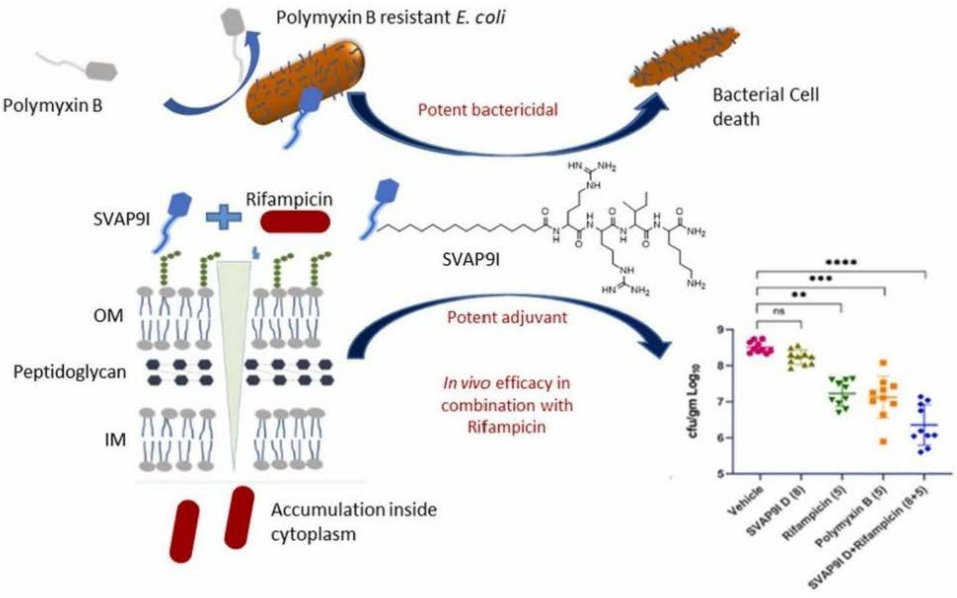
An Antibacterial Ultrashort Lipopeptide Acts as an Antibiotic Adjuvant to Narrow-Spectrum Aantibiotics Against MDR Gram-Negative Pathogens

Apurva Panjla[1], Grace Kaul[1], Sidharth Chopra[2], and Sandeep Verma[1]

[1]Department of Chemistry, IIT Kanpur, Kanpur, India
[2]Division of Molecular Microbiology and Immunology, CSIR-CDRI, Lucknow, India

The decimated antibiotic pipeline against continually rising multi-drug resistant Gram-negative bacteria, MDR-GNB, necessitates alternative strategies to combat infections, especially by WHO-designated critical priority pathogens. Herein, we report an ultrashort cationic lipopeptide, SVAP9I, as a potent antibiotic and adjuvant that potentiates existing antibiotic classes towards GNBs.[1]

The lipidated tetrapeptide exhibited broad-spectrum activity against critical MDR GNBs with fast concentration-dependent bactericidal action at an MIC of 4 mg/L and no detectable resistance in *E. coli*. It showed similar activity against polymyxin B-resistant *E. coli* and a post-antibiotic effect of ~4 h. SVAP9I targeted the bacterial membranes by predominantly binding to LPS in the outer membrane and phospholipids cardiolipin and phosphatidylglycerol prevalent in the inner membrane of *E. coli*.



Membrane damage resulted in ROS generation, depleted intracellular ATP concentration, and a concomitant increase in extracellular ATP in *E. coli.* At sub-inhibitory concentrations, it served as a potent permeabilizing agent for multiple GNBs including carbapenem-resistant *A. baumannii,* CRAB, a WHO critical priority pathogen. In a murine *in vivo* model of thigh infection, SVAP9I and rifampicin synergized to express excellent antibacterial efficacy against MDR-CRAB outcompeting polymyxin B. Taken together, SVAP9I's distinct membrane-targeting broad-spectrum action against multiple MDR GNBs, lack of resistance and strong *in vivo* potency in combination suggest its potential as a novel antibiotic adjuvant for the treatment of serious MDR-GNB infections.

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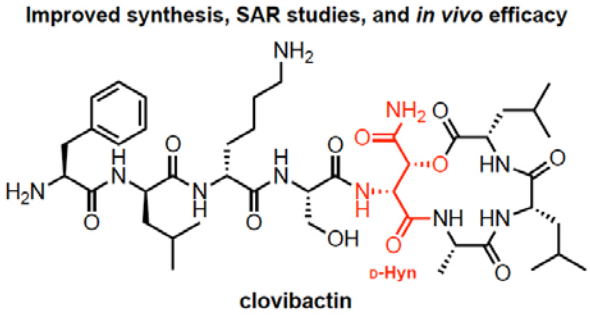
P-120

Development of Clovibactin Analogues as Preclinical Antibiotic Candidates

Jackson Brunicardi[1], James Griffin[2], Jeramiah Small[1], Sophia Padilla[1], Jovanna Carrera Plancarte[1], and James Nowick[1]

[1]University of California, Irvine, Irvine, USA
[2]University of Redlands, Redlands, USA

Clovibactin has emerged as a potent and promising antibiotic against drug-resistant strains of Gram-positive bacteria. Clovibactin is a peptide composed of eight amino acids, the most notable of which is the rare, noncanonical amino acid D-hydroxyasparagine, D-Hyn. Our laboratory recently published an improved, gram-scale synthesis of Fmoc-D-hydroxyasparagine which facilitates the production of clovibactin analogues and enabled structure-activity relationship studies of clovibactin, *J. Org. Chem.* **2024**, 89, 12479–12484.



These SAR studies elucidated the critical features of clovibactin that contribute to its excellent antibiotic activity – from which we produced more synthetically accessible analogues with comparable potency to clovibactin, *J. Org. Chem.* **2025**, Article ASAP. DOI: 10.1021/acs.joc.4c02828. The work presented herein will showcase ongoing efforts to improve the pharmacological properties of clovibactin so it may become a viable preclinical antibiotic.

P-121

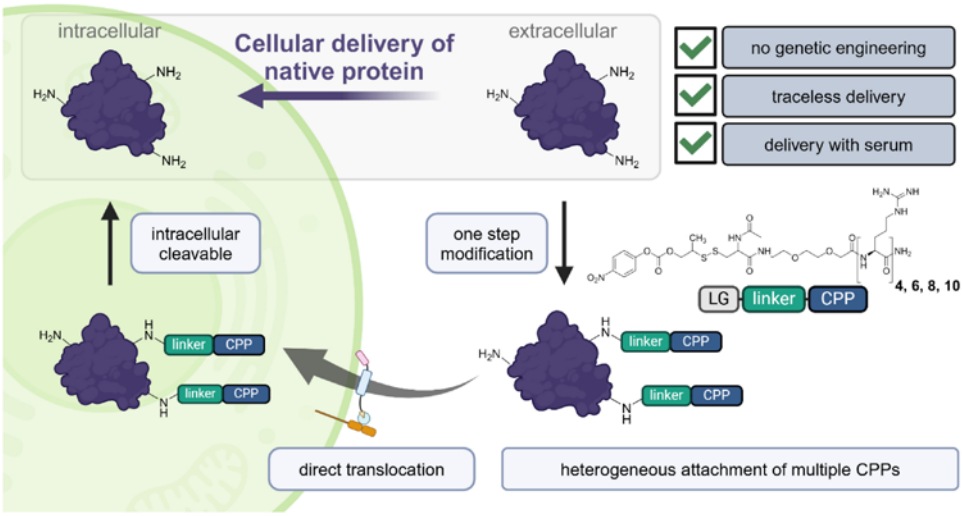
Intracellular Delivery of Native Proteins by Bioreversible Polyarginine Modification

Jonathan Franke, Jan V. V. Arafiles, Christian Leis, and Christian P. R. Hackenberger

Leibniz-Forschungsinstitut für Molekulare Pharmakologie, FMP, Berlin, Germany

Protein-based tools are emerging as innovative solutions to interfere with biological pathways in molecular biology and medicine. They offer advantages over traditional small molecules due to their structural diversity and ability to engage previously inaccessible cellular targets. However, most proteins do not penetrate the lipid bilayer of mammalian cells and are therefore restricted to extracellular targets. Despite recent advances, a universal method for delivery of functional proteins into human cells remains a significant challenge.

In this study, we present a bioreversible protein modification strategy using polyarginines, that enables cytosolic



delivery of native proteins. We optimized the bioconjugate to achieve fast intracellular cleavage by the reductive environment and complete restoration of the native protein. In combination with our previously established additive protocol[1], we show delivery of fluorescent protein and functional RNase A into the cytosol. We enhance efficiency, enabling delivery in the presence of serum using native proteins, thereby broadening the scope for intracellular applications significantly.

[1]A. F. L. Schneider, M. Kithil, M. C. Cardoso, M. Lehmann, C. P. R. Hackenberger, *Nat Chem* **2021**, 13, 530-539; V. V. J. Arafiles, J. Franke, L. Franz, J. Gómez-González, K. Kemnitz-Hassanin, C. P. R. Hackenberger, *JACS* **2023**, 145, 24535-24548.

P-122

Design and Ultrasound-Assisted Synthesis of Cyclic Antimicrobial Peptides Derived from Temporin L

Ida Boccino[1], Bruno Casciaro[2], Marialuisa Mangoni[2], Paolo Grieco[1], and Francesco Merlino[1]

[1]Department of Pharmacy, University of Naples Federico II, Naples, Italy.
[2]Department of Biochemical Sciences, Laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome, Italy

The growing number of antibiotic-resistant bacterial strains has made the development of new therapeutic strategies increasingly urgent. In this context, antimicrobial peptides, AMPs, represent a valid alternative to traditional antibiotics due to their properties.[1] Simultaneously, the increasing emphasis on sustainable synthetic practices has driven interest in environmentally responsible approaches to peptide production.[2,3] Among current methods, the Fmoc-based solid-phase peptide synthesis, SPPS, is one of the most widely used for the preparation of therapeutic peptides, although it presents limitations in terms of cost and sustainability.

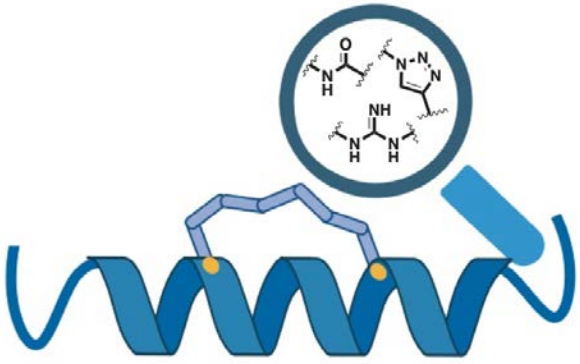


Figure 1. Structural modifications applied to TL peptide sequence to improve antimicrobial properties.

We designed a series of novel AMPs derived from Temporin L, a cationic antimicrobial peptide, with the aim of enhancing antimicrobial activity while reducing cytotoxicity. The new analogues incorporate non-canonical amino acids, which enabled the diverse *side-chain-to-side-chain* cyclization strategies, including lactam, guanidino, and triazole bridges, see figure 1. Peptides were synthesized using ultrasound-assisted SPPS, a promising approach that improves reaction efficiency and reduces environmental burden.[4] These cross-links are expected to positively influence the physicochemical properties such as polarity and increase protease resistance, thereby contributing to improved antimicrobial efficacy. Preliminary results on the antimicrobial activity and cytotoxicity of these derivatives will guide the design of further analogues, whose interaction with bacterial membranes and therapeutic potential against multidrug-resistant pathogens will be further investigated.

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P-123

Synthesis and Structure-Activity Relationship Study of the Antimicrobial Lipopeptide Brevibacillin

Louis-David Guay, Omar Fliss, Florence Henley, Maxime Boucher, Fayanne Nolin, Ismail Fliss, and Éric Biron

Université Laval, Quebec City, Canada

Antimicrobial resistance, AMR, has become a major problem in the prevention and treatment of bacterial infections. Among problematic resistant bacteria of clinical interest are vancomycin-resistant *Enterococcus,* VRE, methicillin-resistant *Staphylococcus aureus,* MRSA, and several Gram-negative bacteria of the ESKAPE group. Faced with this critical situation, the development of new antimicrobials with new modes of action has become a global priority.

Antimicrobial peptides are recognized as an interesting tool to fight AMR as they often show broad spectrum of activity and act via a wide range of original mechanisms of action. The lipopeptide brevibacillin is very interesting as it demonstrates considerable inhibitory activity against several clinically relevant bacteria, including multidrug-resistant strains. To better understand its mechanism of action and optimize its pharmacological properties, we aimed to develop a straightforward chemical synthesis of brevibacillin and conduct structure-activity relationship studies.

In this study, brevibacillin and several analogues have been produced by solid-phase peptide synthesis to evaluate the impact of C- and N-terminal modifications and substitution of specific amino acids. The antimicrobial activity and cytotoxicity of the lipopeptides have been evaluated and some analogues exhibited inhibitory activity comparable to native brevibacillin against Gram-positive and Gram-negative bacteria. The structure-activity study allowed the identification of key features that tolerate modifications without affecting antimicrobial activity while significantly reducing cytotoxicity. This study highlights the great potential of brevibacillin as an antimicrobial and the possibility of making modifications to increase production yields, improve stability, optimize activity and reduce cytotoxicity for further applications in the food, veterinary, and medical sectors.

[1]Guay, L.-D.; Fliss, O.; Fliss, I.; Biron, E., Synthesis and structure–activity study of the antimicrobial lipopeptide brevibacillin. *RSC Medicinal Chemistry* **2024**, 15(12), 4168-4179.

P-124

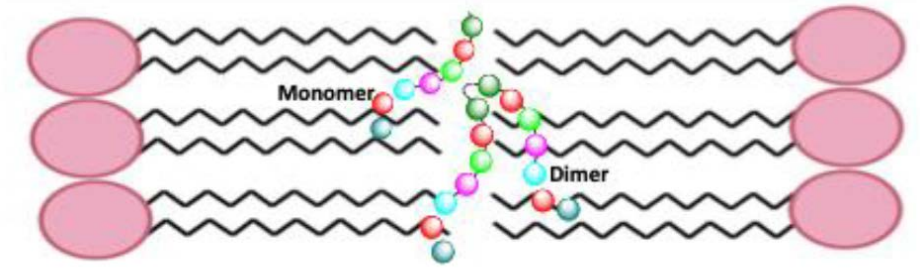
Dynamic Reversible Dimerization of Antimicrobial Peptides: Enhancing Potency Through Dual Functional Forms

Madeline Swanson, Elizabeth Bredice, Skander Abboud

University of North Carolina Wilmington, Wilmington, USA

The rise of antibiotic resistance among bacterial pathogens necessitates the development of novel therapeutic strategies. Antimicrobial peptides, AMPs[1] have emerged as promising alternatives to conventional antibiotics due to their ability to target bacterial membranes and intracellular components. Dimerization has proven to be a powerful strategy to enhance the potency of AMPs by improving their stability, binding affinity, and membrane penetration, thus increasing their effectiveness against pathogens.

In this work, we employ reversible click chemistry[2] to generate dimeric AMPs, creating a dynamic equilibrium between monomeric and dimeric forms. This approach leverages the advantages of both: monomeric peptides cross bacterial membranes more efficiently due to its smaller size and flexibility, facilitating internalization, while the dimeric form,[3] though less effective at membrane crossing, enhances antimicrobial activity by providing stronger membrane disruption and targeting intracellular components once inside. This dual role, monomer for membrane crossing and dimer for enhanced activity, optimizes bacterial targeting and amplifies the peptide's effectiveness.



Synergistic Antimicrobial Effects of Monomeric and Dimeric AMPs

We applied this approach to several AMPs with distinct mechanisms of action, including peptides that disrupt bacterial membranes and others that target intracellular components such as DNA. Our results highlight the versatility and effectiveness of this reversible dimerization strategy in improving AMP performance, underscoring its potential as a powerful tool in the fight against antibiotic-resistant bacteria.

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[2]Chatterjee, S.; Anslyn, E. V.; Bandyopadhyay, A. Boronic Acid Based Dynamic Click Chemistry: Recent Advances and Emergent Applications. *Chem. Sci*. **2021**, 12 (5), 1585–1599.
[3]Lorenzon, E. N.; Piccoli, J. P.; Santos-Filho, N. A.; Cilli, E. M. Dimerization of Antimicrobial Peptides: A Promising Strategy to Enhance Antimicrobial Peptide Activity. *Protein Pept. Lett*. **2019**, 26 (2), 98–107.

P-126

Backbone Expansion via Enzyme-Mediated Side-Chain Transfer "BEST"

Meghna Bajaj

Cornell University, Ithaca, USA

Peptide and protein backbone modifications are crucial for advancement in the field of biomolecular engineering which offers a platform for expanding the functional and structural diversity of natural backbones. By introducing a non-canonical monomer or embedding a desired synthetic polymer, d-aa, PEG, nucleic acids, et cetera, into the backbone, researchers can create novel therapeutically active peptides and proteins with enhanced stability, increased cell penetration, improved binding to the target and more. Post-translational modifications, PTMs, offer the distinct

advantage of bypassing ribosomal machinery and eliminating many of the complex engineering steps required for incorporation of these non-canonical monomers.

Herein, we report a strategy for successful post-translational, site-specific, chemoenzymatic incorporation of monomers that surpass the constraints of existing approaches. Transglutaminase, an enzyme known to catalyze isopeptide bond formation between Glutamine, Gln, and Lysine, Lys, has been employed to perform cyclization in varied peptide constructs. Our three-step methodology involves installation of a desired side chain, or synthetic polymer, with a primary amine onto a click handle followed by an enzyme-mediated intramolecular cyclization between the glutamine and extended amine. Finally, the third step entails the cleavage, via hydrolysis, photo-cleavage, or enzymatic cleavage, of the peptide or protein backbone at the precise residues involved in the cyclization process. This proximity-guided backbone extension represents a new chemical approach to the development of therapeutic agents and biomaterials.

P-127

Substrate-Derived Peptides for Selective Covalent Inhibition of Protein Tyrosine Kinases

Minhee Lee[1], Zijing Wang[2], and Neel Shah[1]

[1]Columbia University, New York, USA
[2]Barnard College, New York, USA

Protein tyrosine kinases are important regulators of cell signaling, and aberrant kinase activity contributes to diseases including diabetes and cancer. Most tyrosine kinase inhibitors are ATP competitive small molecules that offer strong potency and efficacy. However, achieving on-target selectivity across different kinases and specific disease mutants remain challenging due to the highly conserved nature of the ATP-binding pocket. By contrast, the variable substrate-binding site in kinases enables recognition of diverse protein substrates and offers an opportunity for selective inhibition using substrate-derived peptides.

Here, we present a modular strategy to design selective, peptide-based covalent inhibitors of tyrosine kinases with a distinct binding mode from existing inhibitors. Our peptides simultaneously target the unique substrate-binding site and react with non-conserved cysteines near the active site. These peptides were designed through high-throughput substrate profiling and structural modeling, and this pipeline is broadly applicable to most tyrosine kinases. We demonstrate that by optimizing substrate sequence and strategically positioning electrophiles near non-conserved cysteines, selective reactivity across different kinases can be achieved. Additionally, inhibitor potency can be improved through the incorporation of non-canonical amino acids. Building on this approach, we explore its application in developing selective inhibitors for oncogenic kinase mutants with altered substrate specificity or acquired cysteines from the wild-type kinase. Given their distinct mechanism of action, these peptides could also potentially be used alongside small molecule inhibitors to enhance efficacy and suppress the emergence of drug resistant mutations. The modular strategy presented here provides a powerful framework for future drug development efforts targeting protein tyrosine kinases.

P-128

Novel Peptide Inhibitors of SARS-CoV-2 Infection

Nina Raasch[1], Lucas Weißenborn[1], Elie Richel[2], Simon Schäfer[3], Heinrich Sticht[3], Klaus Überla[2], and Jutta Eichler[1]

[1]Department of Chemistry and Pharmacy, Erlangen, Germany
[2]Institute of Molecular andClinical Virology, Erlangen, Germany
[3]Institute of Biochemistry, Erlangen, Germany

Covid-19 remains a threat to global health, causing hundreds of deaths each day. Mechanistically, the infection with the causative virus SARS-CoV-2 is based on the interaction of the viral Spike, S, protein with the ACE2 receptor on the human host cell[1]. With the aim to increase virus neutralizing activity we have generated and characterized a truncated and cyclized peptide variant of the SARS-CoV-2 neutralizing miniprotein LCB1.[2] The 10-fold stronger antiviral activity of this peptide, LW25.13, as compared to LCB1 to the wild-type Spike receptor-binding domain, RBD, as well as its sub-

stantially improved proteolytic stability, make it a better potential candidate for SARS-CoV-2 therapy.[3]

Nevertheless, there is a need for optimization of the peptides in order to facilitate neutralization of newer virus variants, such as BA.1/ BA.2/ BA. 5, omicron variants. Therefore, we have combined reverse mutation strategies with structural and bioinformatic analysis to design peptide variants of LW25.13 capable of efficiently neutralizing omicron virus variants. This approach can be expected to be useful for the adaptation of antiviral peptides to new virus variants of concern that may pose a threat to humans in the future.

[1]Jackson, C. B. et al. *Nature Rev. Mol. Cell Biol*. **2022**, 23(1), 3 - 20.
[2]Cao, L. et al. *Science* **2020**,370(6515), 426 - 431.
[3]Weißenborn, L. et al. *Int. J. Mol. Sci*. **2022**, 23(11), 6309.

P-129

Broad Spectrum Antifungal Drug Synergies With Cryptomycin, a Cdc50 Inspired Antifungal Peptide

Robert Tancer[1], Siddhi Pawar[1], Greg Wiedman[2], and Chaoyang Xue[1]

[1]Rutgers University, Newark, USA
[2]Seton Hall University, South Orange, USA

Fungal infections are challenging to treat with increasing levels of drug resistance and limited treatment options. Our previous studies demonstrated that *Cryptococcus* lipid flippase is required for antifungal resistance and is essential for fungal virulence. This research project endeavored to develop a specific peptide inhibitor of the fungal lipid flippase to help inhibit drug resistance in concerning fungal pathogens.

Several iterative rounds of rational design, synthesis, and screening led to the discovery of Cryptomycinamide, KKOO-NH_2. This peptide has several noncanonical modifications that are important for antifungal activity. Key structural features vital for activity include N′ myristylation, nine amino acid backbone, back-to-back cationic residues, and C′ amidation. The final peptide possesses minimum inhibitory concentrations, MIC, of 8 µg/mL against *C. neoformans* H99, *C. glabrata* 2001, 16 µg/mL against *C. albicans* Sc5413, *C. auris* B11245, and 32 µg/mL against *A. fumigatus* AF293. Excitingly, the peptide demonstrated drug synergy with several commonly used antifungals, potentiating the antifungal activity depending on which species the combination was tested. The peptide localizes to the plasma membrane, promotes reactive oxygen species production, and intracellular calcium signaling. Peptide treatment at sub-MIC sensitizes *Cryptococcus* cells to macrophage killing *in vitro*. In aggregate, we developed a peptide-based inhibitor of fungal lipid flippase function, which demonstrates promising drug synergy with existing antifungal drugs that implies great potential for combination therapy.

P-130

Structural Analysis of a Pain Inducing Peptide from the Australian Stinging Tree

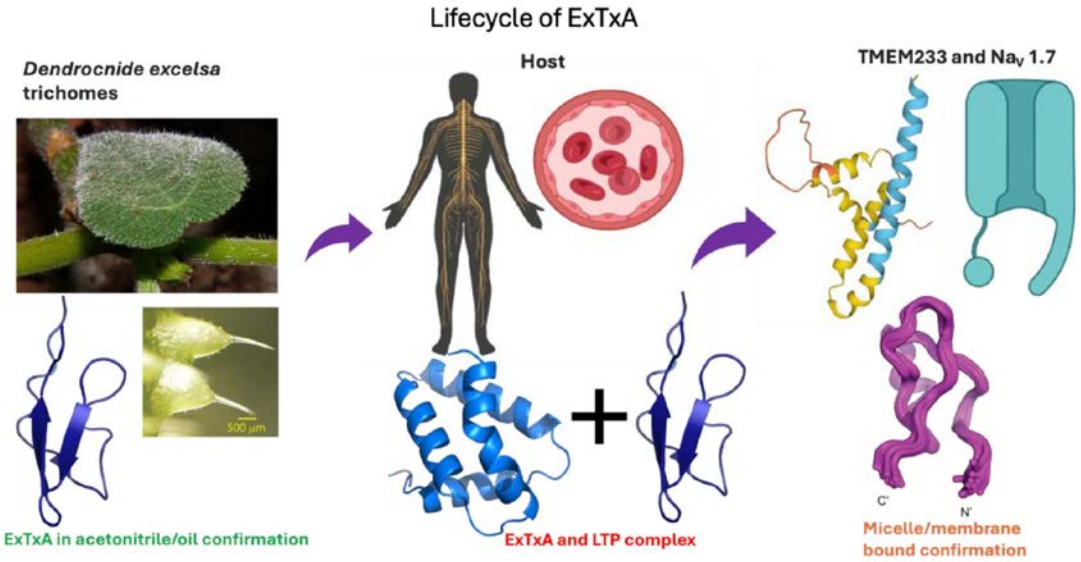
Saipriyaa Purushotham Vasan, Theo Crawford, Yanni Chinn, Jennifer Deuis, Tabea Klasfauseweh, Jennifer Naughton, Yan Zhou, Thomas Durek, Irina Vetter, and Mehdi Mobli

The University of Queensland, Brisbane, Australia

The gympietides are a new class of peptides derived from the venom of the Australian Stinging Tree.[1] Gympietides alter the activity of voltage-gated sodium channels in sensory neurons by inhibiting the inactivation of the channels, leading to a prolonged sensation of pain. Recent studies identified TMEM233, a previously uncharacterised transmembrane protein, as a molecular target of gympietides in modulating NaV activities and potentially a new auxiliary sodium channel protein.[2]

This study aims to investigate the structure of the gympietide ExTxA, *Dendrocnide Excelsa* toxin, using NMR spectroscopy in a membrane model. We solved the 3D structure of isotope-labeled ExTxA in DPC micelles using multidi-

mensional heteronuclear NMR methods. Compared to previous studies in an acetonitrile-water mixture[1], our findings reveal differences in the rearrangement of a hydrophobic amino acid loop in DPC micelles, suggesting a distinct conformation in this environment. Given TMEM233's predicted short extracellular region,[2] this hydrophobic loop is likely crucial for interacting with TMEM233 via the membrane.



Additionally, we explored ExTxA's interactions with trichome-derived components, including a Lipid Transfer Protein, LTP. We demonstrated that ExTxA specifically binds to LTP, providing insights into its stabilization in the host's aqueous environment, potentially facilitating a toxin delivery system.

This study elucidates a comprehensive structural and mechanistic framework for understanding ExTxA's interaction with TMEM233 and other cellular components. Our findings provide insights into the lifecycle of ExTxA once injected into humans, thereby advancing the knowledge of pain modulation by gympietides.

[1]Gilding, E.K., et al., Neurotoxic peptides from the venom of the giant Australian stinging tree. *Sci. Adv.*, **2020**. 6(38): p. eabb8828.
[2]Jami, S., et al., Pain-causing stinging nettle toxins target TMEM233 to modulate NaV1. 7 function. *Nat. Comm.*, **2023**. 14(1): p. 2442.

P-131

Structure-Based Design of Antibody Mimetic Peptides: HIV-1 Antibody PG16

Simon Leukel, Lucas Weißenborn, Manuel Deubler, Heinrich Sticht, and Jutta Eichler

FAU Erlangen-Nuremberg, Erlangen, Germany

The design of synthetic antibody mimetic peptides presenting one or more of the antibody's complementarity determining regions, CDRs, is a promising strategy to preserve antibody binding specificity in smaller molecules.[1] The broadly neutralizing HIV-1 antibody PG16 [2] contacts the HIV-1 envelope gly-coprotein gp120 with its unusually long heavy chain CDR 3, H3, which adopts a characteristic hammerhead-like conformation. Based on the PG16 - gp120 complex structure,[3] we designed and evaluated peptides presenting the H3 of PG16.

Molecular dynamics, MD, simulations indicated that a peptide presenting the H3 of PG16 remains stable in complex with gp120. In order to confirm this notion experimentally, we synthesized different peptide variants of the 33 amino acid long H3, and tested their binding behavior towards gp120. A sul-fotyrosine within the H3 sequence was replaced with phosphotyrosine, which is better stable during the synthesis. In order to stabilize the beta-hairpin conformation of the peptide, disulfide bridges between cysteine residues were in-troduced at various sites of the peptide. While a disulfide bridge connecting the N- and C-terminal ends of the peptide had no effect on the affinity of the peptide to gp120, compared to the linear peptide, introducing a disulfide be-tween positions within the sequence, which stabilized the hammerhead struc-ture, resulted in a five-fold increase of binding strength. MD simulations con-firmed that

a terminal cyclization was not sufficient to stabilize the beta-hair-pin structure of the peptide, whereas introducing a disulfide bridge into the hammerhead region could efficiently stabilize the beta-hairpin structure.[4]

1Haußner, C. et al. Synthetic antibody mimics for the inhibition of protein - ligand interactions. *Curr.Opin.Chem.Biol.*, **2017**, 40, 72-77.
[2] Walker, L. et al., Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target, *Science*, **2009**, 326(5950), 285-289.
[3]Pejchal, R. et al. Structure and function of broadly reactive antibody PG16 reveal an H3 subdomain that mediates potent neutralization of HIV-1. *Proc. Natl. Acad. Sci. USA*, **2010**, 107, 11483–11488.
[4]Deubler, M. et al., Computational Characterization of the Binding Properties of the HIV1-Neutralizing Antibody PG16 and Design of PG16-Derived CDRH3 Peptides, *Biology*, **2023**, 12(6), 824.

P-132

Breaking the Boundaries of a Clinically Approved Cyclic Peptide: Development of Rodent-Active and Long-Duration Compstatin Analogs

Stephanie A. Vogt[1], Alexander J. Lander[1], Roman Aschwanden[1], Christina Lamers[2], Oliver Schwardt[1], Henriette Meyer zu Schwabedissen[1], John D. Lambris[3], Martin Smiesko[1], and Daniel Ricklin[1]

[1]University of Basel, Basel, Switzerland
[2]University of Leipzig, Leipzig, Germany
[3]University of Pennsylvania, Philadelphia, USA

The compstatin family of macrocyclic C3 inhibitors has been continuously optimized and its members are widely used in biomedical research and as complement therapeutics, pegcetacoplan, Apellis. However, the narrow species specificity of compstatins for human/primate C3 prevents their evaluation in many preclinical disease models, limiting translational studies. In addition, there remains room for improvement regarding pharmacokinetic, PK, properties.

By combining experimental and computational models of compstatin interactions with mouse/rat C3, we revealed that the lack of rodent activity is due to a reduced number of target contacts, rather than steric hindrance. We utilized *in-silico* redesign to extend/enhance peptide interactions of compstatin with homologous areas between human and rodent targets. Evaluation of predicted peptides regarding target binding kinetics and *in-vitro* complement inhibition led to the discovery of a promising lead derivative with both affinity and inhibitory activity for rodent C3, while retaining activity for human complement.

First *in-vivo* studies with the new derivatives are being performed in rat models to evaluate efficacy and PK. Additionally, to address potential limitations of PEGylated compstatin used in clinic, we explored alternative approaches to extend plasma half-life. As renal filtration can be reduced by increasing target residence on the abundant plasma protein C3, we developed a new compstatin analog with low picomolar target affinity based on SAR studies. In parallel, we explored lipidation as an alternative strategy to PEGylation to achieve long-acting compstatins. Collectively, this work addresses limitations of clinically approved compstatin peptides and may guide future development efforts.

[1]Ricklin D, et al.: *Nat. Rev. Nephrol.* **2017**; 14: 26-47.
[2]Lamers Ch, et al. *Nat. Commun*. **2022**; 13: 5519.

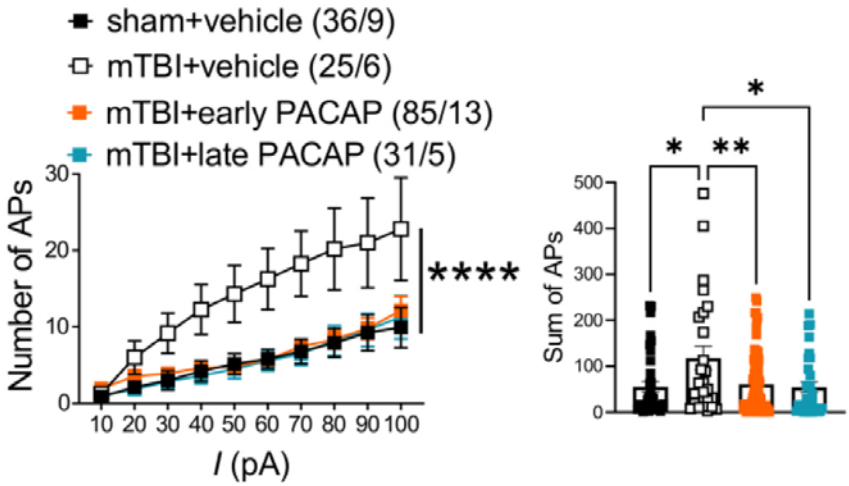
P-133

Synthesis and Neuroprotective Potential of PACAP Glycopeptide Analogues

Troy Smith[1], Fereshteh Nugent[2], and Robin Polt[1]

[1]University of Arizona, Tucson, USA
[2]Uniformed Services University, Bethesda, USA

A global paradigm shift has begun within the pharmaceutical sector; peptides were thought to be poor drug candidates; but drugs such as Semaglutide® providing therapeutic benefits to millions worldwide, this belief has now been rejected. While peptide-based therapeutics can treat a variety of conditions, there has been less development of peptide drugs for central nervous system applications, largely due to challenges crossing the blood-brain barrier, BBB.



PACAP, Pituitary Adenylate Cyclase-Activating Polypeptide, has been recognized for its high potential to treat neurodegeneration– if its half-life and BBB permeability can only be improved. To this end, we have developed glycosides of PACAP with increased BBB penetration and stability. These analogues have also demonstrated neuroprotective effects in Parkinson's, mTBI[1] and stroke models.[2] This talk focuses on the design, synthesis and therapeutic potential for these PACAP glycopeptide analogues with regard to neurodegenerative diseases.

[1]C.R. Apostol, K. Bernard, P. Tanguturi, G. Molnar, M.J. Bartlett, L. Szabo, C. Liu, J.B. Ortiz, M. Saber, K.R. Giordano, T.R.F. Green, J. Melvin, H.W. Morrison, L. Madhavan, R.K. Rowe, J.M. Streicher, M.L. Heien, T. Falk and R. Polt, Design and Synthesis of Brain Penetrant Glycopeptide Analogues of PACAP with Neuroprotective Potential for Traumatic Brain Injury and Parkinsonism, *Front. Drug Discov.* **2022** 1, 818003 (2022). https://doi.org/10.3389/fddsv.2021.818003
[2]K. Bernard, D. Dickson, B.L. Anglin, M.L. Heien, R. Polt, H.W. Morrison and T. Falk PACAP glycosides promote cell outgrowth in vitro and reduce infarct size after stroke in a preclinical model *Neurosci. Lett* **2024** 836, 137883 https://doi.org/10.1016/j.neulet.2024.137883

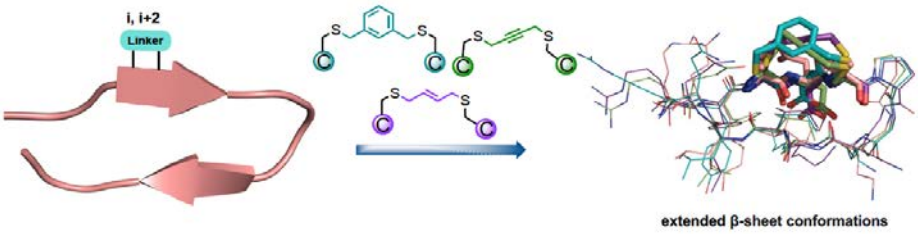
P-135

β-Hairpin Stabilization by Intra-Strand Side Chain Stapling

Abha Dangi, and Juan Del Valle

University of Notre Dame, South Bend, USA

Peptide secondary structures mediate several protein-protein interactions and underlie the high target selectivity of designed probes. Stabilizing these secondary structures through chemical modification is therefore essential for developing peptide-based therapies. β-sheets play an important role in biomolecular recognition. Covalent restriction, side chain alteration, and peptide backbone surrogates are commonly used strategies to stabilize these β-sheet and hairpin folds. The majority of existing techniques rely on an auxiliary strand or turn motif to template haripin conformation.



Here, we describe an intrastrand cysteine stapling approach that dramatically enhances the folded population of model β-hairpin peptides. We synthesized 17 *i*-*i*+2 cysteine-stapled peptides to quantify the effects of distinct linkers on β-hairpin stability. NMR chemical shift deviations were used to calculate the folded fraction in three different model systems. Dicysteine variants featuring *m*-xylyl, butynyl, and *E*-butenyl linkers exhibited superior foldedness compared to parent peptides. Distance restrained NMR solution structures of all three stapled peptides were computed and exhibited native-like conformation and extended backbone torsions at the macrocyclization site. Our results suggest that specific intra-strand cysteine staples are highly effective for stabilizing β-hairpin and β-strand conformation.

P-136

Structure-Guided Design of Lipopeptide Inhibitors of SARS-CoV-2

Ariel Kuhn, and Samuel Gellman

University of Wisconsin, Madison, USA

Despite widespread availability of vaccines against severe acute respiratory syndrome coronavirus type 2, SARS-CoV-2, the emergence of new variants and vaccine hesitancy necessitate the development of alterative antivirals. To meet this end, with the aid of our virology collaborators, we have pioneered an approach for developing peptide-like inhibitors with improved pharmacokinetic properties. SARS-CoV-2 infection is instigated by spike protein-mediated fusion between the viral envelope and the host cell membrane. This mechanism is facilitated by the rearrangement of the spike homotrimer into a stable, 6-helical bundle, 6HB, formed between two heptad repeat domains, HRN and HRC. Peptides derived from SARS-CoV-2 spike HRC can inhibit viral infection, through formation of a stable, hybrid-6HB on the target membrane. However, conventional peptides, composed entirely of α-amino acids, are vulnerable to rapid proteolytic degradation, which severely limits the half-life *in vivo*. Our collaborative team has demonstrated that site-selective incorporation of noncanonical residues, such as D-, β- and ɣ-amino acids, in combination with cholesterol conjugation, can decrease proteolytic sensitivity while maintaining high pan-variant, antiviral potency. Our peptidomimetics have been evaluated in a cell-based assay for S-mediated membrane fusion; this assay is strongly predictive of *in vivo* efficacy. Additionally, we have employed protein crystallography, circular dichroism, and fluorescence polarization to assess structure, stability, and target engagement. These studies have been supplemented with protease assays to rapidly screen the impact of specific modification strategies on stability. Through structure-guided design and multi-pronged assay implementation, these efforts could generate effective, membrane-targeted antivirals against SARS-CoV-2.

P-137

Turn Induction and Nitrogen Chirality in Azapeptides

Chitra Pandiparambil Sadanandhan, Darince Truong, William Lubell

Université de Montréal, Montréal, Canada

The privileged role of turns in peptide molecular recognition has garnered interest in mimicking their backbone orientation and side chain pharmacophores. Introduction of a semicarbazide, as an amino amide surrogate in which Ca is replaced by nitrogen, into a peptide induces turn geometry by stereo-electronic effects due to urea planarity and hydrazine nitrogen lone pair-lone pair repulsion.[1] Furthermore the Na nitrogen of the semicarbazide can in principle adopt planar and pyramidal geometry with pseudo–*R* and -*S* configuration in a dynamic equilibrium.[2,3] Such dynamic chirality can play a key role in modulating binding affinity of azapeptides.[2,3] Our presentation investigates the role of pyramidal nitrogen and turn induction. X-ray crystallographic data of various semicarbazide analogs in peptides will be examined. Furthermore, variable temperature NMR spectroscopic experiments have been used to observe coalescence and determine nitrogen inversion barriers. Relationships between semicarbazide structure, nitrogen pyramidalization and turn conformation have been elucidated with potential for guiding the design of peptide mimics with defined geometry.

[1]Proulx, C.; Sabatino, D.; Hopewell, R.; Spiegel, J.; García Ramos, Y.; Lubell, W.D. Azapeptides and their therapeutic potential. *Future Med. Chem*. **2011**, 3, 1139-1164.
[2]Danelius, E.; Ohm, R.G.; Ahsanullah; Mulumba, M.; Ong, H.; Chemtob, S.; Erdelyi, M.; Lubell, W.D. Dynamic Chirality in the Mechanism of Action of Allosteric CD36 Modulators of Macrophage-Driven Inflammation. *J. Med. Chem*. **2019**, 62, 11071–11079.
[3]Bouayad-Gervais, S.H.; Lubell, W.D. Examination of the potential for adaptive chirality of the nitrogen chiral center in aza-aspartame. *Molecules* **2013**, 18, 14739-14746.

P-138

Azapeptide Atropisomers From Late-Stage N-Alkylations

Molly Helton, Chris Howard, Keisy Prieto, Katelyn Cartrette, Maxwell Bowles, and Caroline Proulx

North Carolina State University, Raleigh, USA

Aza-amino acids are unnatural amino acids that substitute the α-carbon with a nitrogen. This substitution can reinforce secondary structures such as turns and PPII helices, increase stability towards proteases, and alter the hydrogen bonding properties of neighboring NH.[1] However, there are few examples of azapeptoids and *N*1,*N*2-dialkylated azapeptides, despite their interesting conformational properties. Specifically, substitution of azapeptides at the N1 nitrogen has been shown to induce atropisomerism with hindered rotation around the N-N bond.[2] This property could make them interesting candidates for future medicinal applications.[3] Our previous work demonstrated that chemoselective alkylations of internal aza-glycine residues were possible, providing rapid access to azapeptoid and *N*1,*N*2-disubstituted azapeptide derivatives of Leu-Enkephalin.[4] Here, we investigate the scope of amino acid compatibility with our late-stage alkylation procedure using a series of tripeptides where the terminal amino acid is modified. We also study the atropisomeric properties of azapeptoids and *N*1,*N*2-disubstituted azapeptides using variable temperature NMR and dynamic HPLC.

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P-139

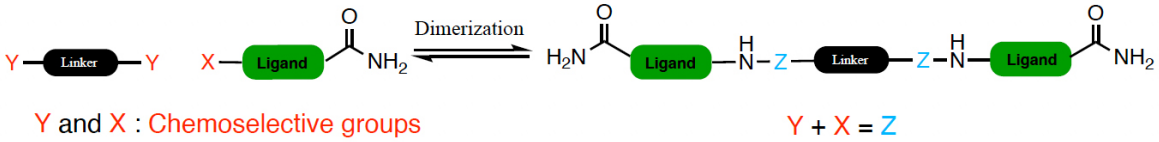
Leveraging Reversible Dimerization to Optimize Peptide and Peptoid Binding and Efficacy

Elizabeth Bredice, Madeline Swanson, and Skander Abboud

University of North Carolina Wilmington, Wilmington, USA

Dimerization of small peptides and peptoids offers significant advantages in drug design, primarily by enhancing binding affinity and specificity for target proteins. Bivalent ligands formed through dimerization can simultaneously engage two binding sites on a target, resulting in improved efficacy, better selectivity, and reduced side effects. While traditional dimerization methods are effective, they often rely on irreversible reactions to link monomers, which can limit bioavailability and pharmacokinetic properties due to the increased molecular weight.

Reversible dimerization, in contrast, utilizes bioorthogonal reactions to enable monomeric units to assemble dynamically into dimers under specific conditions.[1] This approach ensures that the ligands maintain their pharmacokinetic properties, such as solubility and bioavailability, while still achieving high binding affinity and specificity upon reaching the target.



To exploit these benefits, we have developed a novel strategy for generating dimers using different reversible bioorthogonal reactions. Our approach involves a synthetic design wherein a single monomer is modified with a chemoselective functional group and paired with a difunctionalized linker that serves as the dimerization handle. This method reduces synthetic complexity and enhances efficiency.

We successfully applied this strategy to a streptavidin, SA-binding peptide, demonstrating the feasibility and effectiveness of our approach. Additionally, we extended this method to GU40C, a peptoid known to bind vascular endothelial growth factor receptor 2, VEGFR.[2] Preliminary results show that the reversible dimerization of GU40C enhances its binding affinity and specificity. We will also present findings from the application of this strategy to an optimized version of GU40C obtained by a structure-activity relationships study, highlighting further improvements in binding and efficacy. These results underscore the potential of our reversible dimerization strategy for advancing peptide and peptoid-based therapeutics.

[1]S. Chatterjee, E. V. Anslyn, A. Bandyopadhyay, *Chem. Sci*. **2021**, 12, 1585–1599.
[2]D. G. Udugamasooriya, S. P. Dineen, R. A. Brekken, T. Kodadek, *J. Am. Chem. Soc*. **2008**, 130, 5744–5752.

P-140

Crosslinker Stabilization of Peptide Polyproline II Helices

Emma Elsdon, and Nicholas Sawyer

Fordham University, Bronx, USA

Upregulated protein-protein interactions, PPIs, are associated with many diseases, including cancer and neurodegenerative diseases. PPIs are strongly dependent on the three-dimensional structure of the proteins involved. Consequently, many research groups have explored strategies to inhibit PPIs using protein mimics, including peptides whose three-dimensional structures resemble the interacting proteins. One particular three-dimensional structure, the polyproline II helix, is found in many PPIs. Previous studies have recently identified a new chemical crosslinking strategy to create stable peptide polyproline II helices to block these interactions. This strategy was studied exclusively in the context of the EphB2-specific peptide inhibitor SNEW. Based on these studies, we sought to investigate this crosslinking approach in additional peptides to generalize the results for targeting other PPIs.

In order to evaluate the generality of the crosslinking approach, we designed a crosslinkable model peptide, GCPRCP-GY, based on the model peptide GPPRPPGY, Brown & Zondlo, 2012. We synthesized ten variants with different cross-linkers and conducted one-dimensional and two-dimensional NMR spectroscopy to study their three-dimensional structure. Using the central arginine residue as a folding indicator, we found that the 2,7-dimethylnaphthyl crosslinker produced a peptide with the closest match to the characteristic φ dihedral angle of -75° for polyproline II helices. This result is consistent with previous studies of this crosslinker, suggesting that it is generalizable to stabilize peptide polyproline II helices for the development of PPI inhibitors.

P-141

α/Sulfonyl-ɣ-AApeptide Foldamers Mitigate Alzheimer's Disease Pathology by Stabilizing Transient Helical Domains in Aβ

Heng Liu[1], Donghui Zhu[2], Jianfeng Cai[1]

[1]University of South Florida, Tampa, USA
[2]University of Stony Brook, NY, USA

Alzheimer's disease, AD, is characterized by the pathological accumulation of amyloid-β, Aβ, with Aβ oligomers playing a central role in neurotoxicity. These Aβ oligomers disrupt calcium homeostasis, impair neurotransmitter signaling, damage mitochondrial function, and activate neuroinflammation responses, exacerbating neuronal injury. Aβ aggregations are widely recognized to result in mitochondrial impairment, synaptic dysfunction, chronic neuroinflammation, amyloid plaque formation, and neuronal death, culminating in cognitive decline. Thus, identifying potent inhibitors of Aβ aggregation holds significant promise for mitigating AD progression.

Here, we identified a peptidomimetic lead compound, named foldamer M4, as a potent antagonist of Aβ oligomerization. Biophysical assays demonstrated that M4 effectively inhibits Aβ aggregation and disrupts preformed aggregate species through high binding affinity to Aβ. By interacting with the central domain of Aβ, M4 stabilizes its partial helix domain, thereby preventing the formation of cross β-sheet-rich structures and reducing neurotoxicity. In mouse primary neurons, M4 preserves synaptic integrity by restoring presynaptic vesicle protein synaptophysin and postsynaptic scaffold protein PSD-95. M4 also maintains cellular homeostasis by reducing ROS level and preserving mitochondrial membrane potential. Furthermore, M4 attenuates microglial-astrocytic activation, reduces inflammation, and mitigates Aβ pathology, preserving cognitive function in 5xFAD Mice. Given the central role of Aβ aggregation in AD pathology, M4 presents a promising therapeutic strategy to address the multifaceted aspects of Alzheimer's disease, paving the way for transformative interventions that may alter the trajectory of this devastating condition.

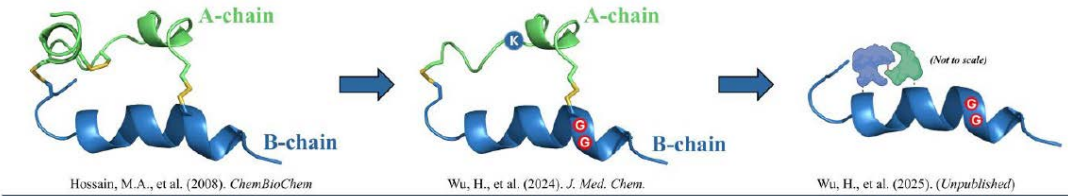
P-142

Developing Simplified Insulin-like Peptide 5, INSL5, Analogues as Colon Motility Regulators

Hongkang Wu, Ruslan Pustovit, John Furness, Ross Bathgate, and Akhter Hossain

The Florey, The University of Melbourne, Melbourne, Australia

Insulin-like peptide 5, INSL5, is a hormone produced exclusively by colonic L-cells of the colon and acts as the natural ligand for the relaxin family peptide receptor 4, RXFP4. This receptor-ligand system has been implicated in the regulation of gut motility, particularly in the control of colorectal propulsion,[1] making it a promising target for treating colon motility disorders such as constipation. However, the clinical application of INSL5 is limited by synthesis challenges,[2] resulting in a low yield, 0.8%, and thus highlighting the need for more accessible yet potent analogues. To address this, our laboratory recently engineered an INSL5 analogue, A13:B7-24-GG, see figure 1, featuring a simplified two-chain, two-disulfide scaffold with 32 amino acids, as opposed to the 45 amino acids found in native INSL5, two-chain, three-disulfide, improving the synthesis yield by 19.5-fold.[3] A13:B7-24-GG also demonstrates approximately four-fold higher potency than native INSL5, establishing it as the lead two-chain, two-disulfide analogue of INSL5 to date.



Native INSL5	A13:B7-24-GG	B-chain-specific analogue
45 residues	32 residues	18 residues
Two-chain	Two-chain	Single-chain
K_i: 3.29 nM	K_i: 2.29 nM	K_i: 1.32 nM
EC_{50}: 4.57 nM	EC_{50}: 1.17 nM	EC_{50}: 8.91 nM
Complex scaffold to modify	Complex scaffold to modify	Easy scaffold to modify

Figure 1. Development of simplified analogues of INSL5

Based on prior structure-activity relationship studies and recently available INSL5/RXFP4 Cryo-Electron Microscopy structure,[4] we identified the B-chain of INSL5 to be the sole determinant for RXFP4 binding and activation. Therefore, we hypothesised that bioactive INSL5 analogues could be designed as B-chain-specific compounds. Here, using the B-chain of A13:B7-24-GG as a template, we present new designs and chemical approaches to generate a series of B-chain-specific INSL5 analogues. By using our recently optimized NanoBiT complementation binding assay,[5] we found that one of these analogues, see figure 1, demonstrated high RXFP4 affinity, ~1 nM. Additionally, the forskolin-induced cAMP assay confirmed its potency at ~9 nM, establishing this new compound as a novel lead for further *in vivo* studies and a promising therapeutic candidate for constipation.

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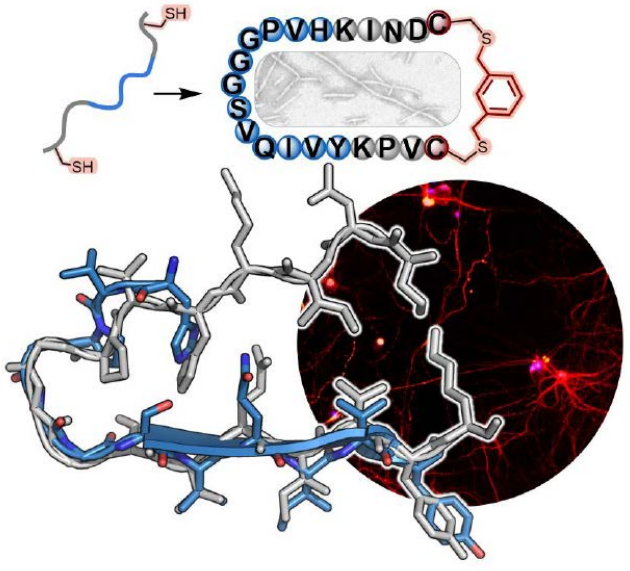
P-143

β-Arch Peptide Macrocycles as Structural and Functional Mimics of Pathological Tau

Isaac Angera[1], Xueyong Xu[2], Benjamin Rajewski[1], Grace Hallinan[3], Xiaoqu Zhang[2], Bernardino Ghetti[3], Ruben Vidal[3], Wen Jiang[2], and Juan Del Valle[1]

[1]University of Notre Dame, Notre Dame, USA
[2]Purdue University, West Lafayette, USA
[3]Indiana University School of Medicine, Indianapolis, USA

Tauopathies are a class of neurodegenerative disorders that include Alzheimer's disease, corticobasal degeneration, chronic traumatic encephalopathy, and many others. A predominant feature of these diseases is tau protein deposits in the brain. Tau is intrinsically disordered and involved in microtubule dynamics but can transition into pathological amyloid structure. Misfolded tau can template or "seed" the aggregation of naïve tau, leading to the prion-like transcellular spread of tau filaments. Tau protomers within filaments exhibit cross-β amyloid structure, but distinct conformations often correlate with specific diseases. An understanding of how tau misfolded conformation impacts seeding activity remains elusive. Identification of the minimal epitopes required for transcellular propagation represents a key step toward more relevant models of disease progression.



Here, we present a diversity-oriented peptide macrocyclization approach toward seed-competent miniature tau, or "mini-tau",

proteomimetics derived from 4R tauopathic folds. Mini-tau macrocycles exhibit several amyloid characteristics including thioflavin T binding, formation of filamentous species observed by transmission electron microscopy, and canonical β-sheet rich circular dichroic spectra. Mini-tau macrocycles induce endogenous tau inclusions in engineered biosensor cells and primary hippocampal neurons. Structural elucidation of potent seed competent mini-tau filaments by cryo-electron microscopy reveals close conformational mimicry of pathological tau misfolds. These studies aid in delineating the structural epitopes necessary for prion-like tau seeding and pave the way for the development of tauopathy specific antibodies.

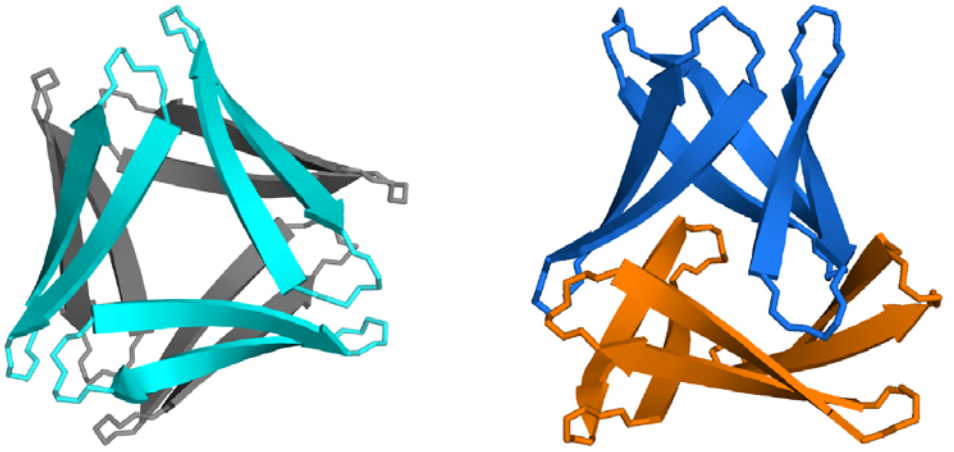
P-144

A β-Hairpin Peptide Derived from Aβ Forms Different Oligomers in the Crystal State and in Aqueous Solution

Jason Zhu[1], Adam Kreutzer[1], Zhiwei Liu[2], Xingyue Li[1], Sabrina Richter[1], Vojislava Pophristic[2], and James Nowick[1]

[1]University of California, Irvine, Irvine, USA
[2]Rowan University, Glassboro, USA

Understanding of the structures of soluble oligomers formed by the amyloid-β, Aβ peptide represents a fundamental challenge in understanding Alzheimer's disease, AD. Soluble Aβ oligomers have emerged as neurotoxic species involved in AD progression and some Aβ oligomers are thought to be composed of β-hairpins.



symmetric hexamer
X-ray crystallographic structure

asymmetric hexamer
solution-phase NMR model

In this work, we report the X-ray crystallographic and solution-phase assembly of a macrocyclic β-hairpin peptide that mimics a β-hairpin formed by $A\beta_{16-36}$. In the crystal lattice, the peptide assembles into a symmetric hexamer composed of two identical triangular trimers. In aqueous solution, the peptide assembles to form an asymmetric hexamer. ^{1}H NMR, TOCSY, and $^{1}H,^{15}N$ HSQC experiments establish that the asymmetric hexamer contains two different species, **A** and **B**. ^{15}N-edited NOESY reveals that species **A** is a cylindrin-like trimer and species **B** is a triangular trimer that collectively constitute the asymmetric hexamer. Diffusion-ordered NMR spectroscopy, DOSY suggests that two asymmetric hexamers further assemble to form a dodecamer. NMR-guided molecular mechanics and molecular dynamics studies provide a model for the asymmetric hexamer and suggest how two asymmetric hexamers can form a dodecamer. Solution-phase NMR studies of analogues show that intermolecular hydrogen bonding and the formation of a hydrophobic core help stabilize the asymmetric hexamer.

These NMR and crystallographic studies illustrate how an Aβ β-hairpin peptide can assemble to form different well-defined oligomers in the crystal state and in aqueous solution, providing a deeper understanding of the heterogeneity of Aβ oligomers and new structural models of Aβ oligomers composed of Aβ β-hairpins.

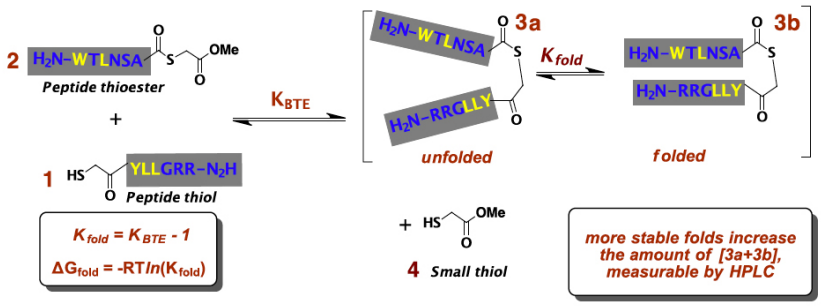
P-145

Exploring Folding Thermodynamics in Irregularly Structured Peptides

Jordyn Pieper[1], Stuart Parnham[2], and Michael Giuliano[1]

[1]College of Charleston, Charleston, USA
[2]University of North Carolina, School of Medicine, Chapel Hill, USA

Small signaling peptides, such as neuropeptides, play myriad critical roles in human physiology. Despite hundreds of known sequences, there is relatively little description of neuropeptides' intrinsic structures found in the Protein Data Bank. In this knowledge gap we seek to better understand the fundamental, intrinsic conformations of these vital components of the human nervous system and the folding of small peptides more broadly. We previously observed that a small fragment of the neuropeptide galanin adopts an "irregular secondary structure," seemingly trapped between wholly discorded and folded states. While this peptide, hGal(2-12)KK,[1] adopts a rigid backbone and hydrophobic clustering of sidechains, it lacks intrachain hydrogen bonding common to canonical secondary structures.



Schematic of a Backbone Thioester Exchange System derived from hGal(2-12)KK

We suspect these structures are common in many other small signaling peptides, and are actively exploring both their generality and, in this work, their stability. Backbone Thioester Exchange, BTE, has previously been used to study canonical peptide folds such as helix bundles, β-hairpins, as well as hybrid structures of peptidomimetics but has never been applied to study irregular secondary structures.[2,3] Herein we describe our efforts to adapt this method to a modified thiodepsipeptide analog of hGal(2-12)KK in an effort to measure the driving force provided by hydrophobic amino acids toward irregular secondary structures which, we hypothesize, act as models for the early collapsed stages of protein folding.

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P-146

N-Aryl Peptide Ligations in Organic Solvent: Preventing Loss of Amino Acid Side Chains During the Oxidative Coupling

Karlee McKinney, Ellen Warner, Hailey Young, and Caroline Proulx

NC State University, Raleigh, USA

Our research program harnesses the reactivity of electron-rich Cα-substituted *N*-aryl peptides in chemoselective ligations with aminooxy and hydrazide substrates, affording highly functionalized ketoxime or kethydrazone peptides under mild conditions.[1-3] Previously, the *N*-(*p*-Me_2Ph)-peptide substrates were accessed either via on-resin submonomer peptoid synthesis procedures using bromoacetic acid derivatives and aniline nucleophiles, or by Pd-catalyzed *N*-arylation of amino acid *tert*-butyl esters.[1]

Here, to further expand side chain diversity at the site of ligation, we report conditions for Pd-catalyzed *N*-arylation of

amino acid methyl esters, where the side chains of amino acids are orthogonally protected with acid-labile protecting groups, for example, Tyr(O*t*Bu)-OMe, Trp(Boc)-OMe, Ser(O*t*Bu)-OMe, et cetera. After hydrolysis of the ester, the *N*-aryl amino acids were coupled to resin bound peptides using solid phase peptide synthesis, SPPS, and subsequently cleaved from the solid support. During the oxime ligation reactions of the *N*-aryl peptides, a loss of side chain at the site of ligation was observed for Trp, Ser, and Tyr. To circumvent this issue, we developed conditions for the oxime and hydrazone ligation of side chain protected *N*-aryl peptides in organic solvent. allowing newfound side chain diversity at the site of ligation.

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P-147

Chloroalkene-Type Dipeptide Isosteres Applicated for Amyloid-Beta Aggregation Inhibitors

Kenichi Yamamoto, Takuya Kobayakawa, Kohei Tsuji, and Hirokazu Tamamura

Department of Medicinal Chemistry, Laboratory for Biomaterials and Bioengineering,Institute of Integrated Research, Institute of Science Tokyo, Tokyo, Japan

The primary cause of Alzheimer's disease is the accumulation of amyloid beta, Abeta, peptide aggregates in the brain. Various Abeta aggregation inhibitory peptides related to fragments of Abeta such as the KLVFF peptide have been developed. Peptides as natural components of bodies with specific physiological functions might offer potentials as relatively safe and highly potent therapeutic agents. However, their low water solubility and high aggregability might pose significant challenges to their use as drugs.

In this study, we designed and synthesized chloroalkene-type dipeptide isosteres, CADIs, in which the peptide bond is replaced by a chloroalkene unit as chemical equivalents of peptide bonds to address these limitations. Diastereoselective allylic alkylation of a (*Z*)-gamma,gammadichloroalpha,beta-unsaturated ester with organocopperzinc reagents brought the successful synthesis of CADIs.[1,2] The CADIs were then introduced into the KLVFF peptide by solid-phase peptide synthesis, followed by the resin cleavage, cyclization, and deprotection to obtain the cyclic peptidomimetics.

Furthermore, the synthesized CADIs were incorporated into the cyclic peptide *cyclo*[KLVFF], and the inhibitory activity of the resulting peptidomimetics was evaluated.[3,4] As a result, a CADI-containing *cyclo*[KLVFF] peptidomimetic was proven to be a superior inhibitor of amyloid-beta aggregation compared to the parent peptide.

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P-148

Selective Inhibition of UGDH with a Series of Novel Peptide and Peptoid Analogs

Kristiana Witte, Skylar Harrelson, Carolynn Davern, Emily Allego, Caroline Proulx, JosephBarycki, and Melanie Simpson

North Carolina State University, Raleigh, USA

Recurrence of therapy-resistant prostate cancer following androgen deprivation therapy currently has few treatment options and a high mortality rate.[1] Dysregulation of UDP-glucose dehydrogenase, UGDH, and its downstream pathways has been implicated in these cases of resistance, and knockdown of UGDH has been shown to resensitize these cancer cells to treatment.[2] Here, we present our initial findings following structure-activity relationship, SAR, studies with several peptide inhibitors of UGDH previously identified through phage display. These studies were initiated with alanine scanning to gauge the importance of each residue. The following phases of these studies employ

peptoids, *N*-substituted glycine oligomers, which have been shown to improve cell permeability and proteolytic stability in comparison to peptides, while boasting a simple synthetic route with a wide variety of incorporable side chains.[3]

These advantages have made peptoids an interesting prospect as drug candidates, however, the tertiary amide, absence of a chiral center at the α-carbon, and lack of backbone hydrogen bond donor, NH, can contribute to increased flexibility of the backbone, incurring an entropic cost which may impede peptide binding. Nevertheless, a number of conformation inducing peptoids have been identified, which promote rigidity of the peptide, overcoming this issue of flexibility. For this we focus primarily on *trans*-inducing *N*-imino- and *N*-alkylamino glycine residues, demonstrating their incorporation using the submonomer method with hydrazones nucleophiles during solid phase peptide/peptoid synthesis.[4]

We also explore the incorporation of *N*-methyl residues, as well as *N*-substituted alanine, NSA, another transinducing peptoid-type monomer.[5] In a recent addition to these studies, we explore the effects of cysteine crosslinking in UGDH inhibitors using a variety of biselectrophilic crosslinking agents. The synthesis and biological evaluation of conformationally constrained analogs is ongoing with the objective of studying the inhibitory potentials of each to establish structure-function relationships.

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P-149

Impact of Backbone-Oxidized Proline Surrogates on the Stability of Complex Polyproline II Folds

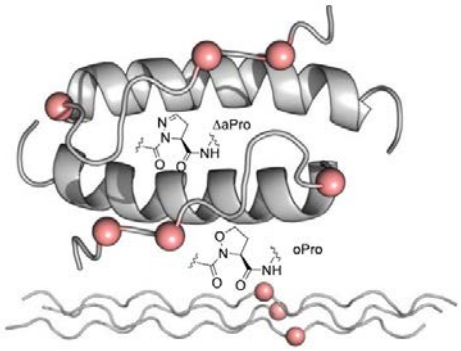
Madison Wright[1], Taylor Gerrein[1], Benjamin Rajewski[1], Natalia Cano-Sampaio[1], Zhiyi Xu[2], Lorna Smith[2], Seth Horne[3], and Juan Del Valle[1]

[1]University of Notre Dame, Notre Dame, USA
[2]University of Oxford, Oxford, UnitedKingdom
[3]University of Pittsburg, Pittsburg, USA

Proline is unique among the canonical amino acids due to its cyclic backbone, φ, constraint and its increased *cis* amide rotamer, ω, propensity, making it an attractive target for protein modification. Surrogates with perturbed backbone torsional preferences are particularly useful as probes of protein folding and stability. Here, we describe the synthesis and conformational analysis of three proline analogues, dehydro-δ-azaproline, ΔaPro, N-methyl-δ-azaproline, ((Me)aPro), and δ-oxaproline, oPro. These backbone oxidized surrogates were incorporated into tertiary and quaternary structures containing polyproline II, PPII, helices to assess their impact on folding.

ΔaPro was shown to stabilize the avian pancreatic polypeptide, aPP, when incorporated at select solvent-exposed positions within the PPII helix. ΔaPro substitution of a conserved Pro in the switch region also resulted in a variant with enhanced thermal stability. Structure determination by NMR and molecular dynamics indicated that ΔaPro-substituted aPP variants maintained the wildtype aPP tertiary fold. Additionally, ΔaPro residues in the PPII helix exhibited increases in the population of PPII backbone conformation relative to wild-type residues. ΔaPro, (Me)aPro, and oPro were also incorporated into a collagen mimetic peptide to ascertain their effects on triple helix assembly and stability.

Thermal denaturation revealed that while ΔaPro and (Me)aPro destabilized collagen, an oPro-containing variant exhibited thermal stability equivalent to that of the parent sequence, despite the higher *trans* amide propensities of (Me)aPro and ΔaPro compared to oPro.



An acyclic alkoxylated analogue of oPro was also shown to destabilize the collagen triple helix, indicating that the cyclic constraint helps maintain favorable φ and ψ backbone torsions. Kinetic studies revealed that incorporation of oPro increased the rate of triple helical folding, likely due to the electron-withdrawing δ oxygen. These results establish δ-heteroatom-substituted prolines as useful unnatural building blocks for the design of stable and fast-folding proteomimetics.

P-150

Structural Characterization of Disulfi de-Linked p53-Derived Peptide Dimers

Magdalena DiGiorno[1], Nisansala Vithanage[2], Clara Victorio[1], Dale Kreitler[3], Victor Outlaw[2], and Nicholas Sawyer[1]

[1]Fordham University, Bronx, USA
[2]University of Missouri, Columbia, USA
[3]Brookhaven National Laboratory, Upton, USA

Disulfide bonds provide a convenient method for chemoselective alteration of peptide and protein structure and function.We previously reported that mild oxidation of a p53-derived bisthiol peptide, CTFANLWRLLAQNC, under dilute non-denaturing conditions led to unexpected disulfide-linked dimers as the exclusive product. The dimers were antiparallel,significantly α-helical, resistant to protease degradation, and easily reduced back to the original bisthiol peptide. Here we examine the intrinsic factors influencing peptide dimerization using a combination of amino acid substitution, circular dichroism, CD, spectroscopy, and X-ray crystallography. CD analysis of peptide variants suggests critical roles for Leu6 and Leu10 in the formation of stable disulfide-linked dimers. The 1.0 Å resolution crystal structure of the peptide dimer supports these data, revealing a leucine-rich LxxLL dimer interface with canonical knobs-into-holes packing.

Two levels of higher-order oligomerization are also observed in the crystal: an antiparallel "dimer of dimers" mediated by Phe3 and Trp7 residues in the asymmetric unit and a tetramer of dimers mediated by Trp7 and Leu10. In CD spectra of Trp-containing peptide variants, minima at 227 nm provide evidence for the dimer of dimers in dilute aqueous solution.Importantly, and in contrast to the original dimer model, the canonical leucine-rich core and robust dimerization of most peptide variants suggests a tunable molecular architecture to target various proteins and evaluate how folding and oligomerization impact various properties, such as cell permeability.

P-151

Beyond Disulfide Bridge Mimetics: Extending the Application of Triazoles Toward Multifunctional Cyclization Moieties

Oscar Shepperson, Michael Malone, and Andrew Jamieson

University of Glasgow, Scotland, United Kingdom

Peptides continue to gain momentum in the pharmaceutical world, bridging the gap between small molecules and larger biologics like antibodies.[1] Yet, despite their promise, peptide-based drugs are often hampered by poor in vivo stability and limited bioavailability. One powerful strategy to address these limitations is peptide macrocyclization, but existing methods frequently interfere

with key peptide-protein interactions, particularly when modifying side chains or the N-terminus.[2] In response to these challenges, we have developed a versatile and efficient synthetic approach that enables precise, single-site functionalisation of peptides.

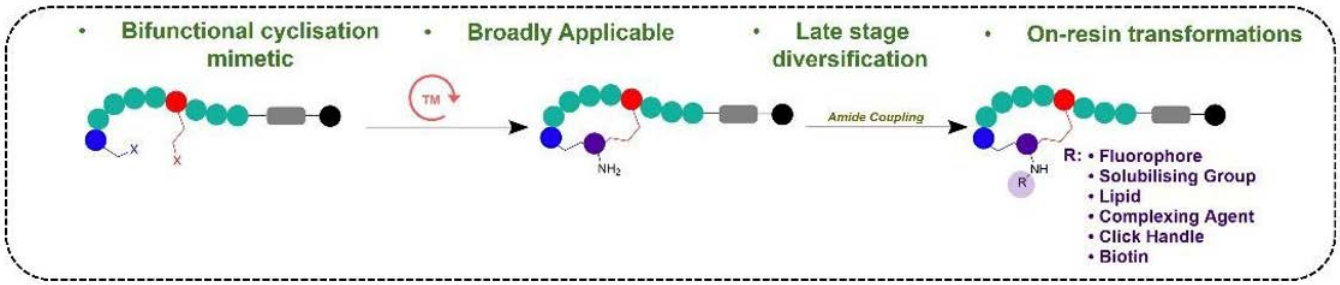


Figure 1. Possible chemical developments employing our novel technology following on-resin peptide cyclisation

This presentation introduces a novel on-resin cyclisation motif that not only mimics the structural and stabilizing features of native disulfide bridges but also incorporates a reactive handle for late-stage transformations.[3] This innovation facilitates rapid and modular peptide diversification while maintaining structural integrity, crucial for therapeutic applications. Our work outlines the design, optimisation, and broad applicability of this chemistry for solid-phase peptide synthesis, SPPS, paving the way for high-throughput generation of modified peptides with enhanced properties and functionality.[4]

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P-152

Impact of Backbone N-Amination on the Stability of Parallel β-Sheets

Syrah Starnes, and Juan Del Valle

University of Notre Dame, Notre Dame, USA

Extensive parallel β-strand associations are the fundamental structural motif of amyloid filaments that underlie several neurodegenerative diseases. Minimalist synthetic approaches to stabilize parallel sheet conformations can inform the design of β-strand mimics and selective ligands of pathological amyloids. Backbone amide substitution can be used to enforce canonical protein secondary structure without sacrificing side chain content. We previously demonstrated that linear N-aminated peptides, NAPs, readily adopt stable antiparallel β-sheet folds.

Here, we investigate the impact of backbone amide N-amination on the folding of a parallel β-hairpin peptide and the stability of a miniprotein that exhibits a βαβ tertiary fold. We synthesized a series of α-hydrazino acid monomers suitable for incorporation into NAP sequences by conventional SPPS. Solution NMR structures of the parallel β-hair-

pins indicate that N-amination of selected residues in the ordered core enhance the folded population. In the βαβ tertiary fold of the DS119 miniprotein, CD spectroscopy showed that N-amination of residues with side chains in the hydrophobic core, aVal31 and aPhe33, resulted in destabilization of tertiary structure while N-amination of solvent-exposed residues, aTrp9, retained the thermal stability of the wild-type sequence. Our study is the first to investigate the impact of α-hydrazino acid substitution on the stability of parallel β-sheet models and should help inform the design of novel backbone-modified proteomimetics.

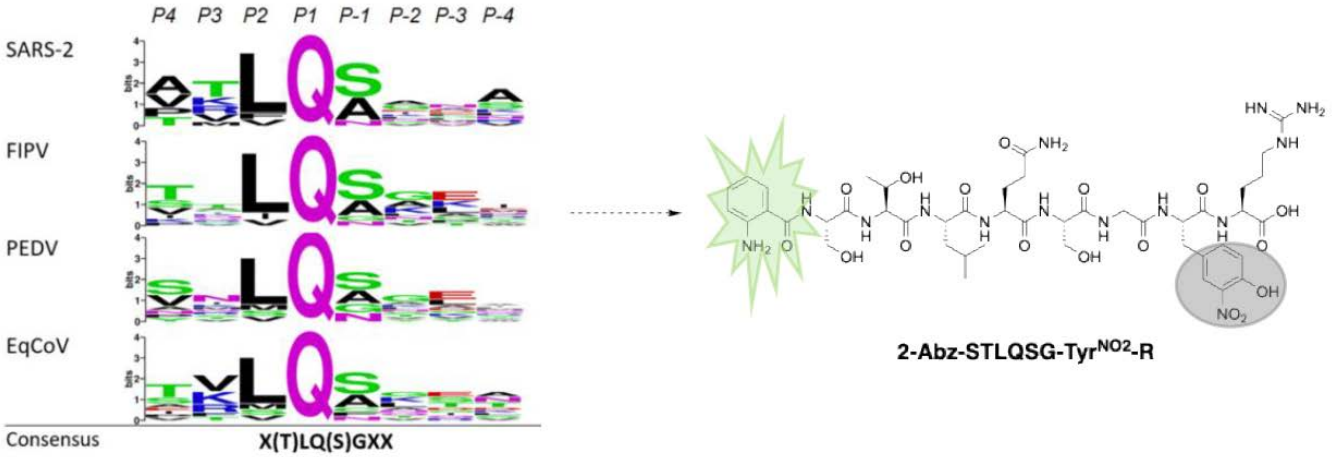
P-153

Designing FRET Substrates and Peptidomimetics for the Development of Broad-Spectrum Inhibitors of Coronaviral MainProteases

Tayla Van Oers, Conrad Fischer, Marco van Belkum, and John Vederas

University of Alberta, Edmonton, Canada

Coronaviruses can infect humans and animals, typically causing respiratory diseases with a high mortality rate. For example, feline infectious peritonitis virus, FIPV, causes severe respiratory disease in cats, and porcine epidemic diarrhea virus, PEDV, gives rise to diarrhetic disease in pigs. Both diseases are nearly 100% fatal for young kittens and piglets once they exhibit symptoms, and no approved treatments are available. Coronaviruses are reliant upon the activity of a cysteine main protease for processing of viral polypeptides, which means inhibition of this protease is a desirable target for preventing replication.[1]



Because the structure and function of the main protease are highly conserved over this family of viruses, we aimed to develop an inhibitor that is effective for several different coronaviruses, including selected animal viruses. In order to effectively standardize the testing of the inhibitors over the panel of coronaviruses, we synthesized and evaluated a variety of Fluorescence Resonance Energy Transfer, FRET, substrates that could be used for all of the coronaviruses in our study. The FRET substrate needs to be recognized and cleaved by all investigated main proteases, so its sequence is based on the consensus cleavage site sequence of the investigated coronaviruses. The FRET substrates in this study ranged from 9 to 11 amino acids in length and contained a fluorophore and quencher moiety. We successfully identified a short FRET substrate that is highly effective for the panel of studied coronaviruses and developed a standardized assay to test our inhibitors against each of the coronaviral main proteases.[2] Using this method, we have designed and identified peptidomimetic inhibitors, that act as lead structures for the development of anti-coronaviral therapeutics.

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P-154

Development of Novel BRD4-PROTACs Possessing HIV-1 Viral Protein R-derived Peptides as E3 Ligase Ligands

Kohei Tsuji[1], Xueyuan Huang[1], Maho Miyamoto[2], Hidetomo Yokoo[3], Takuya Kobayakawa[1], Yosuke Demizu[3], and Hirokazu Tamamura[1]

[1]Institute of Science Tokyo, Tokyo, Japan
[2]Yokohama City University, Kanagawa, Japan
[3]National Institute of Health Sciences, Kanagawa, Japan

Proteolysis-targeting chimeras, PROTACs, recruit a protein of interest, POI to an E3 ligase and facilitate ubiquitination and degradation of the POI via ubiquitin-proteasome system, UPS. Despite the diversity of E3 ligases, current PROTACs mainly utilize CRBN, VHL, IAP, or MDM2 as the E3 ligases. Since E3 ligase expression and activity levels are different among target cells, a novel E3 ligase and its ligands for PROTACs are required. DDB1 and Cullin4 Associated Factor 1, DCAF1, is a substrate-binding protein in the Cullin4-E3 ligase complex.

Recently, small molecule DCAF1 ligands and DCAF1-based PROTACs were reported to successfully degrade the POIs in the CRBN-based PROTAC-resistant cells. An HIV-1 accessory protein, Viral Protein R, Vpr, also interacts with DCAF1 and degrade host cell enzymes via the host cell UPS. Therefore, we envision that Vpr or its fragments can serve as novel E3 ligase ligands for PROTAC development. Bromodomain protein 4, BRD4 regulates transcription by recognizing acetylated lysine residues on histones. Its dysfunction is related to cancer, inflammatory diseases, and viral infections. Accordingly, we attempted to develop novel BRD4-targeting PROTACs, BRD4-PROTACs.

Herein, we will present the design, synthesis, and biological evaluation of the BRD4-PROTACs, which are used Vpr-derived peptides as E3 ligase ligands.

P-155

Rational Design of Sulpho-ɣ-AA Peptides as cMyb Mimics for Disruption of cMyb-KIX Interaction

Yu Yu Win, Jianfeng Cai

University of South Florida, Tampa, USA

cMyb proteins is a transcription factor which plays an important role for hematopoietic cells proliferation. It binds to KIX domain of the transcriptional coactivator CREB-binding protein. Aberrant expression of cMyb causes leukemia. Several research focus on cMyb-KIX disruption to be used as antitumor agents. We designed sulfonyl-ɣ-AA peptide using structure-based rational design to inhibit cMyb/KIX protein protein interaction. Several analogs and wild type cMyb, cMyb 32, were synthesized.

In this study, the designed peptides show favorable binding affinity comparable to wild type. Furthermore, confocal microscopy confirms the better cell permeability of our mimics. To understand more on how peptide mimics binding to KIX relative to cMyb 32, NMR titration experiments were performed using 15N labeled KIX protein. cMyb 32 and mimics cause shifts in the KIX binding sites. Our results suggested that sulfonoyl-ɣ-AA peptides have great potential to be developed as antileukemia agent.

P-156

Advancing Proteomimetic Design: Recreating and Tuning Protein Tertiary Structure with Heterogeneous Backbones

Yuhan Lin, and Seth Horne

University of Pittsburgh, Pittsburgh, USA

The development of artificial synthetic macromolecules with defined folded conformations has proved a fruitful approach to mimicking protein tertiary structure. One strategy to these proteomimetic agents is to strategically place monomers of artificial backbone connectivity throughout a native protein sequence. Prior studies on the resulting heterogeneous backbone variants have explored folding energetics, folding kinetics, folded structure, and biological function.

Protein folding is an intricate process, and backbone modification can sometimes lead to unexpected structural changes. This is particularly the case when substitutions are made in the hydrophobic core of a prototype. Buried residues play an important role in specifying and stabilizing protein structure, and they are often left untouched in design of proteomimetics. Modifying the hydrophobic core, however, has potential benefits, as artificial connectivity in this context can significantly impact structure with minimal chemical change.

This has motivated us to design, synthesize, and characterize a series of miniprotein analogs with backbone modification specifically targeted to the hydrophobic core. Structures obtained via NMR analysis reveal that the hydrophobic core in two distinct prototype systems can accommodate modifications while maintaining the global fold. Biophysical characterization provides quantitative insights into effects of altered chemical composition on folding energetics. These findings broaden the scope of heterogeneous backbone proteomimetic design while also demonstrating how even subtle changes to chain composition can influence tertiary structure.

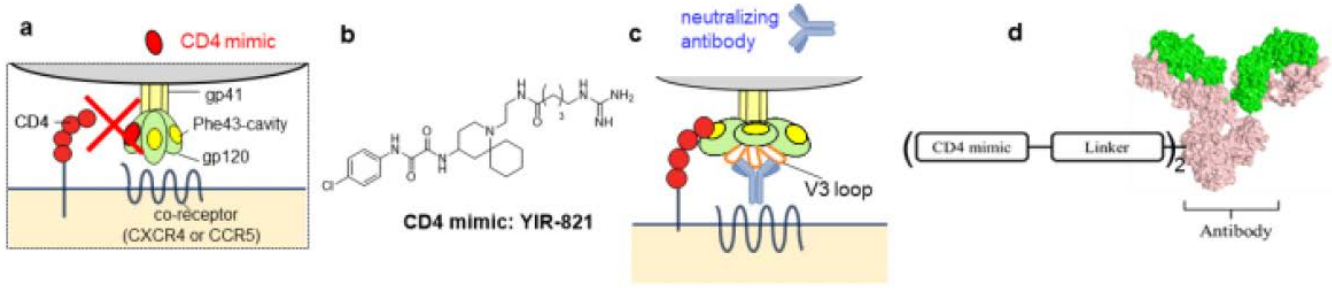
P-157

Anti-HIV Agent-Based Antibody-Drug-Conjugates - ADCs

Yutaro Miura[1], Kohei Tsuji[1], Takuya Kobayakawa[1], Kaho Matsumoto[2], Takeo Kuwata[2], Yuji Ito[3], and Taku Yoshiya[4]

[1]Institute of Science Tokyo, Tokyo, Japan
[2]Kumamoto University, Kumamoto, Japan
[3]Kagoshima University, Kagoshima, Japan
[4]Peptide Institute Inc., Ibaraki, Japan

HIV infection/AIDS is still an incurable disease although combination antiretroviral therapy, cART, which involves a combinational dosage of several anti-HIV drugs, is currently used in clinical treatment. Because of the emergence of drug-resistant strains of HIV, et cetera, development of novel anti-HIV drugs with different mechanisms of action is required. Therefore, this study is focused on anti-HIV agents that interact with several conserved regions of envelope proteins to inhibit viral entry.



To date we have developed small molecule HIV entry inhibitors, CD4 mimics, to bind to an HIV envelop protein, gp120, and compete with the host cell surface protein, CD4, see figure a & b. The binding of CD4 and CD4 mimics to gp120 induces its conformational change, and the co-receptor binding site is then exposed to be recognized by neutralizing antibodies, see figure c.[1] Therefore, we envisioned that antibody-drug-conjugates, ADCs, which are composed of CD4 mimics and neutralizing antibodies, would effectively inhibit virus entry.[2]

We synthesized several ADCs using various linker structures and lengths, and evaluated their anti-HIV activities, see figure c. We adopted site-specific conjugation methods as well as a non-specific modification method using an active ester to conjugate these antibodies and CD4 mimics. The linker length of the ADCs was adjusted by the number of linker unit repetition. As a result, significant activity of the ADCs compared to that of the parent antibodies and CD4 mimics or co-treatment of them were improved. The present data would lead to development of novel HIV entry inhibitors.

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[2]Ohashi, N. et al., *ChemMedChem* **2016**, 11, 940-946.

P-158

Evaluation of Benzoyllysine Isostere Interactions and Removal by Deacetylase Enzyme Hst2

Jake Wilkinson[1], Joseph Treacy[2], Kendall Houk[2], Marcey Waters[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, USA
[2]UCLA, Los Angeles, USA

Benzoyllysine, Kbz, is a post translational modification, PTM, found on all histones that links epigenetics to diet as it can be generated from sodium benzoate, a common food preservative. Sirtuins are a class of enzymes that remove acyl marks from histones through a NAD+ based mechanism and are a promising target for inhibitor development because of their role in tumor promotion of c-Myc driven cancers. Hst2, a yeast Sirtuin, has been found to remove Kbz from histones. The crystal structure of Hst2 binding histone 3, H3, suggests Kbz makes offset π-π stacking with Hst2 F67 and hydrogen bonds with the backbone carbonyl of Hst2 V182.

We report here mechanistic studies to evaluate the contributions of π-π stacking and hydrogen bonding to binding to gain insight into factors that can be exploited for inhibitor design. We investigate the binding of Hst2 to H3K9Bz through a series of benzoyl peptide derivatives with electron-donating or electron-withdrawing substituents to modulate the electrostatic potential of the benzene ring. The free energy of binding has a linear relationship with the electrostatic potential of the benzene ring and the partial charge of the amide NH, with electron-withdrawing groups tightening binding. In addition, we find that a thioamide isostere of Kbz tightened binding to Hst2, likely through strengthening the hydrogen bond donator ability of the ε-NH. We show that the Kbz thioamide isostere is resistant to removal by both Hst2 and Sirt2, providing potential functional groups for chemical probes with improved binding and stability.

P-159

A Versatile Fluorescent System for Studying Diverse Post-Translational Modifications Using Simple Peptide Substrates

Jodi Hintzen, and George Burslem

University of Pennsylvania, Philadelphia, USA

Post-translational modifications, PTMs, are essential regulators of cellular processes, influencing gene expression, protein stability, and protein-protein interactions.[1] Among these, lysine and arginine modifications such as acetylation, methylation, citrullination and other acylation variants are key players in epigenetic regulation.[2, 3] However, the development of assay systems that can adapt to a wide range of PTMs remains a challenge. Here, we present a generalized fluorescent turn-on platform that utilizes simple peptide substrates to study the installation and removal of a diverse set of lysine and arginine PTMs, with a focus on epigenetically relevant ones, Fig. 1.

In addition to synthetic installation of native post-translationally modified residues in peptides, we employed thialysine and thiaarginine analogs to mimic modified lysine and arginine residues, enabling facile introduction of functional PTM mimetics using simple cysteine chemistry.[4, 5] We utilize the cleavage of peptidyl lysine and arginine bonds by trypsin, which are only removed when these residues are in their unmodified state, Fig. 1. Conversely, in their post-translationally modified state, the peptides remain intact leading to internal fluorescent quenching, making the system adaptable to studies of both writer and eraser enzymes, Fig. 1.

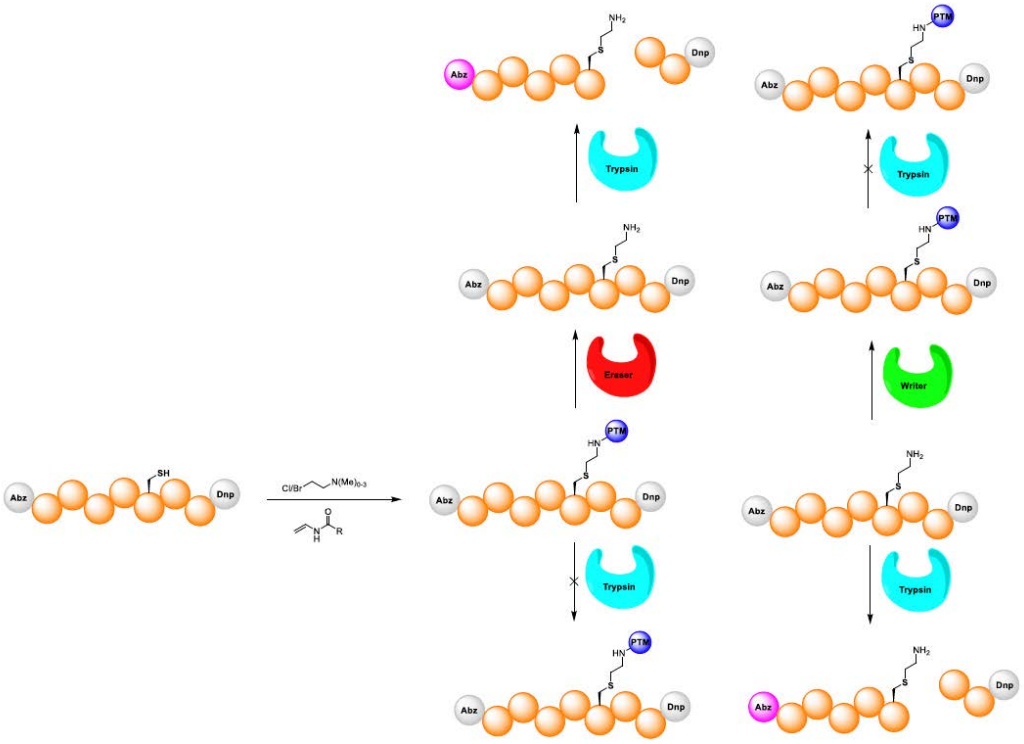

Figure 1: Overview of cysteine chemistry to install PTMs on fluorescent turn-on peptides and enzymatic assays involving writer and eraser enzymes.

Model PTMs that have been studied are removal of lysine acetylation, lactylation and β-hydroxybutyrylation by SIRT3, removal of methylated lysine variants by KDM3A and KDM4A as well as arginine citrullination by PAD4, highlighting the versatility of this approach. By integrating modularity and fluorogenic detection, this system provides an accessible, flexible, efficient, and adaptable tool for PTM studies. Its broad applicability offers significant potential for exploring enzymatic mechanisms, PTM crosstalk, and protein regulation across diverse biological contexts.

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[3]A. H. Shukri, V. Lukinović, F. Charih and K. K. Biggar, *Biochim. Biophys. Acta Gene Regul. Mech.*, **2023**, 194990.
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P-160

Modulating SETD2 Activity via Allosteric Inhibition of its AWSDomain: A Novel Strategy for Epigenetic Cancer Therapies

Katherine Menendez

The Graduate Center and Brooklyn College, City University of New York, New York, USA

H3K36me3 is a critical epigenetic modification involved in transcriptional regulation, DNA damage repair, and genomic stability. SETD2, the primary enzyme responsible for this trimethylation in mammals, is frequently dysregulated in various cancers, making it an attractive therapeutic target. Developing innovative, selective, and potent modulators for SETD2 is an area of growing interest. Here, we report the design, synthesis, and characterization of peptidomimetics

that allosterically inhibit SETD2 by targeting its AWS domain. Computational analyses and molecular dynamics simulations guided the rational design for developing these molecules, which were synthesized using a solid-phase peptide synthesis approach.

The incorporation of hydrocarbon staples enhanced the solubility, stability, and potency of the peptidomimetics, resulting in modulators with low micromolar inhibition capacity *in vitro*. To our knowledge, these peptidomimetics are the first reported allosteric inhibitors of SETD2. By leveraging an allosteric mechanism, this approach provides a promising strategy for selectively modulating SETD2 activity while minimizing off-target effects, offering a novel therapeutic avenue for SETD2-driven malignancies.

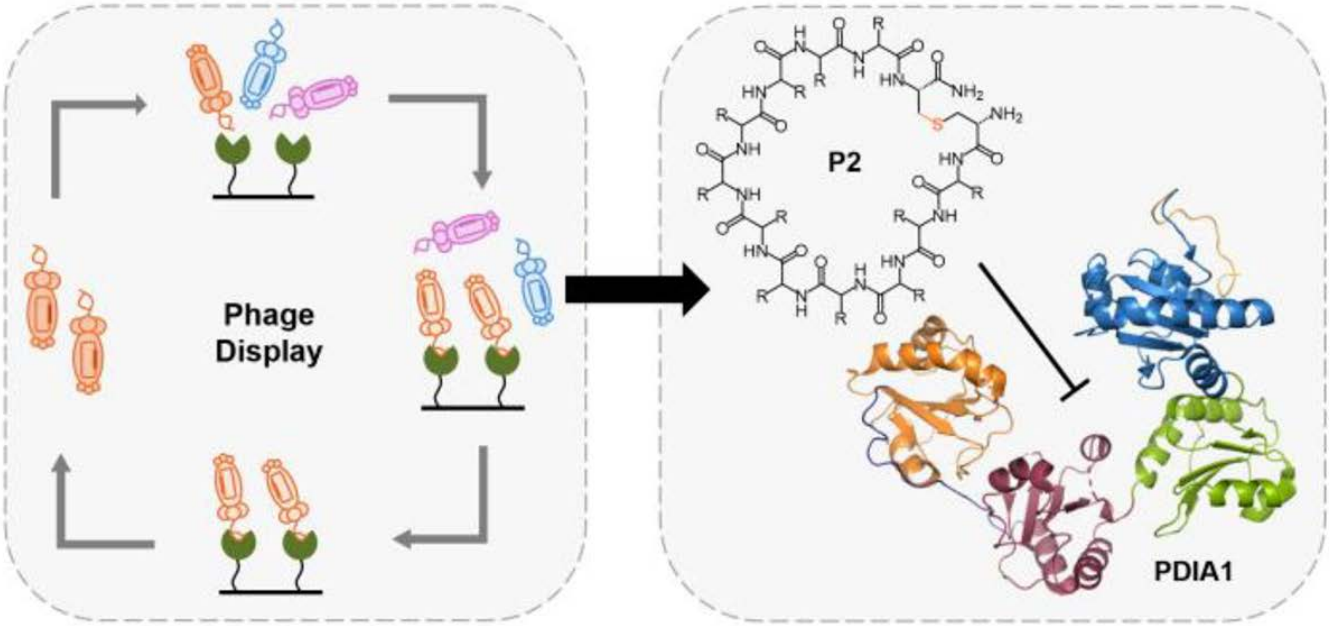
P-161

Discovery of a Protein Disulfide Isomerase 1 Macrocyclic Peptide Inhibitor by Phage Display

Brittney Chau, Fan Yang, Aspen Fain, and Jianmin Gao

Boston College, Chestnut Hill, USA

Cardiovascular disease is the leading cause of death globally. According to the CDC, 60,000 to 100,000 Americans die or experience long-term complications each year due to venous thrombosis. There is a clear need for novel therapeutics to treat thromboembolic diseases. Extracellular Protein Disulfide Isomerase 1, PDIA1, is known to trigger platelet aggregation and thus has been identified as a therapeutic target to treat thrombosis. Most reported PDIA1 inhibitors are small molecules and have struggled to achieve clinical use because they either inhibit intracellular PDIA1 function due to cell permeability, lack of selectivity between PDI family members, or have low potency. Peptide macrocycles overcome this challenge through their cell impermeability, while being capable of generating high specificity.



We report the first PDIA1 peptide inhibitor, P2, kd = 610 nM, discovered by phage display with a disulfide cyclized library. To evaluate the inhibitory activity by insulin turbidity assay, a P2 analog was synthesized to replace the disulfide bond with a thioether linkage, revealing an IC_{50} of 1.2 μM. Furthermore, P2-thioether demonstrates selectivity between PDI family members that exist in the extracellular space. Structure and activity studies will reveal which residues are crucial towards binding to PDIA1 and provide insight to optimize the P2 parent peptide. Our results suggest that P2 can be a promising candidate for extracellular PDIA1 inhibition.

P-162

Chemoselective Sulfonyl-Fluoride Exchange 'SuFEx'-InducedMacrocyclization of Tyrosine-Containing Peptides

Hassan Seyrani, Hossein Heidarzadeh Vazifekhorani, and Victor K. Outlaw

University of Missouri, Columbia, USA

Peptide-based drugs are increasingly employed to modulate a wide range of biological processes due to their ability to mimic endogenous molecules, such as growth factors, neurotransmitters, ion channels, and hormones. Despite their therapeutic potential, peptides face significant limitations, including short biological half-lives, poor oral bioavailability, and susceptibility to proteolytic degradation. Peptide cyclization has emerged as a promising strategy to overcome these challenges. Cyclic peptides possess constrained conformational flexibility, leading to enhanced resistance to proteolysis and increased *in vivo* stability, while often improving target affinity and membrane permeability. The development of new methods for chemoselective cyclization of peptides is important for expanding the chemical diversity of potential peptide therapeutics.

In this work, we present a new method for chemoselective cyclization of native peptides at tyrosine. The method leverages a sulfur-fluoride exchange, SuFEx, reaction of an N-terminal 4-fluorosulfonylbenzoyl cap with the phenolic side chain of tyrosine residues to afford a new class of Sulfonate Tyrosine Ester Macrocyclic peptides, STEMtides. Detailed descriptions of this methodology, as well as its applications in generating biologically active cyclic peptides, will be discussed.

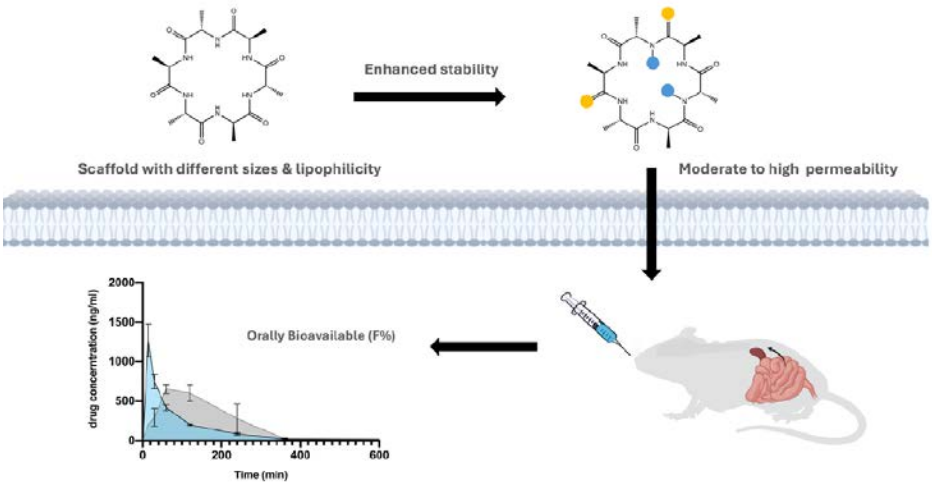
P-163

Editing Macrocyclic Peptides with Backbone Modification to Improve Oral Bioavailability

Nishant Raj[1], Mifune Takhashi[2], Monika Patel[3], Raju Rajmani[1], Pritam Biswas[1], Vartika Gupta[1], Prem Yadav[3], Emile Plise[2], and Jayanta Chatterjee[1]

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, Bangalore, India
[2]Department of Drug Metabolism and Pharmacokinetics, Genentech, San francisco, USA
[3]Neuroscience & Ageing Biology, CSIR-CDRI, Lucknow, Lucknow, India

Macrocyclic peptides are emerging as very potential candidates for drug development. Due to the advancement in display technologies, macrocycles can target undruggable proteins. These molecules have the appropriate biochemical and therapeutic properties to provide advantages for both small molecules and antibodies. Still, most macrocycles cannot be given orally due to their low gastrointestinal absorption and rapid digestion. Our study shows that site-specific editing of the backbone of these macrocycles can enhance its drug-like properties.



We focused on shielding the amide hydrogen bond acceptor, >C = O, and amide hydrogen bond donor, >N-H, to reduce the de-solvation penalty during membrane permeation. Additionally, altering the backbone amide of these macrocycles enhances their metabolic stability. In a proof of concept, we applied our strategy to four different macrocycles with very different physicochemical properties. With our approach, we were able to develop several orally bioavailable macrocycles. In this process, we explored interesting parameters governing the permeability and compared the influence of hydrogen bond donor-acceptor to de-solvation. Finally, we applied these strategies to bioactive molecules to increase their drug-likeness without altering their bioactivity. Our approach provides a unique therapeutic niche to macrocycles that can expand its pharmaceutical landscape.

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P-164

Non-insulin Peptides for Oral Insulin Delivery

Wenchao Li, Terra Lin, and Danny Chou

Department of Pediatrics, School of Medicine, Stanford University, Palo Alto, USA

Diabetes is a global health crisis, affecting millions worldwide. Type 1 diabetes requires lifelong insulin therapy to prevent severe complications such as cardiovascular disease and neuropathy. However, insulin's structural complexity, stability issues, and production challenges limit its therapeutic effectiveness, emphasizing the need for improved alternatives. S597, a single-chain insulin receptor agonist, offers superior stability and more straightforward synthesis than insulin but suffers from reduced potency and efficacy.

Insulin signaling activation (AKT phosphorylation) assay

Entry	Insulin	Analog 1	Analog 2
EC_{50}(nm)	10.0	0.94	5.3
%Max	100	55	84

WWThis study focuses on optimizing S597 analogs to enhance their therapeutic potential. Utilizing recently published cryo-EM structures of the S597 insulin receptor complex,[1] we designed and synthesized 77 analogs through macrocyclization and mutations to optimize receptor interactions and linker length, followed by evaluating their biological activities. Among these, two analogs demonstrated significant improvements—one exhibiting a ~10-fold increase in potency compared to insulin, while the other increased efficacy and potency like native insulin. These findings represent a significant advancement in insulin mimetic development and could lead to new therapeutic approaches, including oral insulin formulations. Our study provides new insights into non-insulin activators of insulin receptor and accelerates the development of next-generation insulin therapies.

[1]Park, J., et al. Activation of the insulin receptor by an insulin mimetic peptide. *Nature Communications,* **2022,** 13, 5594.

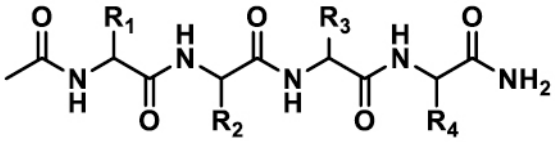
P-165

Development of Novel Antagonists for the Melanocortin-3 Receptor MC3R

An T. Trinh, Mark D. Ericson, Jacob Bouchard, Katie Henning, and Carrie Haskell-Luevano

University of Minnesota, Minneapolis, USA

Obesity is a common, serious, and costly disease that can lead to type 2 diabetes, heart disease, and cancer. While an unhealthy lifestyle may contribute to obesity, genetics also influence body weight. The melanocortin system has five G protein-coupled receptors, known as the MC1–5R. The MC3R and MC4R are centrally expressed in the brain hypothalamus and are involved in energy homeostasis, thus regulating body weight. Therefore, targeting the MC3R and MC4R is a viable strategy to treat not only obesity, but also states of negative energy imbalance, such as anorexia nervosa, and cachexia. While many compounds possess established *in vivo* pharmacological activity to the receptors in the melanocortin system, there is an unmet need to identify selective ligands for the MC3R.



Herein, a library of tetrapeptides is developed as selective antagonists to MC3R. We have identified selective MC3R antagonist tetrapeptide scaffolds from a mixture-based positional scanning library and subsequent characterization of compounds in the lab. The library described here aims to combine these findings to optimize the potency and selectivity of the ligands for the MC3R.

P-166

Retro-Inversion Imparts Antimycobacterial Specificity to Host Defense Peptides

Hugh D. Glossop[1], Gebremichal G. Weindengus[2], Sabiha Sultana[3], Nathan A. Schacht[2], Neela H. Yennawar[4], Diptomit Biswas[4], Anthony D. Baughn[2], and Scott H. Medina[3]

[1]Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA
[2]Department ofMicrobiology and Immunology, The University of Minnesota Medical School, Minneapolis,USA
[3]Department of Biomedical Engineering, Pennsylvania State University, UniversityPark, State College, USA
[4]Huck Institutes of the Life Sciences, Pennsylvania StateUniversity, University Park, State College, USA

The emergence of pan-drug resistant bacterial pathogens has intensified the search for effective alternatives, with peptide-based antibiotics emerging as a promising solution. Among infectious diseases in urgent need of new antibiotics, Tuberculosis, caused by the pathogen *Mycobacterium tuberculosis*, poses a serious threat due to its prevalence in developing countries and its reliance on expensive multi-drug treatment regimens.

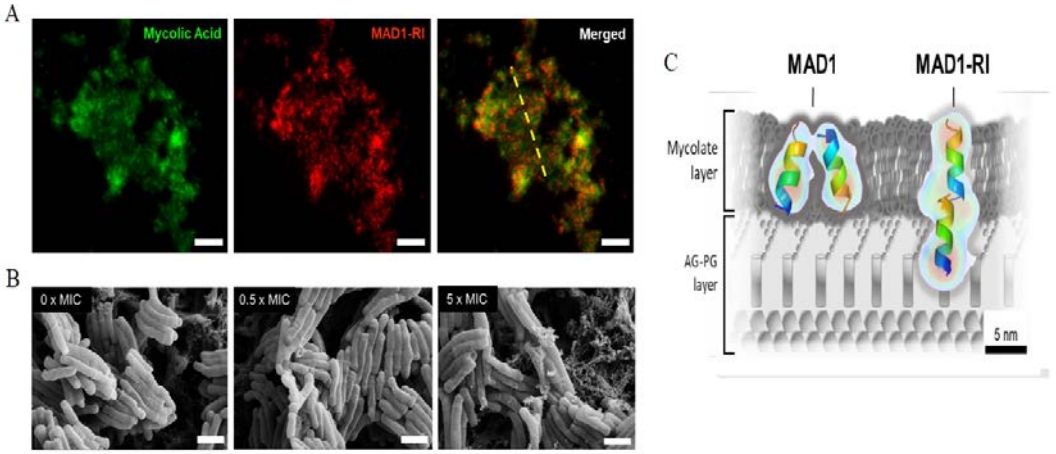


Fig. 1: Mycomembrane dynamics of MAD1-RI. **A** | Fluorescent micrographs of Mtb, mc² 6230, co-stained with Auramine-O, green, and Cy5-labeled MAD1-RI, red. Individual channel and merged image shown. Scale bar = 25 µM. **B** | Scanning electron micrographs of Mtb, mc² 6230, treated with varying concentrations of MAD1-RI, as indicated at the top left corner. Scale bar = 1µM. **C** | Putative arrangement, based on SAXS and AUC simulations, of MAD1 and MAD1-RI within a scaled schematic of the mycobacterial outer membrane.

Unlike conventional antibiotics, host defense peptides, HDPs, exhibit potent antimycobacterial activity while minimizing the likelihood of phenotypic resistance, owing to their diverse and mechanistically distinct modes of action. However, proteolytic instability and low specificity of HDPs have been major obstacles to their clinical application. Attempts to address these barriers through sequence retro-inversion, reversing both backbone direction and amino acid chirality, have shown that gains in peptide stability often come with a penalty of decreased biologic activity.

Here, we show that, contrary to the prevailing paradigm for most bacterial pathogens, retro-inversion of HDPs improves their potency by nearly an order of magnitude against mycobacteria. Bacteriologic assays suggest this enhanced potency is only realized for mycobacterial targets, and that retro-inversion does not appreciably change HDP toxicity towards non-mycobacterial species. Consequently, the lead HDP, MAD1-RI, identified from a library of screened retro-inverted sequences, demonstrated a 4-fold increase in selectivity towards Mtb, and a greater than 30-fold reduction in host toxicity, compared to the original de novo designed MAD1 peptide1. Mechanistic studies suggest this enhanced activity operates through altered mycomembrane thermodynamics of the retro-inverted sequence. These findings establish retro-inversion as an attractive and underexplored strategy that should be considered in the design of anti-mycobacterial HDPs.

P-167

The Cardioprotective Effects of Protein Kinase C Peptides in Myocardial Ischemia-Reperfusion Injury: A Story of Profound Potency of Intracellular Delivery of Cargo Peptides

Kerry-Anne Perkins, Desmond Tanoh, Arjun Nair, Sunit Singh, Kayla Harrell, James Ramsarran, Logan Clair, Taurai Augustin, Juliet Melnik, Annam Humayun, Jennifer Dang, Tameka Dean, Qian Chen, Robert Barsotti, and Lindon Young

PCOM, Philadelphia, USA

Introduction

Restoration of blood flow following myocardial infarction, MI, is necessary to salvage ischemic tissue, but reperfusion is known to cause myocardial ischemia/reperfusion injury, MIR. Protein kinase C epsilon, PKCε, and protein kinase C beta II, PKCβII, signaling is known to activate uncoupled endothelial nitric oxide synthase and NADPH Oxidase respectively, leading to reactive oxygen species, ROS, generation upon restoration of blood flow to previously ischemic myocardium. Both PKC isoforms also induce ROS release from the mitochondria.

Dual conjugation of PKCε and PKCβII peptide inhibitor with myristic acid, myr, and trans-activator of transcription, Tat, (myr-Tat-CC-EAVSLKPT [myr-Tat- PKCε inhibitor] or (myr-Tat-CC-SLNPEWNET [myr-Tat-PKCβII inhibitor]) enhances intracellular delivery and reduces infarct size in *ex vivo* rat hearts. We hypothesize that 0.2mg/kg of myr-Tat PKCε inhibitor or 20ng/kg of myr-Tat-PKCβII inhibitor would exert cardioprotective effects in porcine MIR *in-vivo* by reducing stimulation of ROS and subsequent infarct size.

Methods

Ex vivo: We tested 100nM – 10fM myr-Tat-PKCβII inhibitor and 100nM-1μM myr-Tat-PKCε inhibitor in *ex-vivo* rat MIR. Isolated hearts from anesthetized male SD rats underwent global I (30-min)/R(50-min). Both inhibitors were given during the first 5 mins of R. DP/dt max was measured via a pressure transducer in the left ventricle. 1% TTC staining was used to determine infarct size. Data was analyzed via Fisher's PLSD.

In vivo: Regional I (1 hr)/R (3 hrs) was induced in male Yorkshire pigs. At R, myr-Tat-PKCβII inhibitor, myr-Tat-PKCε inhibitor or scrambled control peptide (20ng/kg; ~100 pM blood volume [vol.] concentration [conc.]) was given via intra-arterial bolus. Ejection fraction, EF, was calculated. Infarct size was determined with Evans Blue dye and 1% TTC staining. Data was analyzed using Student's t-test.

Results

Ex-vivo: Myr-Tat-PKCβII inhibitor (100nM – 100fM; 10-14±3%; n=3-6) significantly reduced infarct size compared to controls (21±3%; n=21; $p<0.05$). DP/dt max was significantly improved (100pM-1pM; 961±274; n=5-6) compared to 100nM (163±77, n=3; $p<0.05$), 100fM (481±86, n=5; $p<0.05$), and 100pM scrambled myr-Tat-PKCβII inhibitor (386±86,

n=4; p<0.05). Myr-Tat-PKCε inhibitor (100nM - 10μM; 5-10±2%) significantly reduced infarct size compared to controls (23.5±1.8%, n=5; p<0.01). Myr-Tat-PKCε inhibitor (100nM) significantly increased dP/dt max values compared to saline control throughout the reperfusion period.

In-vivo: Myr-Tat-PKCε inhibitor (n=5) and Myr-Tat-PKCβII inhibitor (n=4) significantly restored EF to 100±1.2% and 98.8±0.9% of baseline, respectively, compared to controls (94.1±2.5%; n=6, p<0.05). Myr-Tat-PKCε inhibitor and Myr-Tat-PKCβII inhibitor showed significant reduction in infarct size (Myr-Tat-PKCε inhibitor: 9.9±2.1%; n=4 and Myr-Tat-PKCβII inhibitor: 10.0±2.8%; n=4) compared to controls (28.5±8.3%, n=6; p<0.05).

Conclusion
The addition of myr-Tat-PKCε inhibitor or myr-Tat-PKCβII inhibitor during reperfusion with coronary revascularization post-MI exhibits robust cardioprotection and may reduce incident - heart failure after MI. Future studies include a 6-month porcine survival study to evaluate infarct size and cardiac function.

P-168

Designing Active Self-Assembling Peptide Materials with Molecular Dynamics Simulation and Machine Learning

Yuanming Song, Naomy Marrufo, J. Alfredo Freites, Stacy M. Copp, Allon I. Hochbaum, and Douglas J. Tobias

University of California, Irvine, Irvine, USA

Active self-assembling peptide materials hold immense potential for biomaterials, nanotechnology, and bioelectronics, enabling the creation of dynamic, stimuli-responsive systems. Peptides can be designed to self-organize in response to environmental or electronic cues, mimicking biological self-assembly with synthetic control. However, predicting self-assembly from sequence alone remains a significant challenge.

Here, we use molecular dynamics simulations to assess the MARTINI and SIRAH coarse-grained force fields for their ability to predict active aggregation of cystine containing peptides, benchmarking simulations against experimental data. We further introduce a network-based representation of aggregation and a machine learning model trained on simulation data to predict new self-assembling sequences. By integrating molecular simulations, machine learning, and experimental insights, this work advances predictive tools for rationally designing peptide-based materials, accelerating the discovery of functional, programmable biomaterials.

P-169

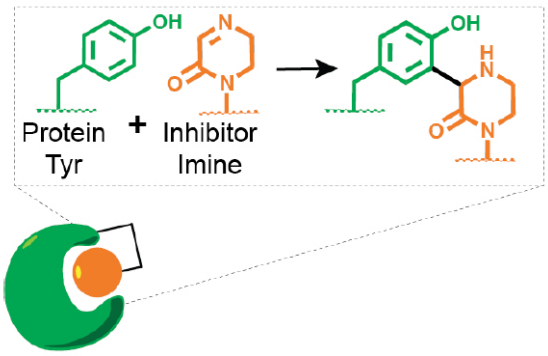
Peptide Inhibitors of the CBX8 Chromodomain that Utilize Covalent Tyrosine Conjugation by the Mannich Reaction and Modulation of Cation-pi Interactions

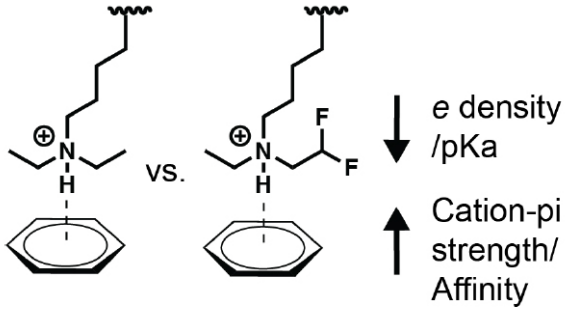
Lei Wang

Purdue University, Lafayette, USA

CBX8 is a member of the Polycomb Group Proteins, PcG, which are required for transcriptional repression of differentiation genes. The chromodomain, ChD, of CBX proteins is necessary and sufficient to recruit the Polycomb Repressive Complex 1, PRC1, to chromatin through binding to the trimethylated lysine at H3K27.

In this study, we have developed modified peptide inhibitors to the CBX8 ChD using two strategies. In the first, we investigate the utility of cyclic imine Mannich electrophiles as covalent warheads to specifically react with a protein tyrosine adjacent to the inhibitor binding pocket. We characterized second order reaction rates for several cyclic imines to tyrosine and tryptophan. Cyclic imine-containing inhibitors react selectively with the targeted tyrosine and improved the potency and selectivity of the inhibitor *in vitro* and in cells. Chemo-proteomic analysis demonstrated selective labeling of CBX8. This is the first demonstration of this chemistry in target covalent inhibitors, TCIs. This chemistry additionally has utility for bioconjugation chemistry applications to tyrosine, tryptophan, and pyrrolated lysine.





In the second approach, we modulated the electron withdrawing properties of the alkylated lysine within inhibitors to increase the strength of the cation-pi interaction to the aromatic cage residues of the CBX8 ChD. We synthesized alkylated lysine analogues with pKa's ranging from 5 to 11. Results showed that decreasing the electron density of the alkyl lysine cation can improve binding affinity of inhibitors up to 10-fold, through increasing cation-pi interaction strength. The pKa optimum was around 8. Additionally, pKa changes affected the log D, solubility, and membrane permeability of inhibitors. We show improved cellular activity of optimal inhibitors. This approach should be generalizable for the modulation of cation pi interactions in medicinal chemistry.

P-170

Environmentally Sustainable Synthesis of Cosmetic Peptides

Lucrezia Sforzi[1], Fosca Errante[1], Anna Maria Papini[2], and Paolo Rovero[1]

[1]Interdepartmental Laboratory of Peptide and Protein Chemistry and Biology, Departmentof Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Sesto Fiorentino, Italy

[2]Interdepartmental Laboratory of Peptide and Protein Chemistry and Biology, Department of Chemistry "Ugo Schiff," Sesto Fiorentino, Italy

In recent years sustainability has become a growing priority in the cosmetic sector, pushing the industry to reduce the environmental impact of both raw materials and production processes. Since peptides are increasingly appreciated as active cosmeceutical ingredients, their production should adapt to these requirements. With the aim of reducing the environmental impact of the synthetic procedures used to produce peptides, we are continuing now in the effort to develop greener solid-phase synthetic protocols.[1-4] Thus, we selected as proof-of-concept two well-known active cosmetic peptides: **i**| Argireline, a neurotransmitter inhibiting peptide with botox-like activity due to the interaction with SNARE complex, Ac-EEMQRR-NH_2, and **ii**| GHK, a carrier peptide, H_2N-GHK-OH. In particular, we are addressing the following aspects:

i| use of greener solvents and solvent mixtures;
ii| specific synthetic problems, that is, methionine oxidation, arginine protection and histidine racemization;
iii| alternatives to trifluoroacetic acid to cleave the peptides from the resin;[5]
iv| replacement of diethyl ether for the precipitation of the crude peptide;[1]
v| alternatives to the classic HPLC purification such as catch and release technology.

[1]O. Al Musaimi, B.G. de la Torre, F. Albericio, *Green Chem*. **2020** 22 996–1018.
[2]C.M. Alder, J.D. Hayler, R.K. Henderson, A.M. Redman, L. Shukla, L.E. Shuster, H.F. Sneddon, Green Chem. **2016** 18 3879–3890.
[3]L. Pacini, M. Muthyala, L. Aguiar, R. Zitterbart, P. Rovero, A.M. Papini, J. Pept. Sci. **2024** 30 e3605.
[4]L. Pacini, M.K. Muthyala, R. Zitterbart, O. Marder, P. Rovero, A.M. Papini, **2025** Under minor revisions.
[5]J. Pawlas, C. André, J.H. Rasmussen, O. Ludemann-Hombourger, Org. Lett. **2024** 26 6787–6791.

P-171

Advancing Cardiovascular Therapies with CVP-350: A P110-Inspired Macrocycle Targeting Drp1/Fis1 Signaling

Mulate Zerihun Workeneh[1], Shulamit Fluss Ben-Uliel[1], Shmuel Silnitsky[1], Rani Vainer[1], Samar Gani[1], Liron Davis[1], Suchismita Dey[2], Moriya Slavin[3], Nir Kalisman[3], Deborah E.Shalev[3], Offir Ertracht[1], Marta De Zotti[4], Shaul Atar[1], Bereketeab Haileselassie[2], and Nir Qvit[1]

[1]Bar-Ilan University, Safed, Israel
[2]Stanford University School of Medicine, Stanford, CA,USA
[3]The Hebrew University of Jerusalem, Jerusalem 9190401, Israel
[4]University of Padova, Via Marzolo 1, 35131 Padova, Italy

Excessive mitochondrial fission disrupts mitochondrial function and energy production, contributing to various pathologies, including neurodegenerative diseases, cancer, and cardiovascular conditions. The interaction between Dynamin-related protein 1, Drp1, and Fission protein 1, Fis1, plays a central role in driving this dysfunction. A decade ago, we developed P110, a linear peptide inhibitor specifically targeting the Drp1/Fis1 interaction, which demonstrated significant efficacy in preserving mitochondrial function. P110 showed neuroprotective effects in Parkinson's disease models and cardioprotective benefits in animal models of ischemia/reperfusion injury, with recent findings indicating potential applications in other neurodegenerative diseases. However, its clinical translation has been hampered by its susceptibility to proteolytic degradation and suboptimal drug-like characteristics.

In this study, we introduce CVP-350, a cyclic, macrocyclic compound inspired by P110. CVP-350 was identified through systematic amino acid substitutions and structure-activity relationship analyses. Our findings demonstrate that CVP-350 is safer and exhibits superior cardioprotective effects compared to P110, with a 6-fold increase in both selective inhibition potency against Drp1/Fis1 signaling and enzymatic stability in the presence of trypsin. These enhanced pharmacological properties position CVP-350 as a highly promising candidate for the treatment of cardiovascular diseases and other conditions associated with mitochondrial dysfunction, offering a novel strategy to address mitochondrial-related pathologies.

P-172

Advancing Peptide Synthesis: Optimizing Coupling Strategies and Green Solvent Systems for Enhanced Efficiency and Sustainability

Mulate Zerihun Workeneh, Lion Davis, and Nir Qvit

Bar-Ilan University, Safed, Israel

Peptide synthesis is fundamental to biochemical and pharmaceutical research, driving the development of therapeutics and biomolecular tools. Achieving high efficiency, yield, and purity while reducing environmental impact remains a key challenge. This study explores an innovative binary solvent system-dimethyl sulfoxide, DMSO, and ethyl acetate, EtOAc, (1:1)-to enhance sustainability and synthetic performance. The efficacy of Diisopropylcarbodiimide, DIC, and a Quaternary Carbodiimides, QCD, coupling reagents were evaluated in the synthesis of linear and cyclic peptides. The linear peptide CVP-763 achieved exceptional yields, 107.23% with DIC, 113.76% with QCD, with 100% purity, HPLC. In contrast, the cyclic peptide CVP-350 exhibited a yield of 89.13%, DIC, and 98.50%, QCD, with corresponding HPLC purities of 73.96% and 66.40%, respectively.

A double coupling strategy using automated, Syro-I, and manual synthesis improved reaction efficiency. Mass spectrometry and HPLC confirmed that QCD enhanced overall yield while significantly reducing cis-trans isomerization, a key limitation in cyclic peptide synthesis. Notably, integrating green solvent systems mitigated isomerization challenges without compromising efficiency. These findings underscore the potential of environmentally conscious peptide synthesis methodologies, offering a sustainable, high-fidelity approach for pharmaceutical and biochemical applications.

P-173

Chemical Protein Synthesis of Mirror-Image Streptavidin

Riley Giesler[1], Peter Woodham[1], Steven Draper[2], Paul Spaltenstein[1], Frank Whitby[1], Christopher Hill[1], and Michael Kay[1]

[1]University of Utah, Salt Lake City, USA
[2]Mayo Clinic-Rochester, Rochester, USA

Due to its nearly irreversible binding to biotin, streptavidin enjoys numerous biotechnology applications. However, two main drawbacks limit its use in, therapeutic and diagnostic applications: high immunogenicity and endogenous biotin interference. We posit that a mirror-image streptavidin and biotin system could solve these problems due to the minimal immunogenicity of D-proteins. We synthesized both L- and D-streptavidin via native chemical ligation with three segments with the help of our helping hand linker needed to solubilize a difficult, aggregation-prone peptide segment. We developed a novel high-efficiency folding protocol and characterized the folded synthetic proteins through circular dichroism and isothermal titration calorimetry.

We found a 200-million-fold difference in affinity between natural and mirror-image biotin, which renders these systems functionally orthogonal. To gain further insight into how mirror-image biotin binds L-streptavidin, we solved high-resolution X-ray crystal structures for the heterochiral and homochiral interactions. This work demonstrates the high degree of stereospecificity of the streptavidin/biotin interaction and the potential utility of a mirror-image biotin/streptavidin system for *in vivo* and diagnostic applications.

P-174

Rapid Screening Platform for Discovering Novel Peptideprenyltransferases to Expand the Structural Diversity of Pseudo-Natural Prenylated Peptides

Yuki Goto

University of Kyoto, Kyoto, Japan

Peptide lipidation is a promising strategy for enhancing the pharmacokinetic properties of peptides. Cyanobactin prenyltransferases, PTases, introduce prenyl groups into peptide substrates with high chemo- and regioselectivity while exhibiting broad substrate sequence tolerance, making them valuable biocatalysts. While several cyanobactin prenyltransferases, PTases, have been characterized, many putative PTases with unexplored activities remain unidentified, highlighting the untapped potential of this enzyme family.

In this study, we aimed to expand the known repertoire of cyanobactin PTases through genome mining and activty-based screening. To achieve this, we developed a streamlined activity screening system utilizing a panel of artificial substrate peptides to efficiently identify novel cyanobactin PTases with unique catalytic properties. This approach led to the discovery of multiple PTases exhibiting diverse and unprecedented prenylation patterns. Structural and biochemical analyses further revealed key determinants governing their substrate specificity and reaction mechanisms. These findings significantly enrich the enzymatic toolkit for peptide lipidation, paving the way for the rational design of lipidated peptides with enhanced functional properties.

P-175

Uncovering Histone Epigenetics Using *De Novo* Protein Design

Katherine Albanese

Wake Forest University, Winston-Salem, USA

The regulation of cellular processes is critically influenced by histone posttranslational modifications, PTMs, which serve as molecular switches modulating protein function, signaling, and chromatin structure. In chromatin biology, histone PTMs regulate essential processes such as gene expression, DNA replication, and repair. Histone PTMs often

act in complex networks of "crosstalk", when combinations of PTMs either positively or negatively influence one another to effect chromatin dynamics and cellular outcomes. Given the important role of histone PTMs in cell homeostasis, it is not surprising that alternations or mutations to PTMs and their surrounding histone sequence are linked to diseases including cancer and neurological disorders. Despite their significance, current methods fall short in decoding the dynamic and combinatorial nature of PTMs in living cells, leaving critical gaps in our understanding of their roles in cellular health and disease.

This proposal seeks to address these challenges by developing a novel set of protein-based tools using computational protein design methods. These proteins, termed protein inducers of proximity, PIPs, are used to investigate how histone PTMs modulate chromatin dynamics and other regulatory pathways. PIPs are *de novo* designed and engineered proteins that selectively bind and manipulate PTMs in *cis,* on the same histone tail, or *trans,* across histones or nucleosomes. **This work is innovative because unlike existing methods, PIPs function independently of endogenous cellular machinery, enabling precise dissection of PTM interactions without disrupting cellular homeostasis.**

Using PIPs, this research aims to: **1|** investigate the mechanisms by which new and putative histone PTMs and mutations influence key cellular processes such as differentiation and stress responses; **2|** decode PTM crosstalk to elucidate how combinatorial PTMs regulate chromatin structure and function; and **3|** develop broadly applicable methodologies for studying PTMs in diverse biological contexts. **This work is significant because we aim to develop tools that enable transformative insights into chromatin biology, PTM-driven regulation, and the molecular basis of diseases linked to epigenetic dysregulation.**

P-176

Investigation into Synthesis of Glycosyl Amino Acids for Incorporation into Glycopeptides

Niki Tran, Ronald Aleman Lemus

California State University, Fullerton, Fullerton, USA

Glycosylation is a posttranslational modification that plays a critical role in the proper structure and function of proteins, as over half of all mammalian proteins bear some form of glycosylation. Dysregulated glycosylation has been implicated in a number of disease states. As such, the synthesis of glycoproteins/glycopeptides and their derivatives is essential for understanding the connection between protein glycosylation and disease at the molecular level.

Herein, we review prior work and share current efforts toward synthesis of Fmoc-protected serine/threonine derivatives modified with *N*-acetylgalactosamine. These building blocks can be incorporated into general solid-phase peptide synthesis to yield corresponding glycopeptides, and we plan to synthesize mucin-like glycopeptides in future work.

P-177

Peptide Chemistry and Antibody Engineering to Probe Receptor Pharmacology and Manipulate Signaling

Shivani Sachdev, Shubhra Saha, Swarnali Roy, Phoenix Davis, and Ross Cheloha

NIH, Bethesda, USA

Peptides and antibodies represent two leading modalities for targeting protein receptors expressed on the surface of mammalian cells. Endogenous peptides often serve as leads for developing tools and therapeutic candidates for probing receptor function, but they often suffer from insufficient selectivity and target residence time, especially when addressing members of large receptor families such as G protein-coupled receptors, GPCRs. Alternatively, antibodies are prized in biomedical research for their high specificity but typically lack any biological effect, inhibition or activation, when targeting receptors with orthosteric sites embedded within transmembrane domains, like GPCRs.

To leverage and expand the favorable properties of peptides and antibodies we have developed methods to create conjugates comprised of synthetic peptide GPCR ligands and antibody fragments, nanobodies. Conjugates are made through a combination of solid-phase peptide synthesis, site-specific labeling of recombinantly expressed nanobodies, and click chemistry. These conjugates show properties superior to those of antibodies or peptides alone, including the induction of pathway specific signaling responses, biased agonism, for a GPCR involved in the treatment of osteoporosis, PTHR1.[1] We are now building on these initial findings to address additional cell surface receptors. One specialized property of nanobody-peptide conjugates is their ability to act through two receptor protomers expressed on the same cell. We are now leveraging this mechanism to create "logic-gated" conjugates that require the expression of two distinct receptors for function. This approach offers the possibility of targeting widely expressed receptors only on a cell type or tissue of choice, potentially enabling therapeutic responses with fewer side effects.

[1]Sachdev, S.; Creemer, B. A.; Gardella, T. J.; Cheloha, R. W. GPCR ligand tethering via nanobody binding promotes highly biased agonism. *Nature Commun.* **2024**. 15, 4687. PMID: 38824166; PMCID: PMC11144202. DOI: 10.1038/s41467-024-49068-5

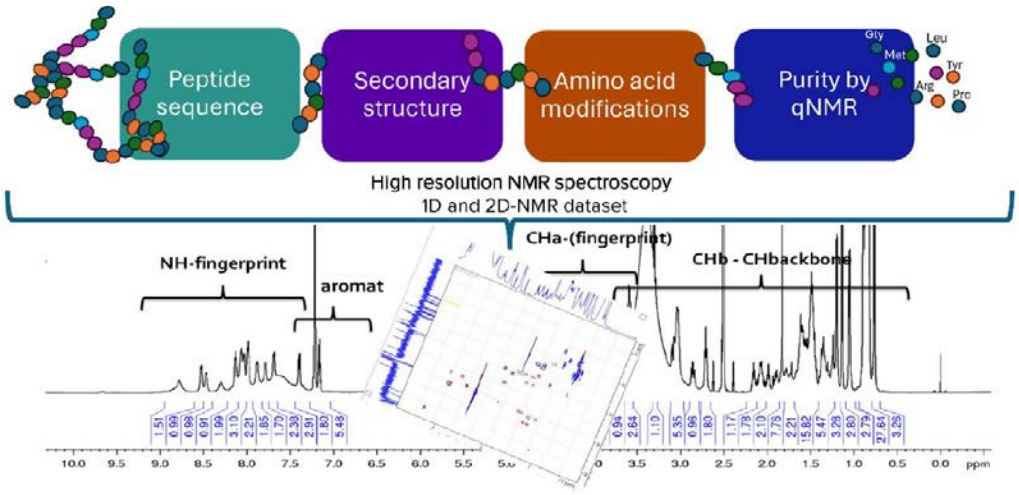
P-178

Comprehensive Peptide Analysis with High-Resolution NMR Spectroscopy: NMR as a Powerful Tool

Nadine Peez, Jakob Waldthausen, and Bernd Diehl

Spectral Service AG, Cologne, Germany

High-resolution nuclear magnetic resonance, NMR, spectroscopy is the most versatile tool for peptide analysis, providing sequence confirmation, secondary structure elucidation, and quantitative assessment.[1] Our approach supports both non-GMP and GMP-compliant workflows for research and regulatory applications. We, one of the world's leading NMR laboratories, want to present our findings from the past years and demonstrate why NMR spectroscopy is such a powerful analytical tool. A key strength is our optimized sample preparation and expertise. Peptides are dissolved in DMSO-d_6 rather than H2O/D_2O, 9:1, to enhance signal resolution and avoid overlap. This ensures high-resolution spectra.



The peptide measurement program includes 1D and 2D NMR, ^{1}H, ^{13}C, and ^{15}N, such as TOCSY, NOESY and ^{15}N-HSQC, enabling full signal assignment for peptides approximately up to 35 amino acids. This allows reliable detection of sequence deviations, truncations, and modifications, surpassing conventional mass spectrometry in resolving complex sequences. Moreover, intentional modifications, such as fluorination or methylation of individual amino acids, pose no challenge for NMR spectroscopy. NMR data evaluation combines automated processing with expert interpretation to extract, for example, chemical shift, and NOE interaction data, supporting batch-to-batch consistency checks or detailed structural analysis. If required, qNMR spectroscopy can also be used for quantification, provided a suitable signal that follows the SCSSRS principle is available.[2]

NMR spectroscopy's adaptability makes it an indispensable tool for peptide characterization in both research and GMP-regulated environments.

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[2]Bernstein, M. *The qNMR Handbook*. tredition. **2023** ISBN: 978-3-7568-7891-0

P-179

A Peptide Can Replace an Essential Enzyme in Yeast

Kira A. Podolsky, Oscar J. Molina, Vivian Y. Long, and Ronald T. Raines

MIT, Cambridge, USA

It is unknown whether peptide catalysts facilitated the emergence and maintenance of cellular biology at the origins of life and if their functions were similar to large protein enzymes in modern cells. Here, we report the first discovery of enzyme mimetic peptide catalysts that sustain eukaryotic life. We designed peptide libraries to complement the function of an essential enzyme, protein disulfide isomerase, PDI, in *Saccharomyces cerevisiae*. Two genetically encoded peptide catalysts, both 24 amino acids long, maintain *S. cerevisiae* survival in the absence of PDI. Their capacity to keep pace with rapid cellular functions therefore implicates peptide catalysts as evolutionary precursors to enzymes.

P-180

Peptide Synthesis in Aqueous Phase with a Reusable Solid Phase

Yong Ma

University of the Pacific, Stockton, USA

A procedure has been developed for synthesizing peptides in an aqueous solution with a reusable solid phase. Specifically designed linker molecule is employed to attach peptides to hydrophilic solid phases, enabling Solid Phase Peptide Synthesis, SPPS, in aqueous solutions. The linker molecule is utilized to connect peptides to an anionic exchange resin during peptide synthesis in an aqueous solution. The general structure of the linker molecule is Fmoc-AA-CH2-Ph-Rx-SO_3^-, the Fmoc, 9-fluorenylmethoxycarbonyl group serves as a protecting group for amino acids.

Amino acids, AA, are linked to the solid phase through a structure of Methoxyphenylcarbonyl group, which is cleavable under strong acidic conditions. The sulfate group is present for forming an ionic bond with the solid resin in an aqueous solution. In this procedure, Fmoc-AA are utilized as building blocks for sequentially adding amino acids in peptide synthesis. Due to Fmoc-AA poor solubility in aqueous solutions, a procedure was developed to enhance the solubility of hydrophobic compounds, with a specific emphasis on dissolving Fmoc protected Amino Acids, Fmoc-AA, in an aqueous solution. This enhancement facilitates SPPS in aqueous conditions with Fmoc-AA as building blocks. Cationic exchange resin, which is reusable, serves as the solid phase.

Our research objective is to shift from the use of organic solvents to an aqueous system while maintaining the existing SPPS practices in organic solvents as closely as possible. This approach is designed to facilitate a more readily acceptable transition for the peptide synthesis industry from using organic solvents to aqueous solution, contributing to greener SPPS and more sustainable synthetic methodologies.

P-181

Building with Precision - Innovative Tools in Peptide Synthesis

Stefan Riedl

Iris Biotech, Marktredwitz, Germany

Iris Biotech offers a broad range of building blocks and reagents specializing in peptide chemistry, with applications spanning life sciences, drug delivery, and linker technologies. True to our slogan "Empowering Peptide Innovation," we deliver cutting-edge technologies and innovative products tailored for diverse needs — from small-scale research to bulk production — serving academia and industry.

Our recent innovations include novel protecting groups designed to enhance peptide synthesis. The tetrahydropyranyl, THP, group protects serine, threonine, cysteine, and hydroxyproline, while the photosensitve 4-methoxy-7-nitroindolin-1-yl, MNI, group shields aspartate and glutamate.[1,2] These orthogonal protecting groups are compatible with Fmoc-SPPS, offering superior stability and enabling versatile residue modifications.

To address peptide stability and functionality, we have expanded our portfolio with homo amino acids, which extend the side-chain carbon backbone. These derivatives of lysine, serine, glutamate, and arginine enhance proteolytic and conformational stability, improve hydrophobicity, and refine binding selectivity.[3]

In the realm of peptide conjugation and cross-linking, we introduce 2-furyl-L-alanine for oxidation-induced furan cross-linking and Diels-Alder reactions, as well as 5-hydroxy-1,5-dihydro-2H-pyrrol-2-one, 5HP2O, building blocks.[4,5] This alternative to maleimides overcomes limitations such as hydrolysis and thiol exchange, offering greater stability and versatility.

Our novel amino-Li resin, a cross-linked polyacrylamide-based solid support, is compatible with both organic and aqueous solvents, providing a robust platform for peptide synthesis.[6] Additionally, we present MYTsA , a ynamide coupling reagent that supports both conventional and inverse peptide synthesis, N to C.[7] MYTsA enhances atom economy and eliminates racemization, setting a new standard in peptide coupling.

[1]Ramos-Tomillero I., Rodriguez H., Albericio F.; *Org. Lett.* **2015**, 17, 7: 1680–1683.
[2]Ramkisson S., Al-Rasheed H. H., Dahlous K. A., De La Torre B. G., El-Faham A., Albericio F.; *Chemistry Select* **2021**, 6: 6626-6630.
[3]Tang S., Cheng J.-Y., Zheng J.-S.; *Tetrahedron Lett*. **2015**, 56: 4582-4585.
[4]Miret-Casals L., Van De Putte S., Aerssens D., Diharce J., Bonnet P., Madder A.; *Front. Chem.* **2022**; 9: 799706.
[5]De Geyter E., Antonatou E., Kalaitzakis D., Smolen S., Iyer A., Tack L., Ongenae E., Vassilikogiannakis G., Madder A.; *Chem. Sci*. **2021**, 12: 5246-5252
[6]Uth C., Englert S., Avrutina O., Kolmar H., Knauer S.; *J Pept. Sci.* **2023**; 29: e3527.
[7]Liu T., Peng Z. , Lai M., Hu L., Zhao J.; *J. Am. Chem. Soc*. **2024**; 146: 4270-4280.

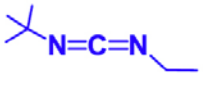
P-182

No-Wash Peptide Synthesis, *UE*-SPPS, with TBEC as a Replacementfor DIC

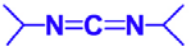
Drew Cesta, Sandeep Singh, Jonathan Collins

CEM Corporation, Matthews, USA

Diisopropylcarbodiimide, DIC, and ethyl (hydroxyimino)cyanoacetate, Oxyma, are widely used for amino acid activation in peptide synthesis. It was recently reported that reaction between DIC and Oxyma generates hydrogen cyanide, HCN.[1] Tert-Butylethylcarbodiimide, TBEC, has been reported as a viable substitute for DIC that eliminates the formation of HCN in presence of Oxyma.[2] *In-situ* carbodiimide-based coupling has shown minimal epimerization and side product formation at elevated temperature.[3]



***Tert*-Butylethylcarbodiimide (TBEC)**



Diisopropylcarbodiimide (DIC)

It was of interest to conduct a comparison study on the use of DIC and TBEC under elevated temperature coupling using microwave irradiation with ultraefficient "no-wash," UE-SPPS, conditions between all reactions. Our initial results with detailed protocols for synthesis and purity analysis will be discussed.

[1]Gui, L., Adjiman, C., Galindo, A., Sayyed, F., Kolis, S., Armstrong, A. *Ind. Eng. Chem. Res.* **2023**, 62, 874.
[2]Manne, S., Akintayo, D., Luna, O., El-Faham, A., de la Torre, B., Albericio, F. *Org. Process Res. Dev.* **2022**, 26, 2894.
[3]Collins, J., Porter, K., Singh, S., Vanier, G. *Org. Lett.* **2014**, 16, 940.

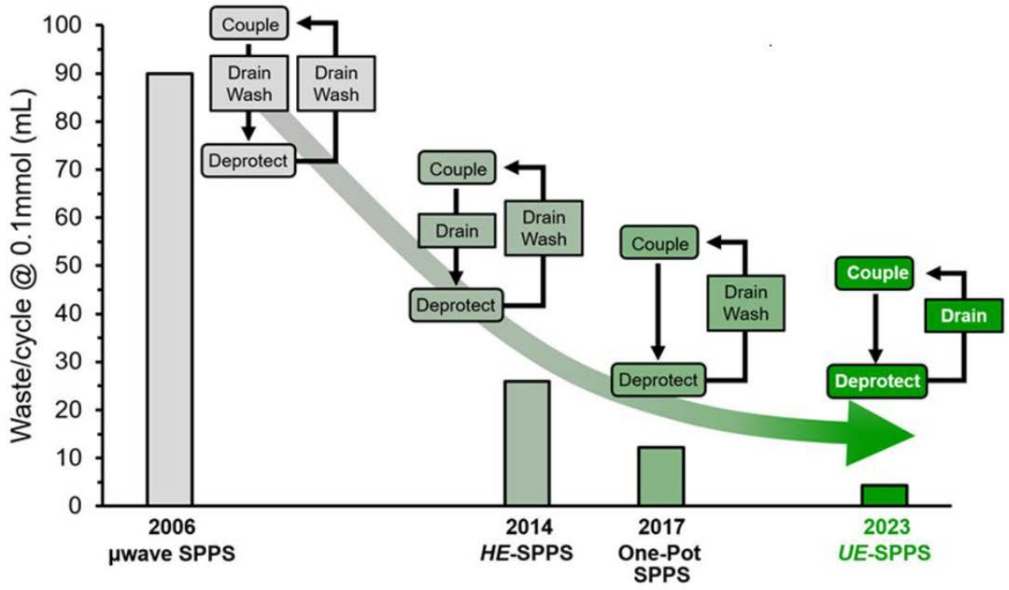
P-183

Ultra-Efficient Solid Phase Peptide Synthesis of Pharmaceutical Peptides

Matthew Kessler, Drew Cesta, Sandeep Singh, Colin Simpson, and Jonathan Collins

CEM Corporation, Matthews, USA

The surge in use of GLP-1 agonists and other therapeutic peptides has increased the demand for solid phase peptide synthesis, SPPS. At the same time, sustainability concerns around the waste and environmental impact of the process have grown. Recently we reported an ultra-efficient methodology for automated SPPS that eliminates up to 95% of the solvent waste from the process by eliminating resin washing steps after each coupling and deprotection.[1]



One key advantage of this wash-free method is that it does not require the use of specialty activators or amino acids, and instead focuses on minimizing the usage of reagents that are commonly available and widely used. This feature makes the methodology easy to implement into existing peptide research and production programs.

Here we have demonstrated the general applicability of the methodology by synthesizing a variety of pharmaceutically relevant peptides ranging in lengths from 18-mer to 86-mer including bivalirudin, exenatide, liraglutide, and pro-insulin. Despite these sequences presenting a range of synthetic challenges, the methodology resulted in high crude purities for each peptide, demonstrating that the method has wide scope. The application of microwave heating also greatly increased the speed of synthesis compared to traditional SPPS. At research scale, 0.1 mmol, each cycle of amino acid coupling and deprotection required less than 4 minutes, and produced ≤ 5.6 mL of waste. The broad scope of this methodology combined with substantial waste reduction makes it a promising strategy to improve the sustainability of peptide-based research and industrial production.

1. Collins J., Singh S., White T., Cesta D., Simpson C., Tubb L., Houser C. Nature Commun 2023, 14, 8168.

P-184

Insights Into the Mechanism of Diatom Peptide-Guided Biomimetic Silica Formation

Christian F. W. Becker

University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Vienna, Austria

Silaffin proteins are key components in the formation of siliceous cell walls in diatoms[1,2] and we have previously taken advantage of silaffin-based peptides to form silica particles in biomimetic processes. The presence of specific amino acid motifs, their connectivity as well as stereochemistry play an important role in their capacity to form silica structures of varying morphologies.[3,4]

Here, we present our current efforts to understand the self-assembly properties of linear, branched silaffin-based peptides and of posttranslationally modified silaffin-1A1.[5] The mechanisms of how peptide assemblies are controlled at the molecular level and how they translate into silica structures are studied by very fast NMR techniques. Employing NMR-derived constraints enables a fractal-cluster formalism that reveals the architecture of the peptide assemblies in atomistic detail. The morphology of the peptide templates is translated into the shape of bioinorganic particles via a mechanism that includes surface coating and particle precipitation.[6]

[1]N. Kroger, R. Deutzmann, M. Sumper, *Science*, **1999**, 286, 1129–1132.
[2]C. C. Lechner, C. F. Becker, Mar. *Drugs*, **2015**, 13, 5297–5333.
[3]Kamalov, M., Capel, P.D., Rentenberger, C., Müllner, A.R.M., Peterlik, H. Becker, C.F.W., *ChemNanoMat*, **2018**, 4, 1209-1213.
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[5]Daus, F., Xie, X., Geyer, A. *Org Biomol Chem* **2020**, 20, 3387-3396.
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P-185

Constrained Peptide Modeling, Conformational Analysis and Property Predictions

Alain Ajamian

Chemical Computing Group

Peptides play an integral role in a myriad of biological pathways. Given their geometric and chemical diversity, peptides can effectively bind a broad spectrum of biological targets. This inherent diversity also presents numerous challenges such as the size of amino acid sequence space, assessment of low energy peptide conformational states and prediction of peptide properties. In this work, we present *in silico* methods for building peptide models, sampling peptide conformations and predicting the properties of peptides.

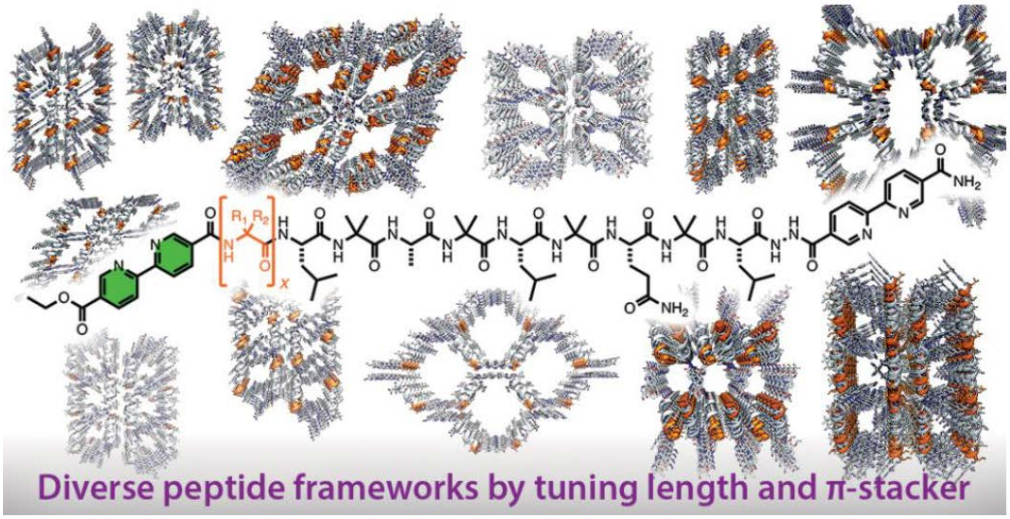
P-186

π-Stacking Peptides as Building Blocks for Evolvable Porous Materials

Andy Nguyen

University of Chicago, Chicago, USA

A longstanding goal is to create crystalline porous materials that mimic protein complexity, evolvability, and dynamics. Towards this end, peptides have been pursued as building blocks for porous materials, but the difficulty of peptide design and structural characterization present major obstacles. To address these challenges, our laboratory has developed a strategy that reliably generates numerous peptide-based porous crystals by leveraging π-stacking and non-canonical moieties.[1-4]



These resulting frameworks have multiple variable positions that enable rapid engineering of complex pore environments reminiscent of protein active sites, and they can utilize flexibility, cooperativity, and site-isolation effects to achieve unique reactivity and host-guest chemistry. Notably, nearly peptide frameworks form single crystal suitable for X-ray diffraction that reveal structural-functional relationships in high detail, laying the foundation for the rational design of complex protein-like materials.

[1]Ganatra, P., Wang, D. F., Ganatra, V., Dang, V. T., Nguyen, A. I. *JACS* **2024**, 146, 22236–22246
[2]Heinz-Kunert, S. L., Pandya, A., Dang, V. T., Oktawiec, J., Nguyen, A. I. *Biomacromolecules*, **2024**, 25, 2016-2023
[3]Hess, S.S., Coppola, F., Dang, V. T., Tran, P. T., Mickel, P. J., Oktawiec, J., Ren, Z., Král, P., Nguyen, A. I., *JACS.* **2023**, 145, 36, 19588-196000
[4]Heinz-Kunert, S. L., Pandya, A., Dang, V. T., Tran, P. N., Ghosh, S., McElheny, D., Santarsiero, B. D., Ren, Z., Nguyen, A. I., *JACS.*, **2022**, 144, 7001–7009.

P-187

Epitaxial Growth of Coiled-Ctoil Peptide Crystals

Ashutosh Agrahari, Andrew Encinas, and Jean Chmielewski

Purdue University, West Lafayette, USA

Epitaxial growth to form layered materials has garnered significant attention in materials science to achieve improved physical and chemical properties. Inorganic molecules and organic polymers have been extensively utilized toward controlled fabrication. However, the application of epitaxial growth in peptide and protein space remains relatively limited due to their complexity.

Previously, coiled-coil peptides, **p2L** and **p2Ida**, containing metal binding ligands have been utilized to generate hexagonal crystals in presence of bivalent metal ions. Along with metal-promoted assembly, the capability to encapsulate His-tagged proteins via co-assembly presents a unique opportunity for metal binding-driven epitaxial growth. The layered peptide material formed as a result of epitaxial growth can broaden the applicability of coiled-coil based crystals with the use of different metals and proteins/enzymes in each layer.

In this work, we present the formation of multicomponent layered material via metal-directed epitaxial growth. The crystals formed with **p2L** and Zn (II) were used as seed crystals that contain exposed metal binding ligands in axial direction. Utilizing these, the second layer of material was grown using **p2Ida** and Cu (II). This process can be extended to grow a third layer. Fluorescent proteins, eGFP and mRuby3, were used to visualize epitaxial growth using confocal microscopy. Control experiments suggested that **p2Ida** and Cu (II) are essential for epitaxial growth. Our findings also revealed that higher temperature, 37ºC, leads to uniform growth. Scanning electron microscopy data further corroborated these findings. Experiments to probe other factors affecting epitaxial growth including the identity of metal ions are in progress.

P-188

Exploring Peptide Secondary Structure Within Protein-Like Polymers

Julia Oktawiec[1], Hassan Nadeem[2], Omar Ebrahim[1], Yu Chen[1], Clara Barbut[1], James Byrnes[3], Matthew Thompson[1], Diwakar Shukla[2], and Nathan Gianneschi[1]

[1]Northwestern University, Chicago, USA
[2]University of Illinois Urbana Champaign, Urbana,USA
[3]Brookhaven National Laboratory, Upton, USA

Protein-like polymers, PLPs, are peptide brush polymers generated by graft-through living polymerization of peptidyl-monomers. They exhibit emergent, proteomimetic properties that make them promising therapeutics, chemical biology tools and adhesive materials. In prior work we have found that the polymer scaffold of these materials promotes the folding of alpha-helical peptide brushes on the polymer. It is also important to understand if the beta-sheet folding of monomer units can be preserved on these polymers.

Towards that end, peptide monomers based on the tryptophan zipper Trpzip2 were polymerized and studied using spectroscopy and scattering. Strikingly, circular dichroism spectroscopy demonstrates that upon polymerization the hairpin structure is partially destabilized, which was further confirmed by infrared spectroscopy. Small-angle X-ray scattering analysis demonstrates that the polymers retain globular conformations in solution, suggesting that the globular conformation might be implicated in disruption of peptide folding.

These results are compared to molecular dynamics simulations that reveal key insight into the folding of the peptide sidechains upon polymerization. Overall, these results show that the beta-hairpin Trpzip2 is exceptionally sensitive to being appended to the polymer environment, suggesting that the polymer scaffold can significantly disfavor folding of this type. This insight is important for the future design of proteomimetics that incorporate beta-sheet segments that may be useful as therapeutics and as components in structural materials.

P-189

Characterizing the Importance of Hoogsteen Face Hydrogen Bonding in Self-Assembling Nucleopeptides

Jillian Smith-Carpenter, Tayana Jones, Morgan Shirley, Bianca Pineiro, and Rishi Black

Fairfield University, Fairfield, USA

Short self-assembling peptides can be modified on the N-terminus with various purines to create nucleopeptides. These nucleopeptides combine the self-assembling properties of the short peptides and hydrogen bonding recognition along the Hoogsteen face of the nucleobase to form nanostructures with different higher-order architectures dependent on their sequence and C-terminus chemistry.

The Smith-Carpenter lab has previously characterized higher ordered guanosine-based structures, such as G-quartets or G-ribbons, as secondary structures within supramolecular nucleopeptides using infrared spectroscopy, ^{1}H-NMR, and circular dichroism spectroscopy. After preliminary data was collected comparing guanosine, adenosine, and inosine purines on the N-terminus indicated that the Hoogsteen face hydrogen bonding is essential for stable self-assembly, we have now expanded our nucleopeptide library to include the addition of an isoguanosine modified nucleopeptide. The synthesis and characterization of these nucleopeptides will help our lab better understand the intermolecular interactions that occur during the assembly process and influence the supramolecular architecture of the nucleopeptides.

P-190

A Next-Generation Device Based on Peptide-Functionalized Silk Fibers for the Treatment of Gastrointestinal Bleeding

Fosca Errante[1], Chiara Bellini[1], Irene Chiesa[2], Andrea Guerra[2], Carmelo De Maria[2], Maria Rachele Ceccarini[3], Luca Valentini[3], Antonino Morabito[4], Paolo Rovero[1], Daniele Bani[5]

[1]Interdepartmental Laboratory of Peptide and Protein Chemistry and Biology, Departmentof Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Sesto Fiorentino, FI, Italy
[2]Department of Ingegneria dell'Informazione and ResearchCenter E. Piaggio, University of Pisa, Pisa, PI, Italy
[3]Civil and Environmental EngineeringDepartment and INSTM Research Unit, University of Perugia, Terni, TR, Italy
[4]Departmentof Pediatric Surgery, Meyer Children's Hospital IRCCS and Department of Neurosciences,Psychology, Drug Research and Child Health, NEUROFARBA, University of Florence,Florence, FI, Italy
[5]University of Florence, Florence, Italy

Gastrointestinal bleeding occurs within the digestive tract, encompassing the esophagus, stomach, small intestine, large intestine, colon, rectum, and anus. The complexity of treatment arises from various factors, including the severity and location of the ulcers, often necessitating invasive procedures such as esophagogastroduodenoscopy or colonoscopy. Silk fibroin, a natural biocompatible and biodegradable polymer extracted from silkworms, has been shown to promote cell adhesion and regulate cellular behavior.[1] Previous studies demonstrated that conjugating an RGD peptide sequence to degummed silk fibroin fibers could modify their mechanical properties and promote cellular interactions.[2,3]

Building on this foundation, we aimed to develop a deployable silk empowered device fabricated using a 4D printing approach designed to chemically and physically interact with bleeding ulcers. To this aim, we selected a peptide sequence capable of binding to P-selectin, a cell adhesion molecule expressed by activated endothelial cells and platelets involved in ulcer inflammation.[4] In addition to adhering to the inflamed site, the device will be engineered to wirelessly communicate and promote tissue regeneration at the injured location, offering a multifunctional solution for GI bleeding treatment.

This work was supported in the framework of the project Prin2022 Prometheus "4D printing selfdeploying bioenabled polymer scaffolds for the non-invasive treatment of bleeding intestinal ulcers", grant: 2022BZLTTK, CUP: I53D23002200006.

[1]D. Lin, M. Li, L. Wang, J. Cheng, Y. Yang, H. Wang, J. Ye, Y. Liu, *Adv Funct Materials* **2024**, 2405255.
[2]L. Valentini, L. Pacini, F. Errante, C. Morchio, B. Sanna, P. Rovero, A. Morabito, *Molecules* **2022**, 27, 4605.
[3]M. R. Ceccarini, V. Palazzi, R. Salvati, I. Chiesa, C. De Maria, S. Bonafoni, P. Mezzanotte, M. Codini, L. Pacini, F. Errante, P. Rovero, A. Morabito, T. Beccari, L. Roselli, L. Valentini, *IJMS* **2023**, 24, 947.
[4]Y. Zhang, X. Zhu, X. Chen, Q. Chen, W. Zhou, Q. Guo, Y. Lu, C. Li, Y. Zhang, D. Liang, T. Sun, X. Wei, C. Jiang, *Adv Funct Materials* **2019**, 29, 1806620.

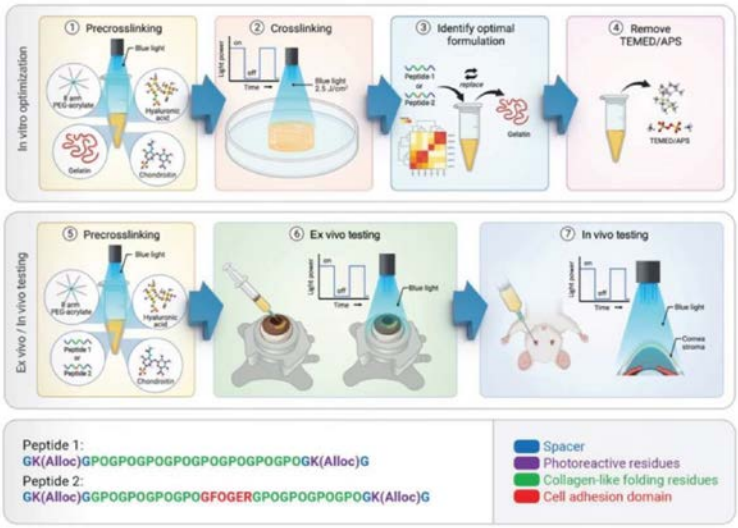
P-191

Photoactive Peptide Injectable Materials for Restoring Thinning Corneas

Marcelo Munoz[1], Aidan MacAdam[1], Jinane El Hage[1], Alex Ross[1], May Griffith[2], Isabelle Brunette[2], and Emilio Alarcon[3]

[1]University of Ottawa Heart Institute, Ottawa, Canada
[2]Département d'Ophtalmologie,Université de Montréal, Montreal, Canada
[3]University of Ottawa, Ottawa, Canada

Many alternatives to human donor corneas are being developed to meet the global shortage of donated tissues. However, corneal transplantation remains the gold standard for diseases resulting in thinning corneas. In this work, transparent low-energy photoactivated extracellular matrix peptide-mimicking materials are developed for intrastromal injection to restore stromal thickness. The injectable biomaterials are comprised of short peptides and glycosaminoglycans, chondroitin, hyaluronic acid, that assemble into a hydrogel when pulsed with low-energy blue light. The dosage of pulsed-blue light needed for material activation is minimal at 8.5 mW cm^{-2}, thus circumventing any blue



light cytotoxicity. Intrastromal injection of these light-activated biomaterials in rat corneas show that two iterations of the formulations remain stable *in situ* without stimulating significant inflammation or neovascularization for up to 6 weeks. The use of low light intensities and the ability of the developed peptide-materials to stably rebuild and change the curvature of the cornea tissue make these formulations attractive for clinical translation.

P-192

Highly Specific Crosslinked Peptide Inhibitors of the EphB2 Receptor

Brian Garcia, Sophie Epstein, Jenna Cain, Jessica Tennett, Nicholas Sawyer

Fordham University, Bronx, USA

The EphB2 receptor, EphB2, is a member of the largest family of receptor tyrosine kinases and plays key regulatory roles in tissue development and homeostasis. EphB2 receptor signaling is stimulated through binding to ephrin protein ligands, leading to bi-directional signaling in ephrin- and receptor-bearing cells. EphB2 signaling impacts various cell processes, including cell proliferation and migration, and EphB2 upregulation has been associated with many different cancers.

Nonetheless, therapeutic targeting of EphB2 is challenging. Because the fourteen human Eph receptors have similar composition and structure, it is difficult to identify molecules that target individual receptors. Also, the interacting EphB2-ephrin surface is large and thus challenging to target with small molecules. Phage display has been applied successfully to identify a highly specific EphB2-binding peptide called SNEW, but its modest, micromolar, binding affinity requires further optimization for effective Eph receptor targeting.

We hypothesized that covalent crosslinking of flexible regions of the SNEW peptide would stabilize favorable interactions with EphB2 to improve receptor binding affinity. We independently introduced crosslinkers at both the N- and C-terminal regions of the SNEW peptide to create new SNEW variants and determined that these variants exhibit greater binding affinity for EphB2 while maintaining EphB2 binding specificity. These crosslinkers could also be combined to yield potent EphB2 inhibitors. Interestingly, peptide variants with N-terminal crosslinkers were found to inhibit EphB2 signaling more potently in cell-based assays. These variants now serve as leads for investigating the molecular origins of EphB2 signaling inhibition and improvement of inhibitory potency for treatment of EphB2-associated cancers.

P-193

Cytosolic Delivery of Biomacromolecules via Micro-Coacervates

Yoshimasa Kawaguchi, Megumi Kiyokawa, Ayumi Kikkawa, and Shiroh Futaki

Kyoto University, Uji, Japan

We have previously developed the cationic intracellular delivery peptide L17E for cytosolic delivery of macromolecules such as antibodies.[1] Furthermore, it was found that micro-coacervates, which are droplet-like particles, are formed by multimers of L17E and the negatively charged fluorescently labeled IgG, Alexa488-IgG, leading to the cytosolic diffusion of Alexa488-IgG.[2,3]

Based on this finding, we aimed to develop a more versatile and practical IgG delivery method without need for Alexa488 modification of IgG. We designed IgGs modified with negatively charged tags, capable of forming micro-coacervates with L17E multimers, without the need for Alexa labeling, and successfully achieved efficient cytosolic delivery of these IgGs. Furthermore, we explored mRNA, which has a strong negative charge, to evaluate its coacervate formation with L17E multimers and subsequent mRNA delivery. While mRNA alone aggregated with L17E trimers instead of forming micro-coacervates, the addition of ssDNA enabled their formation. Treating cells with these micro-coacervates resulted in protein expression, and *in vivo* mRNA delivery was also successfully achieved.

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[2]Iwata T., Hirose H., Sakamoto K., Hirai Y., Arafiles J. V. V., Akishiba M., Imanishi M., Futaki S. *Angew. Chem. Int. Ed.* **2021**, 60, 19804-19812.
[3]Michibata J., Kawaguchi Y., Hirose H., Eguchi A., Deguchi S., Takayama K., Xu W., Niidome T., Sasaki Y., Akiyoshi K., Futaki S. *Bioconjug. Chem.* **2024**, 35, 1888–1899.

P-194

Optimization of IEDDA Reaction Parameters

Rasheda Samiha, Arundhati Nag

Clark University, Worcester, USA

Inverse Electron Demands Diels-Alder, IEDDA, reaction is a biorthogonal reaction that potentially can be used for various applications such as bioconjugation, imaging, screening et cetera. The reaction kinetics of the IEDDA reaction varies based on the nature of the substitutions on the tetrazine and diene. So far, IEDDA reaction studies have focused on the use of electron-withdrawing heterocyclic aromatic ring containing tetrazines with various dienophiles, due to the fast kinetics of the reactions.

There have been no detailed studies on S, S-disubstituted tetrazines with various dienophiles. While we anticipate these reactions would have slower kinetics, incorporation of S,S-disubstituted tetrazine in biomolecules such as peptides is synthetically much less challenging than the incorporation of an aromatic N,N-tetrazines, and detailed studies of the S,S-tetrazines with various dienophiles would offer valuable knowledge that can lead to new applications that do not need fast kinetics.

We recently developed S, S-tetrazine linked molecules in biocompatible systems in our laboratory that can be easily synthesized. We are studying their reaction kineties with moderately reactive dienophiles like norbornene-containing or cyclopropane-containing molecules under various organic and aqueous conditions using various spectroscopic and analytical techniques and will present our results.

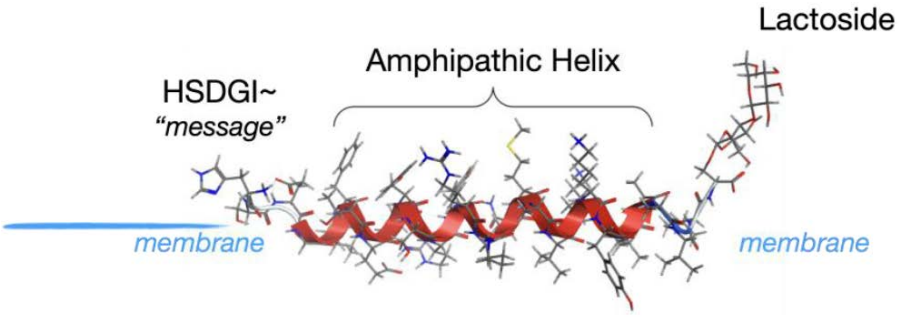
P-195

PACAP Glycosides for the Treatment of Ischemic Stroke

Fereshteh Nugent[1], Troy Smith[2], Lajos Szabò[2], Fahad Al-Obeidi[2], Minying Cai[2], Torsten Falk[2], and Robin Polt[2]

[1]Uniform Services University, Bethesda, USA
[2]The University of Arizona, Tucson, USA

PACAP, Pituitary Adenylate Cyclase Activating Peptide, is a pituitary hormone secreted as either a 27-mer or 38-mer. It has pleiotropic effects at 3 cognate receptors PAC1, VPAC1 or VPAC2. This hormone is far older than the pituitary



gland, mammals, or even vertebrates. Creatures 700MYA produced PACAP, and while it's receptors have evolved, the hormone remains largely unchanged and is identical in most mammalian life forms.

Glycosylated variants of PACAP have been shown to shift PC12 cells from growth phase to development, and more importantly, these O-linked glycopeptides show extended lifetimes *in serum* and penetrate into the CNS to produce therapeutic effects in mouse models of stroke,[1] mTBI and Parkinson's disease.[2] Truncation of the glycosides produces selective PAC1 agonists.

[1]Bernard K, Dickson D, Anglin BL, Leandro Heien M, Polt R, Morrison HW, Falk T. PACAP glycosides promote cell outgrowth *in vitro* and reduce infarct size after stroke in a preclinical model. *Neuroscience Letters* **2024**, 836, 137883.
[2]Apostol CR, Bernard K, et al. Design and Synthesis of Brain Penetrant Glycopeptide Analogues of PACAP with Neuroprotective Potential for Traumatic Brain Injury and Parkinsonism. *Frontiers in Drug Discovery,* **2022**, #818003

P-196

A Conformationally Neutral Proline Substitution to Increase Macrocycle Permeability and Oral Bioavailability

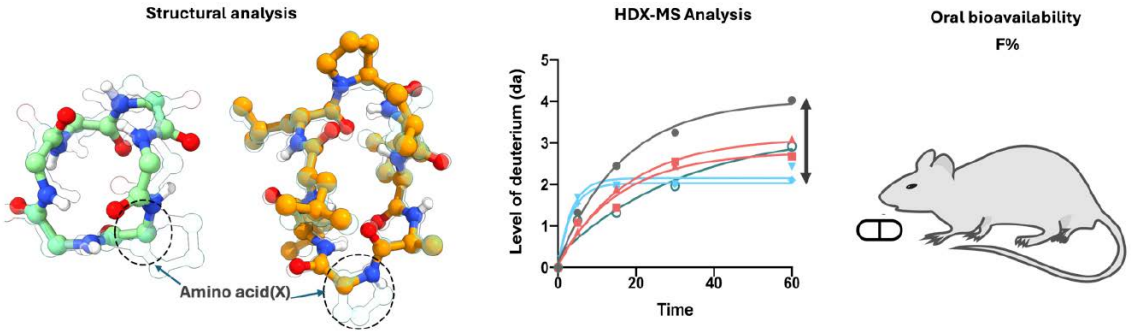
Nishant Raj, Joyati Das, and Jayanta Chatterjee

Indian Institute of Science, Bangalore, India

Macrocyclic peptides are emerging as a novel modality in drug discovery against a wide range of targets. The structure adopted by small peptide macrocycles, owing to its conformational freedom in the φ,Ψ, and χ torsion angles, unachievable by small molecules, allows them to engage complex target surfaces efficiently. However, the cellular permeability of a peptide macrocycle, a significant barrier to drug development, is dictated by its conformation, which undergoes differential solvation while traversing the plasma membrane.

Here, we show how a helicogenic amino acid incorporation constrains the macrocycle backbone and improves the passive permeability of a macrocycle. Our study was guided by MALDI mass spectrometry-assisted Hydrogen Deuterium Exchange, which reliably estimates the solvation of the macrocycle amide protons within polar and nonpolar environments, mimicking the extracellular space and membrane interior, respectively. We also note that this amino acid can conveniently substitute the D-/Lproline within macrocycles to achieve a near-identical conformation with enhanced passive permeability.

Finally, the enhanced macrocycle permeability increases oral bioavailability due to this conformationally neutral proline substitution, advocating the incorporation of the helicogenic amino acid in designing membrane permeable macrocycles.



P-197

Designing Peptides that Bind 14-3-3ε and Inhibit Cutaneous Squamous Cell Carcinoma

Sandor Lovas, Seraphine Kamayirese, Laura Hanson

Creighton University, Omaha, USA

14-3-3 proteins play a critical role in various cellular processes by regulating the activity and function of other proteins. In cutaneous squamous cell carcinoma, cSCC, the overexpressed 14-3-3ε forms heterodimers with the ɣ and ζ isoforms and interacts with CDC25A protein to suppresses apoptosis. Thus, inhibition either the heterodimerization of 14-3-3ε or its interactions with CDC25A are promising strategy for development of therapeutics for cSCC.

We have designed a virtual tetrapeptide library for *de novo* identification of optimal peptide ligands that binds to the *N*-terminal dimerization domain of 14-3-3ε which contains a unique Tyr^9 residue. Molecular dynamics, MD, simulations and Markov state modeling of hundreds of MD trajectories revealed the binding path and the negative free energy of binding, ΔG_b, of the peptide. The synthetic tetrapeptide blocked 14-3-3ε heterodimerization, prevented its interaction with CDC25A, killed cSCC cells and significantly decreased growth of skin tumor xenografts.

In the second strategy, from different regions of CDC25A, we designed two 14-residue phopsopeptide fragments containing either phospho-Thr, (pT(502-515) or phospho-Ser (pS(173-186)) residue. Both peptides bind 14-3-3ε and induce cell death of cSCC cells, although, at high micromolar IC_{50}. Using MD simulations, peptides were shortened to 9-residue analogs. Steered and umbrella sampling MD simulations showed that both peptides bind to 14-3-3ε with large negative ΔG_b. Based on *in silico* experiments various substitutions were introduced in both peptides.

Experimental K_D of the synthetic peptides for recombinant 14-3-3ε was determined using surface plasmon resonance, SPR. K_D values of specific residue replacement resulted in analogs with a range of 2.0 –144.3 nM. Determination of cSCC cell apoptosis induced by peptide analogs is in progress.

P-198

A Cyclic Peptide Appears to Inhibit Growth of Ovarian Cancer Cell Lines by Different Mechanisms

Laura Hanold

University of Florida, Gainesville, USA

The American Cancer Society has estimated that over 20,000 new ovarian cancer cases and over 12,000 ovarian cancer-related deaths will occur in the United States in 2025. Although survival rates have been improving, primary

and acquired resistance and severe side effects remain major limitations of current chemotherapies. Thus, there is a need to develop alternative treatment options for patients who do not respond to current therapies and to limit side effects. Cancerous inhibitor of protein phosphatase 2A, CIP2A, which inhibits tumor suppressor protein phosphatase 2A, PP2A, is overexpressed in over 65% of ovarian cancer cases and is associated with poor prognosis. Knockdown of CIP2A decreased cell proliferation and increased sensitivity to cisplatin in ovarian cancer cells. Thus, downregulating CIP2A and/or its associated proteins may be a promising approach to treat ovarian cancer and overcome chemoresistance.

Our laboratory has identified a lead cyclic peptide that decreases proliferation in ovarian cancer cell lines, with selectivity for these cells over a non-cancer control. The peptide appears to be more potent in cell lines expressing high levels of the oncoproteins CIP2A and/or c-Myc. However, its effects on CIP2A, c-Myc, and PME-1 protein levels, PP2A post-translational modification, and Akt phosphorylation differ between two ovarian cancer cell lines despite having similar anti-proliferative potency in both, suggesting that this peptide may have different mechanism/s of action in different cell lines. Multiple analogs of this peptide also demonstrated anti-proliferative activity in ovarian cancer cell lines with high levels of CIP2A. Thus, these anti-proliferative cyclic peptides may have potential for downregulating CIP2A and/or associated proteins and treating CIP2A- and c-Myc-overexpressing ovarian cancer.

P-200

Stalking Elusive Pathogenic Bacteria: Diving into Cells to Treat Infections

Alysha Reichel[1], Andrew Encinas[1], Reena Blade[1], Manish Nepal[1], Mohamed Seleem[2], and JeanChmielewski[1]

[1]Purdue University, West Lafayette, USA
[2]Virginia Tech, Blacksburg, USA

A significant challenge in the development of effective antibacterial agents arises from bacterial pathogens that have evolved to inhabit mammalian cells, such as phagocytic macrophages. Within these intracellular safe havens the bacteria reproduce and form a repository, and are able to evade the host immune response as well as a number of antibiotic drugs. Therefore, there is a great need to develop antibiotics with the ability to enter mammalian cells and target intracellular pathogens at their specific sub-cellular site.

We have developed a class of molecules, cationic amphiphilic polyproline helices, CAPHs, that enter mammalian cells through both direct transport and endocytosis. We have determined that CAPHs also have potent antibacterial activity *in vitro* with a non-lytic mechanism of action. This dual mode of action, non-lytic antibacterial activity with the ability to localize within mammalian cells, provided us with agents with a pronounced ability to target and kill pathogenic intracellular bacteria within human macrophages.

P-201

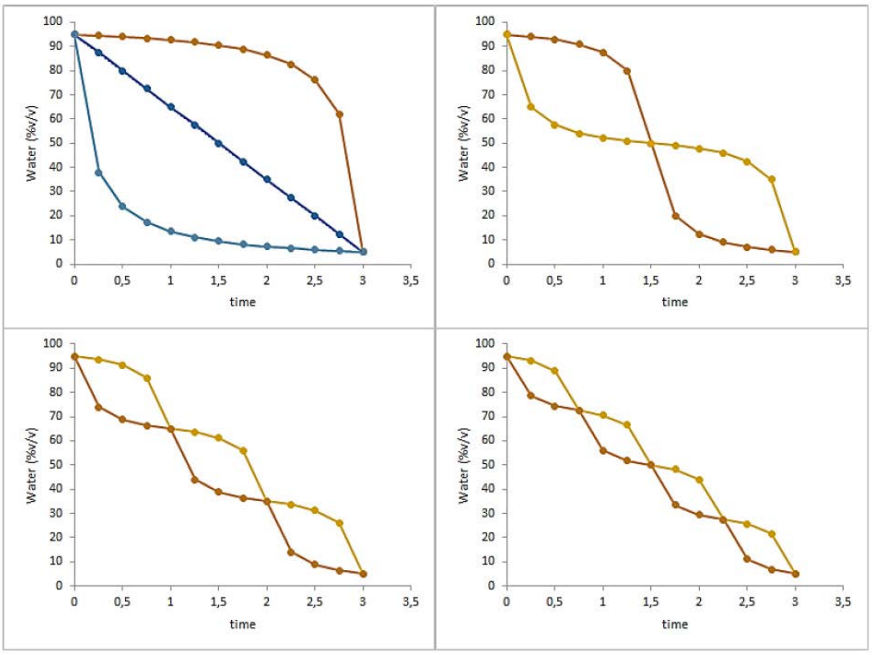
Separation of Synthetic Peptides Using Constant Volume Fraction Gradient HPLC

Mohammadreza Taheri, Stephan Uebel

Biochemistry Core Facility, Max Planck Institute of Biochemistry, Martinsried, Germany

Chromatographic separation remains the gold standard for peptide purification, but synthetic peptides can pose a challenge due to the presence of multiple peptides with similar structures in the crude mixture.[1]

To address this issue, we developed a set of fast elution methods based on constant volume fraction gradients. These methods were designed to improve the separation and purification of synthetic peptides. We compared the performance of our developed method with conventional linear gradient elution using a standard mixture of peptides as the analyte. The critical parameters including retention factor, resolution, number of theoretical plates, tailing and symmetry were investigated.



The results showed that the constant volume fraction gradient method achieved promising separation and purification results. Furthermore, the method was applied to real synthetic crudes, demonstrating its effectiveness in real-world applications

[1]Al Musaimi O.; Jaradat D. S. *Separations*. **2024**, 11, 233-250.

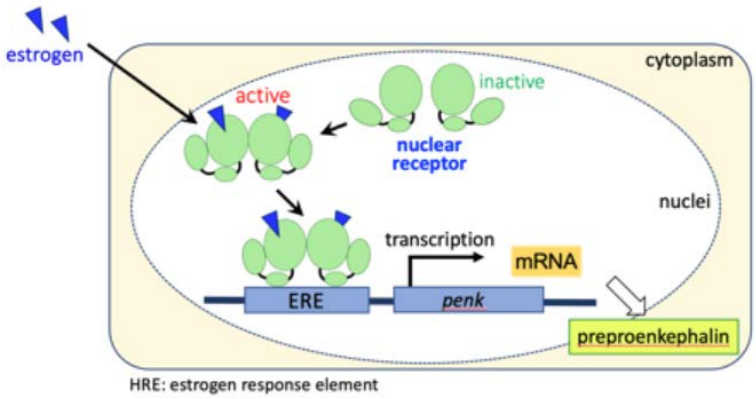
P-202

Identification of Estrogen-Response Elements Regulating the Expression of the Proenkephalin Precursor Gene

Keita Nakamura, Takeru Kajiyama, Kohei Kanazaki, Kyota Shirane, Ayami Matsushima

Kyushu University, Fukuoka, Japan

Studies have shown gender-based disparities in pain sensitivity. While testosterone is linked to pain signaling, it does not fully explain the differences in pain modulation between sexes. Variations in opioid peptide expression related to analgesia are also observed between males and females. Given these findings, we theorize that estrogen receptors play a role in regulating opioid peptide expression. Two types of estrogen receptors exist: α-type, ERα, and β-type, ERβ, both present in the brain and reproductive system. ERβ has been studied less compared to ERα.



Our research explored whether ERα and ERβ influence opioid peptide gene transcription by using existing ERα databases and chromatin immunoprecipitation sequencing, ChIP-seq, on mouse brains for ERβ. We examined ERα binding to the opioid peptide gene through gel shift testing. ChIP-seq data for both receptor types indicated binding near the enkephalin gene, *penk*, an opioid peptide.

We assessed the transcriptional activity of this region using a reporter gene assay, revealing mild estrogen-responsive activity. Additionally, we analyzed potential ER response sequences using three methods: human observation, JASPER, a transcription factor binding prediction tool, and AlphaFold3, an advanced structure prediction AI. We verified direct ERα binding to ERE near the *penk* gene, albeit weakly, suggesting that this subtle interaction is crucial for physiological processes. This discovery may contribute to understanding novel functions of ERs in the central nervous system and explain gender differences in pain perception.

[1] A. Dance, *Nature* **2019**, 567, 448-450

P-203

Phage-Encoded Noncanonical Amino Acids for Peptide-Based Drug Discovery

Wenshe Liu

Texas A&M University, College Station, USA

The conventional phage display technique, while a powerful tool for drug discovery, is limited by its reliance on the 20 genetically encoded amino acids. To expand the chemical diversity and enable unique chemistries for phage-displayed peptides targeting drug interactions, we have developed an innovative phage display system integrated with noncanonical amino acids, ncAAs, encoded by the amber stop codon.

Utilizing genetically encoded ncAAs, we demonstrated that phage-displayed peptides can undergo simultaneous cyclization. This approach enabled us to construct phage-displayed macrocyclic peptide libraries, facilitating the identification of potent ligands for selected drug targets.

Additionally, we developed a novel technique called phage-assisted, active site-directed ligand evolution, PADLE. In PADLE, a genetically encoded ncAA acts as a ligand to bind the active site of an epigenetic protein, guiding phage-displayed peptides towards enhanced binding. Using PADLE, we successfully identified ultra-high potency ligands for SIRT2, HDAC8, ENL, and BRD9. Some of these ligands have also shown strong cellular potency in inhibiting these targets.

[1]Hampton J.T. and Liu W.R., "Diversification of Phage-Displayed Peptide Libraries with Noncanonical Amino Acid Mutagenesis and Chemical Modification", *Chem. Rev.* **2024**, 124(9):6051-6077.
[2]Chen P.-H.C., Guo X.S., Zhang H.Z., Dubey G.K., Geng Z.Z., Fierke C.A., Xu S., Hampton J.T., and Liu W.R., "Leveraging a Phage-Encoded Noncanonical Amino Acid: A Novel Pathway to Potent and Selective Epigenetic Reader Protein Inhibitors", *ACS Cent. Sci.* **2024**, 10(4): 782-792.
[3]Morse J.S., Shen Y.J., Hampton J.T., Sylvain L.D., Das S., Alugubelli Y.R., Chen P.C., Yang K.S., Xu S., Fierke C.A., and Liu W.R., "Phage-Assisted, Active Site-Directed Ligand Evolution of a Potent and Selective Histone Deacetylase 8 Inhibitor", *Protein Sci.* **2022**, 31(12): e4512.
[4]Tharp J.M., Hampton J.T., Reed C.A., Ehnbom A., Chen P.C., Morse J.S., Kurra Y., Perez L.M., Xu S., and Liu W.R., "An Amber Obligate Active Site-Directed Ligand Evolution Technique for Phage Display", *Nat. Commun.* **2020**, 11(1):1392.

P-204

De Novo Designed Proteins and Synthetic Derivatives Clear Small Molecule Drugs *In Vivo*

Guilin Chen[1], Kai Zhao[1], Mengjiao Li[1], Yuefei Zhang[1], Lei Lu[2], Dan Liu[1], Jesús Valdiviezo[3], Nicholas Polizzi[3], William DeGrado[2], and Bobo Dang[1]

[1]Westlake University, Hangzhou, China
[2]UCSF, San Francisco, USA
[3]Dana-Farber Cancer Institute, Boston, USA

Small-molecule-binding proteins can selectively bind and eliminate toxins or cytotoxic drugs withside effects. Despite their significance, the generation of such proteins using traditional approachesposes challenges. While *de novo* protein design has successfully yielded small molecule-binding proteins, there remains to be shown that any designed protein is useful to bind complex drug molecules for *in vivo* pharmacology, serving as an antidote to clear toxins or cytotoxic drugs. Apixaban is an anticoagulant drug with bleeding as a major side effect, and rucaparib is a cytotoxic PARP inhibitor for cancer treatment.

Here, we present the *in vivo* pharmacological efficacy of *de novo* designed proteins targeting apixaban or rucaparib for clearance in mice, potentially mitigating the side effects of these small molecule drugs. The achiral nature of apixaban allows for the integration of conventional medicinal chemistry techniques, such as mirror-image synthesis and cyclization, with *de novo* protein design to generate apixaban-binding mirror-image protein and apixaban-binding cyclic protein. Both variants exhibited the intended *in vivo* functionality, rapidly clearing apixaban. Our findings validate the feasibility of directly designing *in vivo* functional small molecule-binding proteins, which can be seamlessly integrated with traditional medicinal chemistry strategies for tailored *in vivo* pharmacology. The concurrent generation of drugs and their binding proteins with embedded *in vivo* functionality opens new avenues for extended pharmaceutical chemistry, offering innovative solutions for targeted drug clearance.

G. Chen, K. Zhao, M. Li, T. Li, N. F. Polizzi, W. F. DeGrado, B. Dang, De novo-designed natural and unnatural proteins clear apixaban *in vivo*. *Nat. Biomed. Eng*, **2024**

P-205

Synthesis and Purification Strategies for Complex and Challenging Peptides

Beibei Meng

WuXi TIDES, Shanghai, China

Solid-phase peptide synthesis, SPPS, is a well-established and efficient method for peptide production. However, in practical applications, certain long or complex peptides tend to adopt secondary structures, both in solution and when attached to solid-phase resins. This structural behavior can lead to peptide aggregation, which poses significant challenges during synthesis by hindering chain elongation and causing solubility issues. Such issues not only complicate the purification process but also interfere with accurate yield determination. As a result, synthesizing and purifying complex peptides remains a significant technical hurdle. This poster presents two case studies that illustrate these challenges and the strategies we employed to overcome them.

The first case involves a 57-amino acid peptide containing two free cysteine residues. By methodically optimizing each step of the synthesis, we were able to minimize aggregation and ensure a high-purity final product suitable for downstream activity assays. In addition, a two-step optimized purification strategy enabled an improved separation of impurities and resulted in a higher overall yield. The second case involves the implementation of alternative coupling and cyclization protocols to enable synthesis and cyclization of a large library of hydrophobic macrocyclic peptides containing numerous unnatural amino acids. These cases demonstrate practical approaches to overcoming common synthetic and purification obstacles in peptide chemistry.

P-206

Advanced Synthesis and Purification Technology Enhances GLP-1 Receptor Agonist Production for Improved Diabetes and Obesity Treatment

Stephan Lüdtke[1], Robert Zitterbart[2], Dominik Sarma[1], Lorenzo Pacini[3], Anna Maria Papini[3], and Paolo Rovero[3]

[1]Gyros Protein Technologies, Tucson, USA
[2]Gyros Protein Technologies, Teltow, USA
[3]Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, Departments of Chemistry "Ugo Schiff " and NeuroFarBa, University of Florence, Florence,Italy

GLP-1 receptor agonists like Semaglutide and Liraglutide stand out as effective solutions for managing blood glucose while promoting weight loss to treat diabetes and obesity. However, their large-scale production faces challenges in achieving high purity and yield sustainably. Our project focused on optimizing their synthesis and purification using advanced techniques.[1]

We explored scalable catch-and-release, C&R, methodology,[2] and different late-stage modification patterns. The strategies we tested yielded high purities before the final HPLC purification both for Liraglutide and Semaglutide while also reducing the ecological impact of the process. The positive outcomes from our trials demonstrate the potential of advanced peptide purification technologies, such as C&R, to improve and expedite the availability of GLP-1 receptor agonists.

Notable, using a lipidated amino acid building block for the linear synthesis and purification of Semaglutide resulted in better purity and yield than the late-stage modification methods we tested successful for Liraglutide. The results emphasize the need for innovative purification techniques and multidimensional synthesis strategies to enhance green production processes for GLP-1 therapeutics and address future production challenges.

[1]*Int. J. Pept Res Ther* **2025**, under minor revisions
[2]R. Zitterbart; N. Berger; O. Reimann; G. T. Noble; S. Lüdtke; D. Sarma; and O. Seitz. *Chem. Sci.*, **2021**,12, 2389-2396.

P-207

High-Throughput Peptide Library: Rapid, Purification-Free Synthesis of Linear and Cyclic Peptides

Yuyan Chen, Fengping Xiao

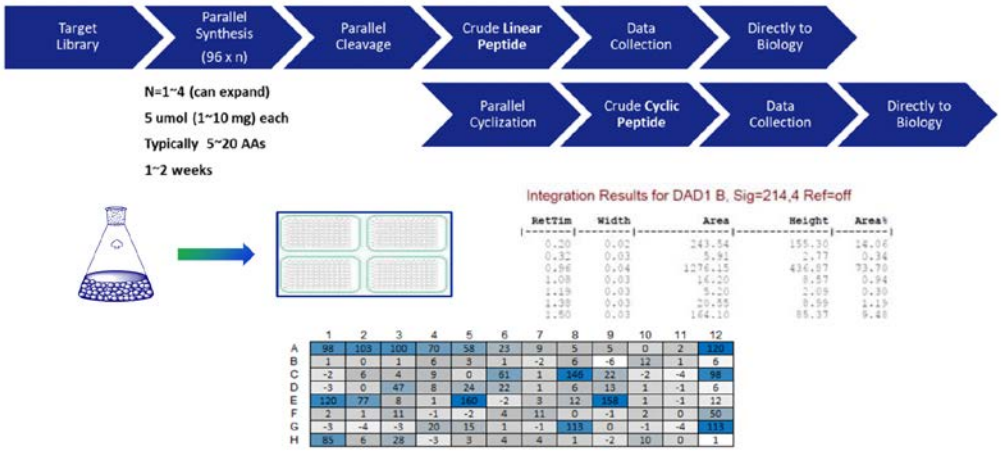
Bioduro, Irvine, USA

Introduction

Peptide therapeutics are a rapidly expanding drug class, yet traditional library synthesis methods are slow, costly, and hampered by the number of parallel synthesis sequences. This significantly hinders early drug discovery. Therefore, innovative and efficient methodologies are critically needed to rapidly and cost-effectively generate and screen diverse peptide libraries, accelerating the identification of promising therapeutic candidates.

Methodology

Our High-Throughput Peptide Library Platform leverages an advanced methodology centered on optimized Solid Phase Peptide Synthesis, (SPPS. This includes rapid synthesis protocols performed in pre-activated amino acids, significantly reducing synthesis time. The platform employs a dual-use synthetic strategy, enabling the generation of both linear peptides via standard amide coupling and cyclic peptides through specialized cyclization techniques, such as thioether, disulfide, lactam. The platform provides LCMS and reliable peptide concentration data. Conduct qualitative and rough quantitative analyses. The platform can achieve crude peptide purity of 70~80% UV purity at 214nm, enabling their direct use in biological assays without additional purification. This direct-to-biology approach supports diverse binding assays, SPR, radioisotope binding, and functional assays, such as fluorescence and absorbance.



Results

This High-Throughput Peptide Library Platform enables up to 45-fold increase in speed compared to traditional peptide synthesis methods. Furthermore, the platform reduces the cost per peptide by over 70%. The platform exhibits high success rates, achieving ~95% success for linear peptide synthesis and ~80% for cyclic peptide synthesis at a 384-peptide scale. The generated crude peptide libraries are validated for extensive direct compatibility with a range of bioassays.

Conclusions

This novel High-Throughput Peptide Library Platform represents a significant advancement, effectively addressing critical bottlenecks in peptide drug discovery.

The rapid, cost-effective, and purification-free screening approach streamlines workflows and accelerates the identification of bioactive peptides. Critically, this platform bridges the gap between combinatorial synthesis and functional validation, reducing discovery timelines from months to weeks.

P-208

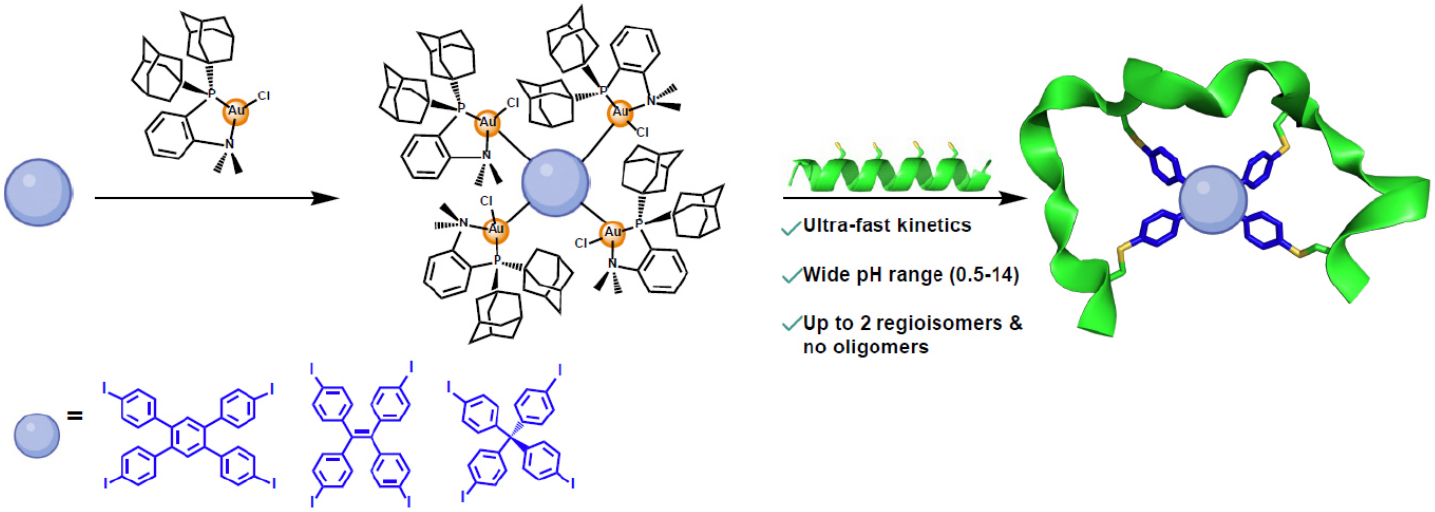
An Organometallic Strategy for Peptide Macrocyclization

Nima Adhami, Michael Rebelo, Rebecca Jenkins, Eileen Olivares, Rachel Loo, Jeff Qu, Reanne Coutinho, Tyler Kerr, Brendan Mahoney, Joseph Loo, James Tilden, Jose Rodriguez, and Alexander Spokoyny

UCLA, Los Angeles, USA

Constrained peptide macrocycles are molecular hybrid systems generated when the native structure of a peptide is rigidified through inter or intra- molecular crosslinking. This structural modification yields unique abiotic morphologies with potentially enhanced therapeutic possibilities. Over the years, researchers have developed many elegant strategies towards constraining a linear peptide into singly or doubly looped macrocycles. However, the synthesis of peptide macrocycles containing greater than two loops remains challenging and additional methods are needed to expand the library of accessible peptide polycycles.

Herein, I will show how organometallic Au(III) chemistry can address these challenges by enabling the synthesis of complex peptide tricycles. By performing the oxidative addition of (P,N)-supported Au(I) complexes with an aryl iodide scaffold bearing four reactive halide handles, we first generate and isolate unique tetrametallic Au(III) reagents. These reagents undergo reductive eliminations with peptides containing four cysteine handles, resulting in the generation of hybrid peptide tricycles. The resulting sulfur-aryl linkages between cysteine sidechains and an aromatic core confer exceptional air, water, and thermal stability.



By leveraging the rapid kinetics and chemoselectivity of these Au(III) reagents towards soft nucleophiles, such as cysteine, we have generated a library of abiotic peptide tricycles. Furthermore, by rigidifying peptides around known solid-state fluorophores, such as tetraphenylphenylethylene, we have generated constrained peptide macrocycles with pronounced solution-state fluorescent profiles that are able to act as robust luminescent markers in cell assays. Overall, this work showcases the power of organometallic chemistry and how it can provide access to previously inaccessible peptide geometries.

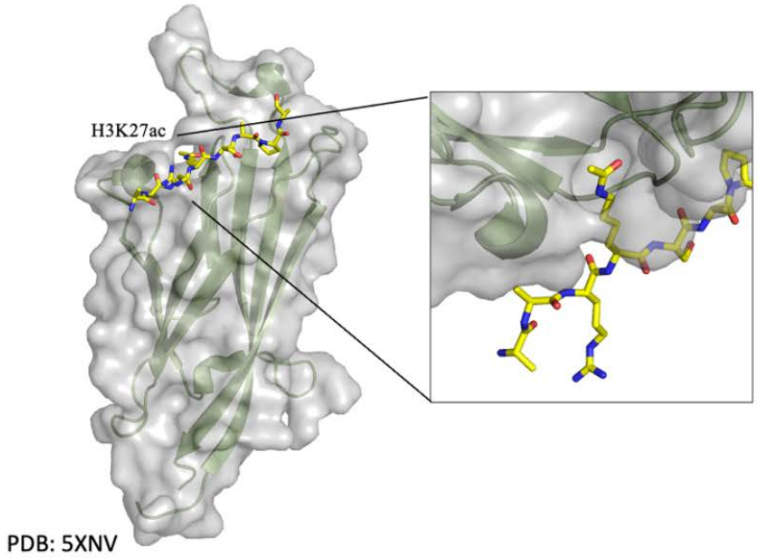
P-209

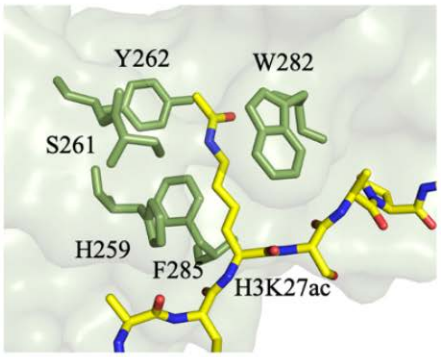
Genetic Code Expansion-Enhanced Phage Display for Discovery of Peptide Inhibitors Targeting the Epigenetic Reader YEATS2

Shivangi Sharma, and Wenshe Liu

Texas A&M University, College Station, USA

Epigenetic regulation is orchestrated by three major classes of proteins—writers, readers, and erasers—that detect or catalyze post-translational modifications on histone tails. Dysregulation of these proteins has been implicated in various human diseases, including cancer, neurodegenerative disorders, and developmental abnormalities. In this study, we aim to develop peptide-based inhibitors targeting the YEATS2 epigenetic reader protein using phage display technology.





Key residues of the YEATS2 proteins that shape the binding pockets

Phage display is a powerful technique for identifying high-affinity peptides from large combinatorial libraries. Its unique mechanism, which physically links each displayed peptide to its encoding DNA, enables the rapid enrichment and identification of binders against therapeutic targets. However, a key limitation of this approach is its reliance on bacterial hosts, which restricts the displayed peptides to the 20 canonical amino acids, thereby limiting chemical diversity. To overcome this constraint, we employed genetic code expansion, utilizing the pyrrolysyl-tRNA synthetase (PylRS)-tRNA pair to incorporate non-canonical amino acids, NCAAs, at amber codons.

Our target, the YEATS2 epigenetic reader, recognizes acylated lysines on histone proteins, promoting the recruitment of regulatory complexes to chromatin. Unlike other epigenetic readers that primarily recognize acetylation, YEATS domains can accommodate bulkier lysine acylations, such as butyrylation and crotonylation. This versatility enables them to regulate key cellular processes, including transcriptional activation and chromatin remodeling, and has been linked to the proliferation of multiple cancer types. By leveraging this approach, we have successfully identified peptides with promising binding affinity for YEATS2. Further optimization and characterization of these peptides could lead to the development of potent and selective YEATS2 inhibitors, offering potential therapeutic strategies for treating cancers driven by epigenetic dysregulation.

P-210

Manufacturing of Anisotropic Gold Nanostructures by Peptide-Based Mineralization With a Gold Reduction Sequence

Shuhei Yoshida, Yoshiki Shitamukai, Koki Yoshida, Makoto Ozaki, Takaaki Tsuruoka, and Kenji Usui

Konan University, Kobe, Japan

The anisotropic gold nanostructures with surface plasmon resonance, SPR-derived absorption in the near-infrared region has attracted much attention for use in cancer therapy and bioimaging. Therefore, it is expected to be used to medical and biological applications. However, reducing agents and surfactants with high environmental hazards and biotoxicity were necessary in conventional methods for manufacturing anisotropic gold nanostructures. Biomineralization is one of the most powerful approaches to solving these problems. Some peptides can selectively and mildly reduce gold ions without reducing agents.[1-3] Additionally, it was reported that peptides were also used to manufacture gold nanostructures in cells because of their low biotoxicity[4] and that peptides were shown to have dispersion-protective properties.[5]

In this study, we attempted to manufacture anisotropic gold nanostructures by mineralization using a gold reduction peptide. At first, we selected and synthesized a gold ion reducing peptide sequence reported in previous studies.[1] When the peptide was reacted with gold chloride acid and silver nitrate, a red shift in SPR absorption was observed by UV-Vis measurements. TEM observation showed that the sample had a petal-like structure. The manufactured anisotropic nanostructures were preliminarily confirmed to be free of biotoxicity by the cell viability assay. We are now conducting ICP-AES and TEM-EDX measurement to analyze the nanostructured elemental species. This method would be expected to be a promising technique for the fabrication of biocompatible anisotropic gold nanostructures.

[1]Munro, C. J.; Hunghes, Z. E.; Walsh, T. R.; Knecht, M. R. *J. Phys. Chem. C* **2016**, 120, 18917-18924.
[2]Yoshida, S; Tomizaki, K.-Y.; Usui, K. *Chem. Commun.*, **2023**, 59, 13239-13244.
[3]Yoshida, S.; Yoshida, K.; Isozaki, T.; Oura, M.; Ozaki, M.; Tsuruoka, T.; Usui, K. *Molecules*, **2025**, 30, 1689.
[4]Ozaki, M.; Yoshida, S.; Tsuruoka, T.; Usui, K. *Chem. Commun*., **2021**, 57, 725-728.
[5]Ozaki, M.; Yoshida, S.; Oura, M.; Tsuruoka, T.; Usui, K. *RSC Adv*., **2020**, 10, 40461-40466.

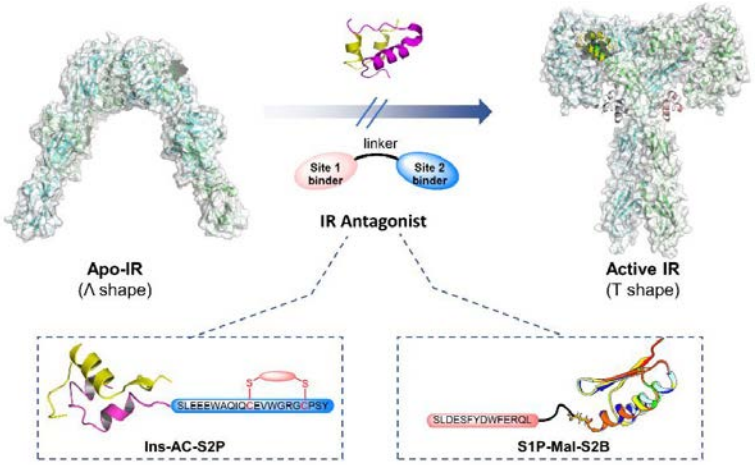
P-211

Developing Potent Antagonistic Insulin Conjugates for Treating Congenital Hyperinsulinism

Yuankun Dao, Terra Lin, Danny and Hung-Chieh Chou

Stanford University, Palo Alto, USA

Congenital hyperinsulinism, CHI, is a genetic disorder characterized by excessive insulin release, leading to persistent hypoglycemia—the most common cause of low blood sugar in early childhood—which can result in severe brain damage or even death.[1]



Insulin receptor, IR, antagonists represent a promising therapeutic approach for CHI. Based on the principle of conjugating IR site 1 and site 2 to stabilize its inactive form, we describe two distinct classes of IR antagonists: **1|** an insulin and S2 peptide conjugate, Ins-AC-S2P, and **2|** an S1 peptide and S2 minibinder conjugate, S1P-Mal-S2B. Through chemical synthesis, we generated a diverse library of IR antagonists and identified two lead compounds with greater potency than the parent compound, Ins-AC-S2 via an *in vitro* pAKT cell assay.[2] Furthermore, these lead antagonists effectively inhibited IR activation and elevated blood glucose levels in mice, demonstrating significant potential for the development of novel therapeutics for CHI treatment.

1 Rahman, S. A.; Nessa, A.; Hussain, K. J. *Mol. Endocrinol*. **2015**, 54, R119–R129;
2 Park, C.; Zhang, Y.; Jung, J. U., Buron, L. D.; Lin, N. P.; Hoeg-Jensen, T.; Chou, D. H. *J. Med. Chem*. **2023**, 66, 7516–7522.

P-212

The Multimodality of the Antibacterial Effect of Peptides; Can They Solve AMR?

Emilia Oueis

Chemistry department, Khalifa University, Abu Dhabi, UAE

Antimicrobial resistance, AMR, has become one of the biggest health challenges of this era, causing yearly millions of deaths worldwide.[1] The overuse and misuse of antibiotic drugs since their discovery have exacerbated the problem, as bacteria evolved and survived through developing resistance mechanisms. Hence, there is currently an urgent need for the development of novel antibiotics with different modes of action.[2,3] Peptides and hybrid peptides have recently gained momentum as efficient therapeutics, not the least as antibacterial drugs.[4]

In our work, we propose multiple methodologies for the discovery and design of peptidic compounds with antibacterial activities. These different strategies lead to high peptide diversity, while resulting in different therapeutic

modalities and modes of action. Among those, we will discuss the progress we are making in advancing nature-derived antimicrobial peptides into drug-like hybrid peptides with improved activity against both Gram-positive and Gram-negative bacteria. Multiple rounds of design and optimization have led to a lead bactericidal molecule with a rapid onset of action.

We will also highlight our recent work on targeting a novel bacterial target with peptide-based therapeutics using two modalities: allosteric binding and protein-protein interaction inhibition. These narrow spectrum potential antibiotics target the Gram-negative *Acinetobacter baumannii* in pneumonia infections. Aided by *in silico* design, these novel molecules are expected to address a long standing hurdle in overcoming AMR in the hospital setting.

[1]Murray, C. J. L. et al. *The Lancet* **2019**, 399 (10325), 629–655.
[2]Theuretzbacher, U., Bush, K., Harbarth, S. et al. *Nat. Rev. Microbiol.* **2020**, 18, 286–298.
[3]WHO Bacterial Priority Pathogens List 2024; *World Health Organization: Geneva*, **2024**.
[4]Zampaloni, C., Mattei, P., Bleicher, K. et al. *Nature* **2024**, 625, 566–571.

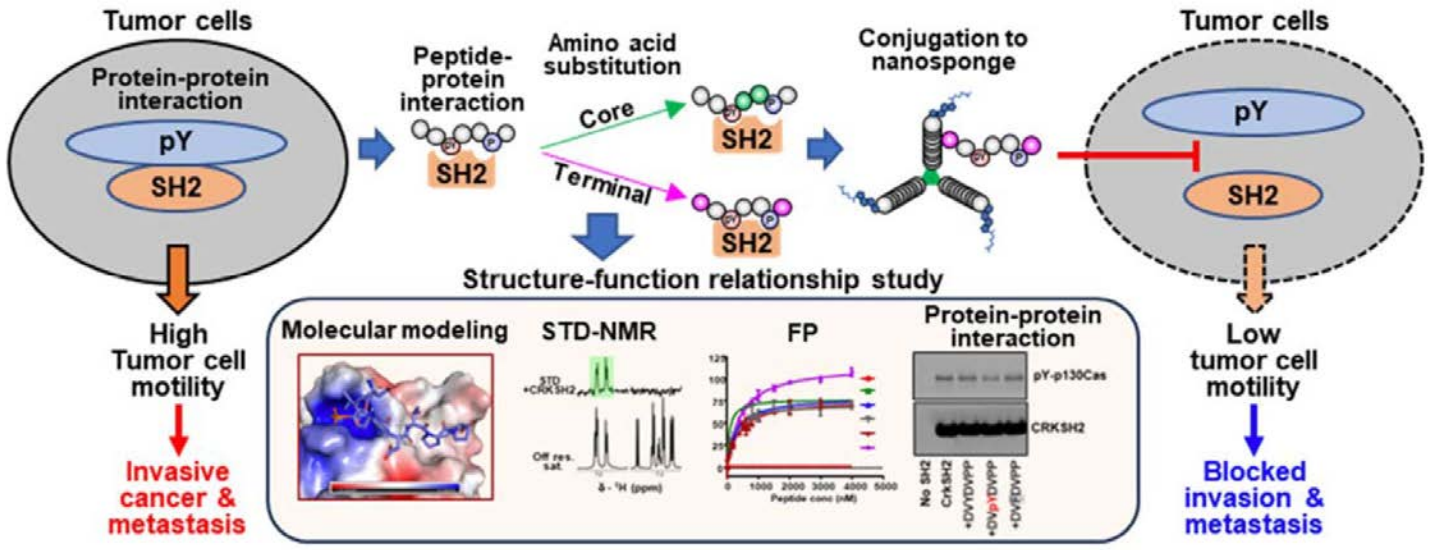
P-213

Rational Design of Peptides and Conjugating Peptides to Nanosponges to Inhibit the Phosphotyrosine-SH2 Interaction

Taeju Park[1], David Johnson[2], Anuradha Roy[2], Justin Douglas[2], Obdulia Covarrubias-Zambrano[3], Neka Large[1], Sarah Neuenswander[2], Harned Laurie[2], Melinda Broward[3], Stefan Bossmann[3]

[1]Children's Mercy Kansas City, Kansas City, USA
[2]University of Kansas, Lawrence, USA
[3]University of Kansas Medical Center, Kansas City, USA

Protein-protein interactions through the phosphotyrosine-SH2 domain interaction play critical roles in many signal transduction pathways. CT10 regulator of kinase, CRK, and CRK-like, CRKL proteins are structurally and functionally similar adaptor proteins containing a SH2 domain. Elevated protein levels of CRK and CRKL are associated with poor prognosis in human cancers, including glioblastoma, and CRK and CRKL have been proposed as therapeutic targets. We demonstrated the essential overlapping roles of CRK and CRKL in glioblastoma cell migration *in vitro* by inducing gene knockdown of CRK and CRKL. However, the lack of CRK and CRKL inhibitors makes it challenging to apply the knowledge to therapeutic intervention.



To develop CRK/CRKL-antagonist peptides that bind to the SH2 domains of CRK/CRKL and interfere with the CRK/CRKL-mediated protein-protein interactions, we analyzed the 15 pYXXP motifs in p130Cas, a well-known CRK/CRKL-binding protein, and designed a phospho-p130Cas-mimicking peptide, PCP, containing conserved phosphotyrosine, pY, and proline, P. The PCP specifically bound to CRKSH2. To improve the activity of PCP, we carried out PCP-SH2 mo-

lecular docking and substituted the core or terminal amino acid residues of the PCP. We determined binding epitopes and affinities of the peptides by conducting saturation transfer difference (STD)-NMR spectroscopy and fluorescence polarization, FP. The inhibitory effects of the peptides on the CRK/CRKL-p130Cas interactions were confirmed. These structure-function relationship studies led to development of CRK/CRKL-antagonist peptides.

We conjugated the CRK/CRKL-antagonist peptides to nanosponges consisting of cholesterols and cation/anion oligopeptides to facilitate uptake by tumor cells. The nanosponge-coupled CRK/CRKL-antagonist peptides showed sustained inhibition of glioblastoma cell motility. We are determining *in vitro* and *in vivo* effects of the nanosponge-coupled peptides.

Our study demonstrates rationally designing and optimizing peptide antagonists of phosphotyrosine-SH2 interactions and taking advantage of nanosponges to deliver the antagonist peptides into tumor cells.

P-214

Internalization of Cell Penetrating Peptides and Secretion by Extracellular Vesicles

Shinya Nakai, Nami Matsuhiro, and Ikuhiko Nakase

Osaka Metropolitan University, Osaka, Japan

Exosomes are extracellular vesicles, EVs, approximately 30-200 nm in diameter, characterized by containing physiologically active molecules, and contributing greatly to intercellular communications. In addition, EVs are known to have high advantages from pharmaceutical perspectives, and are expected to be a next-generation drug delivery tool. In this study, we are evaluating the transfer of cell-penetrating peptides, CPPs, into multivesicular endosomes, MVEs, which are the production sites for EVs, during their cellular uptake, and the effects on EVs secretion.

Using a confocal laser microscope, we observed cellular uptake of fluorescently-labeled octaarginine, R8-Alexa568, a typical CPP, into HeLa cells expressing the EVs marker protein CD63-GFP. By pretreatment with the hydrophobic anion pyrenebutyrate, PyB, highly efficient cell membrane penetration of the R8-Alexa568 and promotion of diffusion into cytosol were observed. In addition, colocalization of the CD63-GFP and R8-Alexa568 was observed over time, suggesting accumulation of the peptides to the MVEs.

After cellular uptake of the R8-Alexa568 into HeLa cells, EVs secreted from the cells were isolated using ultracentrifugation. As results of evaluating the encapsulation of isolated EVs using the quenching effect of trypan blue, TB, the R8-Alexa568 was judged to be encapsulated within EVs. Cryo-TEM observation showed multivesicular formation in the isolated EVs, suggesting possible induction of membrane fusion in MVEs that affect encapsulation of delivered CPPs. Furthermore, the isolated EVs were taken up by other cells, it became clear that the R8-Alexa568-encapsulating EVs also taken up into other cells after their secretion. These results provide basic technologies for artificial inclusion in EVs using the CPPs and intercellular communication via the CPP-encapsulated EVs.

P-215

Transforming Proteins into Bioactive Peptides with PepGenesis™ and PepFusion™ - A Case Study of an IL-6 Inhibitor

Alexander Pisarchik[1], Noreen Gervasi[1], Charles W. Johannes[1,2], Edmund Nesti[1]

[1]Alcamena Stem Cell Therapeutics, LLC, Halethorpe, MD 21227
[2]EPOC Scientific LLC | Alcamena Stem Cell Therapeutics, LLC

Alcamena, a clinical-stage biotechnology company, pioneers next-generation medicines with universal druggability. Our proprietary directed evolution platform compresses millions of years of natural evolution into weeks, enabling rapid discovery of peptides and proteins with optimized binding affinity, enzymatic activity, and biophysical properties. This closed-loop system integrates PepGenesis™, a generative AI engine for rational sequence design, with PepFusion™, a modular library platform for tunable sequence diversification.

Alcamena leverages this platform to address chronic health disorders affecting over 60% of the US population, approximately 150 million people.[1,2] Our lead clinical-stage therapeutic peptide for obesity and diabetic neuropathy exhibits ideal pharmacological properties: stability across pH 1–10 for oral delivery and cell permeability to modulate gene transcription in pancreatic beta cells and neural tissue, enhancing glycemic control and nerve regeneration.

We validate our platform through a case study of a topically administered anti-inflammatory peptide targeting interleukin-6, IL-6, a cytokine implicated in inflammatory diseases such as rheumatoid arthritis and Castleman disease. PepFusion™-derived libraries generated high-affinity leads, outperforming conventional random peptide libraries. PepGenesis™-directed mutagenesis optimized these leads, yielding peptides with sub-nanomolar affinity and low nanomolar EC_{50} values in cell-based assays.

In a humanized mouse model of chemically induced psoriasis, an acylated peptide variant demonstrated potent anti-inflammatory effects, significantly reducing local inflammation and serum levels of IL-6, IFN-γ, and IL-17.

These results validate the PepGenesis™ + PepFusion™ platform as a transformative AI-driven directed evolution engine, combining AI precision with evolutionary adaptability to develop high-performance therapeutics.

P-216

Oral Peptide Therapeutics for Gut Disorders

Markus Muttenthaler

The University of Queensland, Brisbane, Australia

Irritable bowel syndrome, IBS, and inflammatory bowel diseases, IBD, are chronic gastrointestinal disorders affecting over 10% of the global population. Despite their growing prevalence, effective treatments remain limited, and disease mechanisms are poorly understood. Our research focuses on new therapeutic strategies to treat these disorders using orally active and gut-restricted peptides, a new frontier in peptide drug development.[1,2]

In recent work, we targeted oxytocin receptors upregulated in colonic nerves in IBS/IBD-associated abdominal pain. Using state-of-the-art medicinal chemistry, we developed gut-stable oxytocin-based drug leads that potently alleviated pain in preclinical mouse models when administered orally.[2]

We are also interested in the underlying causes of gut disorders. In a clinical study, we revealed a high prevalence of bacterial biofilms in IBS and IBD patients linked to gut microbiome dysbiosis and disease development.[3,4] We are now targeting these gut biofilms with nature-derived antibiofilm peptides and developing them for oral administration.

Another strategy is understanding how endogenous peptides can function in the hostile environment of the gut. The trefoil factor family, TFF, is particularly interesting as these disulfide-rich mini-proteins play key roles in gastrointestinal protection and repair.[5] Studying the TFF and other stable peptide scaffolds has led to new strategies for developing gut-specific peptide therapeutics,[6] representing a major step forward in the field of oral peptide drug development and holding great promise for improving the lives of patients suffering from chronic gastrointestinal disorders.

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P-217

Cyclic Peptide-Doxorubicin Conjugates to Overcome Multi-Drug Resistance and Reduce Toxicity

Keykavous Parang

Chapman University, Irvine, USA

The use of doxorubicin, Dox, an anthracycline chemotherapeutic agent, is associated with cardiotoxicity and inherent acquired resistance. Thus, there is an immediate need for delivery systems to minimize toxicity and deliver Dox to sensitive and resistant cells. We have previously shown several cyclic peptides containing alternate tryptophan, W, and arginine, R, residues act as efficient molecular transporters.

The peptides were conjugated with Dox via a glutarate linker to afford cyclic peptide-Dox conjugates. While the LC_{50} values of free and conjugated Dox were comparable to those in wild-type MDA-MB-231 cells, 0.45 vs. 0.56 μM, respectively, peptide-conjugated Dox was significantly more effective in both Dox-resistant MDA231R cells, LC50 of 2.3 vs. 14 μM, respectively, and MES-SA/MX2 cells, LC_{50} of 4.3 vs. 20 μM, respectively.

Free Dox, 5 μM, reduced the viability of the kidney, LLCP-K1, ATCC CRL-1392 and rat myocardium, H9C2, ATCC CRL 1446, cells by 85% and 44%, respectively. $[R_5K]$W7A-Dox and $[R_5K]W_7$C-S-S-Dox showed minimal toxicity to LLCP-K1, 5-7%, and H9C2, <9%, cells at similar or higher concentrations.

Fluorescence micrographs were consistent with cytotoxicity studies, indicating minimal uptake of $[R_5K]$W7A-Dox in heart cells. The total concentration of Dox, conjugated and released, in the nucleus after 4h exposure to $[W_9R_8K$-β-A]-Dox was higher than free Dox. The mechanistic data indicated endocytosis independence and suggested direct trans-membrane localization. Our data show that appropriate peptide-Dox conjugates can effectively internalize into resistant cells that pump out the free drug and do not cause similar toxicity in normal non-cancerous cells.

P-218

Enhancing Antibacterial Activity with Membrane-Active Macrocyclics: Broad-Spectrum Design, Synergistic Combinations, and Mechanistic Insights

Keykavous Parang

Chapman University, Irvine, USA

The main aim of this study was to create and assess a new group of antimicrobial peptides, AMPs, that possess a broad-spectrum activity. These peptides have the potential to be used as standalone antibiotics or in conjunction with other antibiotics. Despite numerous efforts by various research teams to establish AMPs as a viable alternative to traditional antibiotics, only a few have successfully progressed through clinical trials. This can be attributed to challenges such as their relatively large molecular size, toxicity to mammalian cells, and vulnerability to degradation by proteolytic enzymes, which hinder their drugability.

To address these clinical limitations, our research group has been dedicated to developing small cationic AMPs that exhibit greater selectivity towards bacterial membranes and increased stability against peptidases. We report the synthesis and antibacterial activities of a library of amphiphilic membrane-active peptides. Lead cyclic peptides showed broad-spectrum activity against drug-resistant Gram-positive, MIC=1.5-6.2 μg/mL, and Gram-negative, MIC=12.5-25 μg/mL, bacteria. In combination with commercially available antibiotics, tetracycline, tobramycin, clindamycin, kanamycin, levofloxacin, polymyxin B, metronidazole, and vancomycin, lead peptides showed remarkable synergism against a large panel of resistant pathogens.

Cytotoxicity study showed the predominant lethal action of the peptides against bacteria as compared with mammalian cells. A plasma stability study revealed approximately 2-fold higher stability of lead cyclic peptides as compared to

their linear counterparts after 24h incubation. Calcein dye leakage and scanning electron microscopy studies revealed the membranolytic effect of peptides.

Nuclear magnetic resonance spectroscopy and molecular dynamics simulations studies of the interaction of the peptides with phospholipid bilayer provided a solid structural basis explaining the membranolytic action of the peptides with atomistic details. *In vivo* animal studies were used to determine the pharmacokinetics and efficacy of the lead peptide utilizing a mouse methicillin-resistant *Staphylococcus aureus,* MRSA, septicemia model. These results highlight the potential of newly designed amphiphilic peptides as the next generation of peptide-based antibiotics.

J. Med. Chem. **2023**, 66, 855-874; *J. Med. Chem.* **2022**, 65,15819-15839; *Eur. J. Med. Chem*., **2022**, 17, 235:114278; *J. Med. Chem*. **2022**, 65, 303-322; *Eur J Pharm Sci*. **2024**, 197:106776; *Antibiotics* (Basel). **2024**, 13(6):555; *Antibiotics* (Basel). **2025**, 14(1):82;

P-219

Medicinal Chemistry of Huntingtin-Binding Peptides from mRNA Display: Exploring Non-Canonical Sidechains, Backbones, and Stereochemistry to Discover Potent, Stable Aggregation Inhibitors

Christopher R. Hughes, J. Mario Isas, Anoop Rawat, Kaori Noridomi, Terry T. Takahashi, RalfLangen, and Richard W. Roberts

University of Southern California, Los Angeles, USA

Inhibitors of pathological protein aggregation are of basic and therapeutic interest, but simultaneously achieving "druglike" potency, specificity, and bioavailability is challenging. In Huntington's disease, HD, inherited CAG expansions in the Huntingtin gene produce polyglutamine-containing proteins that form toxic oligomers and fibrils, leading to untreatable, fatal neurodegeneration. As a model amyloid disease, HD has attracted significant attention, but existing compounds targeting Huntingtin aggregation are insufficiently potent, specific, or bioavailable. To overcome the characteristic difficulties of small molecules and biologics, we previously used mRNA display to identify small peptide ligands of mutant Huntingtin, which are specific vs. other amyloids, bind seeding-competent species, and inhibit aggregation.

Here, we report key aspects of the medicinal chemistry of these peptides, including accessible sidechain modifications that increase potency, overall tolerance to backbone modifications, N- and Cα-methylation, β^3 homologation, effects of conformational constraints, cyclic/hindered residues, stapling, and identification of a stereoinverted variant that retains some function. Affinity, SPR, and inhibition, EPR, assays show that binding to an intermediate species drives overall aggregation blockade, with the best analog to date being ~1000x as potent as the parent from selection; to our knowledge, the most potent reported inhibitor.

Notably, these studies were performed without a 3D structure of the complex, suggesting that peptides from naïve libraries can benefit from secondary structure-agnostic optimization. Overall, we demonstrate efficient, substantial progress from a linear, standard-alphabet mRNA display hit, towards potent, stable peptidomimetics targeting Huntingtin aggregation, and highlight features of general interest to peptide medicinal chemistry.

P-220

Novel Peptide Therapeutics for Neurodegenerative Diseases

Dorothy Wai[1], Liam Koehn[1], Joseph Nicolazzo[1], David Finkelstein[2], and Raymond Norton[1]

[1]Monash University, Parkville, Australia
[2]Florey Institute of Neuroscience and Mental Health, Parkville, Australia

Neuroinflammation plays a key role in the pathogenesis of incurable neurodegenerative diseases, including Alzheimer's disease, AD, and Parkinson's disease, PD.[1] Despite extensive development efforts, effective therapies for these diseases are limited, and there is an urgent need for novel drugs that target different pathological mechanisms in AD and PD. The voltage-gated potassium channel $K_V1.3$ is upregulated in pro-inflammatory microglia that mediate

neuroinflammation in AD and PD. K_V1.3 blockade has been shown to be therapeutically beneficial in animal models of these diseases by shifting microglia away from a pro-inflammatory phenotype.[2,3]

HsTX1[R14A] is a potent peptide blocker of K_V1.3, IC_{50} 45 pM, that is highly selective, >2000-fold, for K_V1.3 over closely-related K_V1 channels.[4] HsTX1[R14A] reduces microglial activation *in vitro* and in an animal model of neuroinflammation.[5,6] Compared to other peptide inhibitors of K_V1.3, HsTX1[R14A] is more potent and selective for K_V1.3, simpler to make, and more stable chemically and proteolytically. It thus represents a highly promising molecule for therapeutic development.

HsTX1[R14A] at 1 mg/kg is able to improve cognitive function in a mouse model of sporadic AD.[7] I will report on our characterisation of the biodistribution and brain access of HsTX1[R14A] in different AD mouse models using PET imaging, and describe our efforts towards improving the brain uptake of HsTX1[R14A], with the aim of enabling treatment before the blood-brain barrier is disrupted by neuroinflammation. Additionally, we are currently evaluating the efficacy of HsTX1[R14A] in genetic and chemically-induced mouse models of PD. Collectively, this work will position HsTX1[R14A] as a novel brain-penetrant drug lead for further clinical development in neurodegenerative diseases.

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P-221

Bridging Structure and Function: Bifunctional Macrocyclic Peptidomimetics as Chemical Biology Probes

Andrew Jamieson

University of Glasgow, Glasgow, United Kingdom

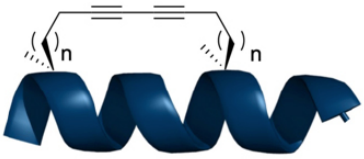
Peptidomimetics are synthetic molecules that mimic the structure and function of a natural peptide. These molecules are generally designed to retain key interactions with biological targets but often feature a single chemical modification that enhance their bioactive conformation. They have greatly impacted the field of chemical biology and peptide drug discovery by overcoming many of the physicochemical limitations associated with linear peptides.

We recently developed a novel peptidomimetic strategy where synthetic modifications not only serve as conformational constraints but also introduce functional elements, such as chromophores, that enhance their utility. In this presentation, the development of two new classes of peptidomimetics that embody this dual-purpose design will be reported.

We developed a Diyne-Girder stapling strategy that enhances peptide helicity, cell permeability, and resistance to protease degradation.[1] Notably, the diyne-girder constraint serves as a bifunctional Raman chromophore, enabling cellular visualization through Raman spectroscopy. This innovative stapling approach was applied to the MDM2/MDMX α-helical binding region of the p53 transactivation domain, resulting in a diyne-girder stapled peptide with improved helicity and selective binding affinity for MDM2, exhibiting an approximately 100-fold reduction in affinity for MDMX.[2]

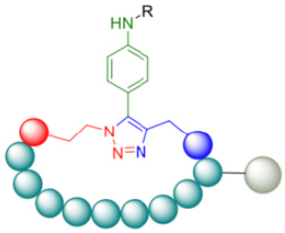
Furthermore, we explored the unique capability of the diyne-girder—a distinctive feature among stapled peptide analogues—for cellular visualization within the "cell-silent" region using Raman spectroscopy.

Diyne Girder Stapled Peptide



- α-Helix constraint
- Protease resistant
- Cell permeable
- MDM2 selective binding

Disulfide bridge mimetic



- Disulfide bridge mimetic
- Protease resistant
- Functional handle
- Functional RGD tool compounds

We have also developed a disubstituted 1,2,3-triazole-based surrogate for a disulfide bridge that effectively mimics its structure.[3] This peptidomimetic strategy offers enhanced stability while preserving the biological activity of disulfide-containing peptides, such as urotensin-II, and α-conotoxins.[4] In this presentation we report the development of a novel solid-phase chemical methodology to synthesise trisubstituted triazole disulfide bridge mimetics, incorporating a handle for further functionalisation.[5]

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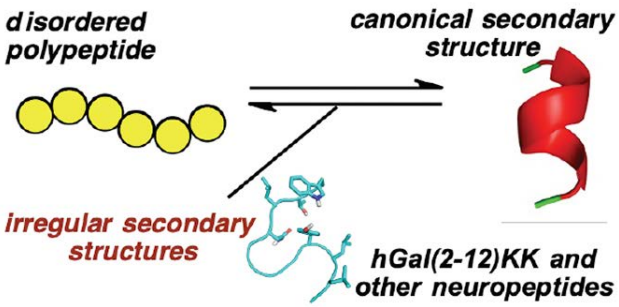
P-222

Exploring the Generality and Stability of Irregular Secondary Structures in Human Signaling Peptides

Michael Giuliano

College of Charleston, Charleston, USA

Small signaling peptides have been the inspiration for generations of drug design efforts, and among these the neuropeptides have historically featured prominently. However, many of the ordered states adopted by small bioactive peptides are poorly predicted by contemporary computational methods, including machine learning algorithms.[1]



Our group's work in the NMR and biophysical characterization of human neuropeptides suggests that at these typically smaller peptide lengths, less than 30 residues, solvation of sidechains is the dominant driving force for protein folding, often leading to environmentally-sensitive and/or irregular secondary structures.[2] In the case of the neuropeptide galanin for example, nearly identical hydrophobic displays, which overlay with the peptide's bound state, are retained across five distinct backbone configurations of its binding sequence in solution. In other words, side chain properties appear to direct the formation of minimal folded states, and these appear to be enough to impart function to small peptides even without ordered, highly regularized backbones.

Presently, we are exploring the generality of this observation through NMR solution study of synthetic analogues of the storied neuropeptide somatostatin. Further, we have developed a system to measure the folding thermodynamics of a representative of these minimal folded states and are corroborating their presence in solution via collaborative 2D IR experiments.

[1]McDonald, E.F.; Jones, T.; Plate, L.; Meiler, J.; Gulsevin, A. *Structure*, **2023**, 31, 111-119.
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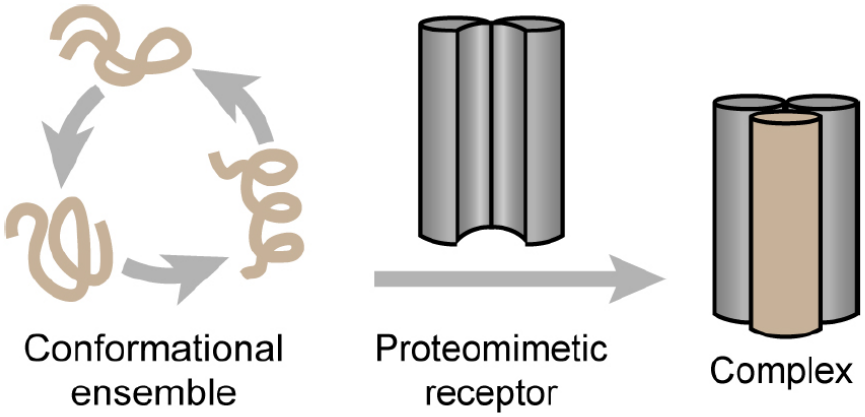
P-223

A Proteomimetic Strategy for Modulation of Intrinsically Disordered Protein MYC

Thu Nguyen, Seong Ho Hong, and Paramjit Arora

New York University, New York, USA

Traditional approaches to target proteins have relied on the paradigm that the unique three-dimensional folds of proteins provide ligandable binding sites. Conformationally dynamic proteins increase the level of difficulty in ligand design and the challenge is further exacerbated for proteins that are intrinsically disordered. We hypothesized that one avenue for the development of binders for a disordered region would be to trap one of its thermodynamically accessible conformations with a receptor.



Here we show the application of this approach to MYC, which represents a critical therapeutic target but has not yielded to small molecule inhibitors due to its conformationally dynamic nature. MYC adopts a helical configuration when it binds its cellular partner MAX. We rationally designed a proteomimetic scaffold, termed Crosslinked Helix Dimers, CHDs, to trap this conformation.

We show that MYC can be directly engaged both in biochemical and cellular assays. Overall, this work demonstrates a general method to capture and trap intrinsically disordered proteins with a propensity to adopt α-helical conformations.

P-224

Using Explore Covalent Peptide Inhibitors Chemical Probes to Investigate PRMT1 Interacting Proteins

Terry Nguyen, Tran Dang, and Y. George Zheng

University of Georgia, Athens, USA

In recent years, a growing interest in arginine methylation has been observed as the dysregulation of this post-translational modification has been implicated in various cancers. Owing to its disease significance, PRMT1 has garnered great attention of researchers as it is responsible for approximately 85% of arginine methylation events in mammals. In the family of protein arginine methyltransferases, PRMTs, PRMT1 is recognized as a type I PRMT from which monomethylation and asymmetric dimethylation of arginine residues is catalyzed. Its reported substrates include proteins that play key roles in cellular processes such as DNA repair or transcriptional regulation.

With its activity having a wide variety of consequences in the cell, it was observed that aberrant PRMT1 activity is associated with breast and colorectal cancer, among others. Though much progress has been made in the realm of proteins that are affected by PRMT1, there have only been a handful of PRMT1 interacting proteins discovered. This suggests the need to further study the interactome of PRMTs in order to better understand the regulatory nature of the enzyme as well as develop effective therapeutics .

This project aims to unraveland profile novel PRMT1 interacting proteins through the use of covalently reactive substrate mimetics. Peptides resembling the sequences of key nascent substrates of PRMT1 were synthesized using solid phase peptide synthesis and have been modified to contain reactive warheads that will bind to PRMT1 and allow for the interactions of PRMT1 with other proteins. The probe then acts as a tag to allow for the pulldown of this complex. Upon identifying the novel PRMT1 interacting proteins, the relevance of this relationship can be investigated as well as the downstream consequences of the protein's interaction with PRMT1.

P-225

Exploring the Impact of Staple Length and Location on the Conformation Stability of PEG-Stapled Proteins

Joshua Price

Brigham Young University, Provo, USA

Macrocyclization or stapling is an important strategy for increasing the conformational stability and/or target-binding affinity of peptides and proteins, especially in therapeutic contexts. Despite impressive achievements in the development of stapled peptide therapeutics, our understanding of the fundamental impact of stapling on peptide/protein biophysics remains incomplete. For example, choosing an appropriate staple location and linker length mostly relies on trial and error.

Here we describe our use of a well-understood disulfide-bound heterodimeric coiled-coil as a molecular ruler for benchmarking the relationship between staple location, linker length, and staple-based changes to conformational stability, as assessed by experiment, variable temperature circular dichroism experiments, and theory, parallel-temperature replica-exchange molecular dynamics simulations.

Observed changes in melting temperature T_m and folding free energy ΔG upon stapling correlate closely with those predicted by our simulations; the simulations provide atomic-level insights into the mechanism of staple-based stabilization and suggest useful guidelines for choosing the right staple length based on the distance between two prospective stapling sites.

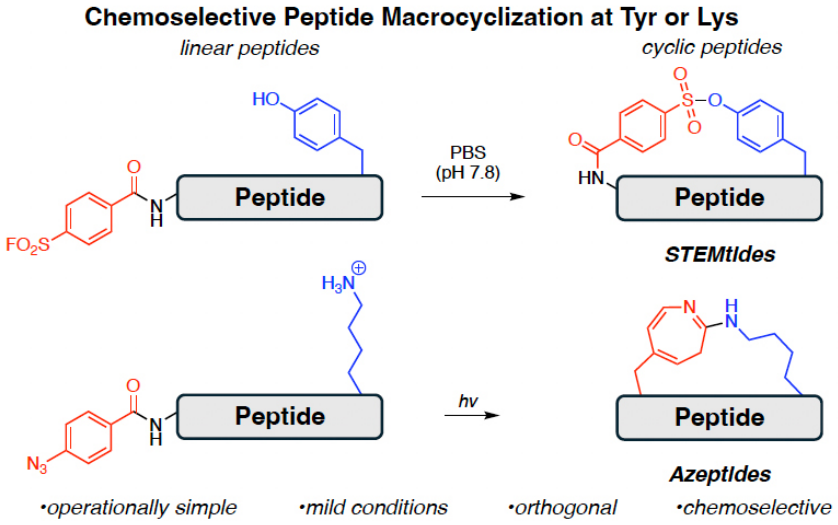
P-226

New Orthogonal Strategies for Chemoselective Peptide Macrocyclization

Hassan Seyrani, Hossein Heidarzedeh Vazifehkhoran, and Victor Outlaw

University of Missouri, Columbia, USA

Although peptides are ideal candidates to modulate protein function and disrupt protein-protein interactions, their biological applications have been limited by low intracellular stability, poor cell permeability, and rapid *in vivo* clearance. Peptide macrocyclization has been shown to be an effective strategy for mitigating these unfavorable pharmacokinetic characteristics. The chemical diversity of macrocyclic peptides, however, is limited by the scarcity of orthogonal synthetic methods for chemoselective cyclization of peptides.



The Outlaw Lab has developed two new, orthogonal, and chemoselective methods for peptide cyclization. The first method operates via a sulfur-fluoride exchange, SuFEx, reaction and proceeds with selective cyclization at tyrosine to afford a new class of Sulfonate Tyrosine Ester Macrocyclic peptides, *STEMtides*.

The second method utilizes aryl azide photolysis to generate a singlet nitrene, which rapidly undergoes ring expansion to an electrophilic ketenimine followed by selective intramolecular reaction with lysine. This cascade ultimately affords a new class of Azepine-linked macrocyclic peptides, *Azeptides*. The described methods for peptide macrocyclization expand current capabilities by being **1|** chemoselective, **2|** operationally simple, and **3|** progressing under mild and sustainable conditions. We exploit the chemoselective control of these methods for the synthesis of cyclic and stapled peptide analogs and demonstrate enhanced serum stability and bioactivity against a wide array of biological targets.

P-227

Synthesis of Opioid Cyclic Tetrapeptides: Analogs of *Cyclo*[Pro-Sar-Phe-D-Phe]

Bijan Shoushtarizadeh, Ashley Smith, Rafael Uriarte, and Michael Ferracane

California State University, Fullerton, Fullerton, USA

In 2023, approximately 81,000 Americans died from an overdose involving an opioid. The cyclic tetrapeptide *cyclo*[Pro-Sar-Phe-D-Phe] was found to treat pain and block drug-seeking behavior with reduced liabilities. Herein, we describe preliminary synthesis, characterization, and analysis of related cyclic tetrapeptides to explore the structure-activity relationship of this scaffold. Synthesis of analogs was performed using solid-phase peptide synthesis

followed by solution-phase cyclization. The resulting products were purified via reverse-phase flash chromatography and characterized via HPLC and LCMS. Analysis of peptide conformation, permeability, and opioid activity is ongoing. Once complete, this data will improve our understanding of the structure-activity relationship of this scaffold and its potential use as a pain or opioid addiction treatment.

P-228

Enhancing Solid-Phase Peptide Synthesis with Green Chemistry Principles

Symone Carty, Elizabeth Denton, and Austin Schlirf

Biotage, Charlotte, USA

As the demand for peptide-based therapeutics rises, developing sustainable synthesis methodologies becomes increasingly important. The bioactive decapeptide ACP_{65-74} is known for its challenging synthesis, making it a benchmark for evaluating novel synthesis approaches. In this study, ACP and two antimicrobial peptides, AMPs, C18 and hepcidin, were synthesized using traditional DMF-based protocols and a greener alternative solvent method to explore the application of green chemistry principles in solid-phase peptide synthesis, SPPS.

The sustainable method utilized a 7:3 BtOAc:DMSO solvent mixture with 20% 4-methylpiperidine, pip, and a single wash post-deprotection. This approach achieved a crude purity of 72.73% for ACP, compared to 88.95% under default conditions. Applying this sustainable protocol to C18 and hepcidin resulted in crude purities of 21.76% and 42.24%, respectively, compared to 29.97% and 56.72% under default conditions. The reduction in purity aligns with the expected impact of minimized post-deprotection washes rather than a fundamental limitation of the solvent system, indicating that proper solvent selection and wash steps play a crucial role in synthesis efficiency. Transitioning toward greener SPPS is critical for reducing environmental impact while maintaining efficiency.

This study demonstrates that the 7:3 BtOAc:DMSO with 20% pip method is a promising alternative to DMF, achieving only a 22.15% average reduction in crude purity with minimal needle washes, effectively cutting solvent consumption in half. While AMPs present unique challenges due to hydrophobicity and steric hindrance, further optimization can expand the applicability of green chemistry in peptide synthesis. Future work will focus on adjusting post-deprotection washes. This effort will contribute to a more comprehensive sustainable approach to peptide manufacturing while maintaining high synthesis efficiency.

P-229

Peptide API Purification by Simulated Moving Bed Chromatography with Small Particle Size Reversed-Phase Polymer-Based Adsorbents

Timothy OMara[1], and Shingo Kusano[2]

[1]Itochu Chemicals America, Inc., New York, USA
[2]Mitsubishi Chemical Corp, Tokyo, Japan

The study demonstrates how an Improved Simulated Moving Bed Chromatographic Separation System, MMD-Sep™ with small particle size reversed-phase polymer-based adsorbents can improve peptide purifications compared to single column chromatography. A case study with the commercial peptide icatibant demonstrates that the MMD-Sep™ can simultaneously achieve high purity, >99%, high recovery, >97%, and reduced solvent usage, <0.45 mL/mg product.

Author Bio:
Tim has worked in chromatographic separation and purification for over 25 years. First with an equipment manufacturer, then a silica gel manufacturer, and currently providing ion exchange resins, reverse phase polymeric beads, enzyme immobilization resins, and chromatography silica. Itochu provides purification solutions for small and large molecules, peptides, oligonucleotides, insulin analogs, precision fermentation-based compounds, CBD/THC, and food applications including dairy proteins, collagen, gelatin, sweeteners, flavors, oils, and juices.

P-230

Effect of Antifreeze Activity and Lipid Interaction of Leucine Scanning of 11-Residue Lysine Homopeptide Based on its Secondary Structure

Roberto Rojas[1], Mónica Aróstica[2], Constansa Cardenas[3], Luis Felipe Aguilar[4], Fernando Albericio[5], Fanny Guzman[2]

[1]UDLA, Viña del Mar, Chile
[2]Núcleo de Biotecnología Curauma, PUCV, Valparaiso, Chile
[3]Núcleo de Biotecnología Curauma PUCV, Valparaiso, Chile
[4]Instituto de Química, PUCV, Valparaiso, Chile
[5]Department of Organic Chemistry, University of Barcelona, Barcelona,Spain

Antifreeze peptides and proteins are essential for the survival of organisms inhabiting cold environments, such as polar fish.[1] These biomolecules fulfill various roles, particularly in cryopreservation, enabling organisms to withstand freezing temperatures. Due to their diverse primary and secondary structures, peptides exhibit a broad spectrum of functionalities related to cold adaptation.

In this study, an 11-residue lysine homopeptide, K11, was synthesized, along with a systematic leucine-scan series, Scan-Leu, in which each lysine residue was individually substituted by leucine along the peptide chain.

Peptide synthesis was performed using solid-phase methodology with Fmoc protection. The resulting peptides were purified and characterized by high-performance liquid chromatography, HPLC, and mass spectrometry. Their secondary structures were analyzed by circular dichroism, CD, spectroscopy across a temperature range of 5°C to 50°C. Antifreeze activity was evaluated via differential scanning calorimetry, DSC, focusing on the inhibition of ice recrystallization in a non-colligative manner, a property referred to as thermal hysteresis, THA.

The results showed that antifreeze activity increased as the number of remaining ice nucleation points decreased, regardless of the peptide analyzed. Substitution with leucine disrupted the polyproline type II helix of the peptide chain, but had a relatively minor impact on antifreeze activity except for slight increases observed near the N- and C-end. Overall, this work provides insights into the mechanisms by which short synthetic peptides may inhibit ice crystal growth during freezing.

[1]Bang J., Lee J., Murugan R., Lee S., Do H., Koh H., Shim H., Kim H. Antifreeze peptides and glycopeptides, and their derivatives: potential uses in biotechnology. *Drugs* **2013** 11(6): 2013-2041

[2]Houghten RA. General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proceedings of the National Academy of Sciences of the United States of America*, **1985**, 82: 5131-5135

P-231

The Mathematics of Positional Scanning Combinatorial Library Use and Optimization

Radleigh Santos[1], Mark Erikson[2], Carrie Haskell-Luevano[2], Marc Giulianotti[2], Richard Houghten[3], and Clemencia Pinilla[2]

[1]Nova Southeastern University, Fort Lauderdale, USA
[2]University of Minnesota, Minneapolis, USA
[3]Independent Scholar, Port St. Lucie, USA

As we recently passed the 30-year anniversary of the first publication of positional scanning combinatorial libraries, the importance of mathematical modeling of mixture behavior and statistical analysis of positional scanning profiles remains at the forefront of research in this area. The harmonic mean model, first applied to chemical mixtures in 1971 and first used to describe the behavior of mixture-based combinatorial libraries in 2011, has lead to current work describing the interactions of agonists and antagonists in mixtures.

Such mathematical modeling of mixture activity behavior is vital in both explaining the success of such approach-

es and the prediction of how successful the approach is likely to be. These models go hand-in-hand with positional scanning profile analysis, such as indices of differentiation, which can be vital in the successful use of such libraries in general, and the use of peptide libraries in antigen discovery in autoimmune disorders, infectious diseases, and cancer in particular.

This presentation aims to describe new work modeling the behavior of the activities of mixtures in both agonist/antagonist relationships and T-cell specificity in antigen discovery, presented in the context of the existing mathematical framework around mixture-based combinatorial libraries. From checkerboard assays to orthogonal pooling to combinatorial libraries, mixtures are ubiquitous, and exploring the mathematics behind their use is highly beneficial.

P-232

Profiling the Substrate Specificity and Activation Conditions of Inflammatory Caspases Using Fluorogenic and FRET-Based Peptide Substrates

Tess C. Boyd, Sophie F. Young, Maya Edgu-Fry, Cathrine A. Southern, and Caitlin E. Karver

DePaul University, Chicago, USA

Inflammatory caspases, caspase-1, -4, and -5 in humans, are aspartate-specific cysteine proteases and are key players in the innate immune response, although the individual role of each enzyme is not well understood. All three enzymes have been shown to cleave essential pro-inflammatory substrates, implicating them in many inflammatory disease states. The optimal conditions for activation of each enzyme appear to be distinct and require further investigation to determine whether their roles are interconnected or independent.

Caspase-1 is the most active and the most studied family member. It is thought to be a dimer in its active form, while caspase-4 and -5 have been reported to be dimers or high molecular weight oligomers when active. The activation conditions required for isolated enzyme assays using fluorogenic peptide substrates were investigated for each caspase. The optimized conditions were then used in a Förster resonance energy transfer, FRET-based peptide cleavage assay in order to create a substrate specificity profile for all three caspases.

P-233

Synthesis and Analysis of a 2,304-Membered Macrocyclic Hexapeptide Library

Conor Galvin, and Gregory Copeland

BRT Biotechnologies, Inc., Claremont, USA

Macrocyclic peptides, MCPs, hold great promise as therapeutics due to their ability to target protein-protein interactions and other challenging biological targets with high affinity and selectivity. Despite their potential, a major limitation to their broader use in drug discovery is the lack of large and diverse MCP libraries suitable for highthroughput screening, HTS. At the same time, with thousands of unique commercially available building blocks, the number of synthetically accessible macrocyclic hexapeptides is greater than 10^{18} – a number far too large to comprehensively screen in functional assays.

We developed **BRiTeCycle** to enable the sparse synthetic sampling of this 10^{18} space, followed by the rapid exploration of the chemical space around any primary hits. **BRiTeCycle** uses a novel spatially addressed, microfluidicsbased synthesis approach that has broad chemical compatibility and high stepwise efficiencies. To assess **BRiTeCycle**, we synthesized a 2,304-membered library of cyclic hexapeptides, in quadruplicate. Sixty amino acid building blocks were incorporated into the library. The purity of 116 samples, 5% of the total, was assessed from 220 nm UPLC chromatograms. Between replicates, crude LC/MS traces are highly reproducible, suggesting reproducible robustness in our synthetic approach. In addition to the desired cyclic hexapeptide, a common side-product was the corresponding cyclic dodecapeptide that results from the cyclic dimerization of two linear hexapeptide precursors. While the linear product was rarely observed, less abundant side-products included larger cyclic peptides, 18- and 24-mers.

This work demonstrates that the **BRiTeCycle** platform can efficiently synthesize large libraries of diverse, cyclic hexapeptides, enabling high-throughput screening for novel drug candidates.

P-234

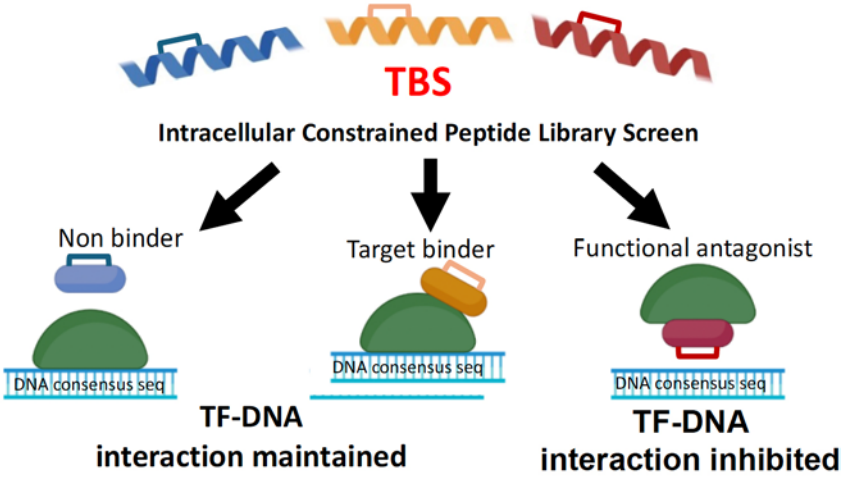
Switching off Transcription Factors Using Intracellular Library-Derived Peptides

Jody Mason

University of Bath, Bath, United Kingdom

Protein-protein interactions, and in particular Transcription Factors, TFs, remain compelling drug targets, yet are often intractable to small molecules and inaccessible to larger biologics. Peptides occupy an attractive middle ground if they can become suitable ordered for target engagement. We utilise intracellular peptide library screening approaches to identify selective peptide-based inhibitors that can functionally antagonise TFs. There are two novelties to our approach:

i| Our *Transcription Block Survival*, TBS, peptide-library screening platform in which TF consensus sites are placed directly into the coding region of an essential gene. Subsequent TF binding within the gene directly blocks gene transcription leading to cell death under selective conditions. Cell survival is therefore only possible if antagonists bind to the TF, but more importantly can prevent it from binding to its consensus sequence, thus shutting down TF function. TBS is an entirely tag-free genotype-to-phenotype approach, selecting desirable attributes such as high solubility, target specificity, biostability and low toxicity within the complex environment of the cell. TBS facilitates rapid library screening to accelerate identification of therapeutically valuable sequences.



ii| Concomitant deployment of cell penetrating crosslinkers. These enter cells to post-translationally constrain every library member into conformations not possible via genetic encoding alone, to select only those in which crosslinking translates into improve target antagonism. Screening ultra-structured biostable peptide libraries, where entire libraries are constrained during the search is highly desirable as it prevents a slow and costly retrospective trial-and-error search for beneficial crosslinkers, positions, and sequences. Using several different exemplars, I will discuss how library-derived constrained peptide antagonists are derived and discuss their characterisation using a range of biophysical and cancer cell-based assays.

Brennan A., Leech J.T., Kad N.M., and Mason J.M. An Approach to Derive Functional Antagonists of Transcription Factor Activity. *JACS Au.* **2022** 2, 4, 996-1006

Brennan A., Leech J.T., Kad N.M., and Mason J.M. The Effect of Helix-Inducing Constraints and Downsizing Upon a Transcription Block Survival Derived Functional cJun Antagonist. *Cell. Rep. Phys. Sci.* **2022** 3, 101077

Tang, T.M.S., and Mason, J.M. Intracellular application of an asparaginyl endopeptidase for producing recombinant head-to-tail cyclic proteins. *JACS Au.* **2023** 3, 12, 3290-96

P-235

Holistic Admissions in 2025: Historical Challenges and Evidence-Based Practices to Recruiting the Best Students

Ethriam C. Brammer and Anna K. Mapp

University of Michigan, Ann Arbor, USA

All graduate programs share the goal of admitting, (and recruiting, outstanding students their department. The rapidly shifting landscape of higher education and research presents unique and significant challenges to achieving this goal.

For example, the 2024 SCOTUS decision 'SFFA v Harvard' ruled race-based admissions processes to be unlawful, leading to significant changes in many states. The University of Michigan has operated under a stricter legal framework since 2006, due to the passage of a Michigan state law that forbids us from **1|** admitting students or **2|** providing financial aid based, in whole or in part, on race/ethnicity, sex, or national origin. To achieve our goal of admitting and recruiting top talent into our doctoral programs while being legally compliant, we have implemented evidenced-based holistic admissions across the campus. Here we will outline the tactics and strategies that we have found to be most effective in doctoral admissions and discuss the outcomes.

P-236

Integrating Social Justice Learning Objectives into Chemistry Courses

Jillian Smith-Carpenter

Fairfield University, Fairfield, USA

At Fairfield University, all undergraduate students are required to complete the ***Magis*** core curriculum, which includes three courses with social justice, SJ, designations. The course, Chemistry and Social Justice, CHEM 2291, was designed to integrate the SJ learning outcomes, including the analysis of the historical and contemporary context of power, inequity, and systematic racism, with the skills suggested by the American Chemical Society's Committee on Professional Training, such as chemical literature skills, communication skills, and ethics.

In this course, students learn about and reflect on the systematic racism and sexism that exist in STEM, as they analyze their own social identities and privilege. Additionally, social justice learning outcomes were integrated into the first semester General Chemistry course, by identifying values and practices across multiple perspectives and asking critical questions about assumptions and biases throughout the course. Specific social justice learning outcomes and assessments for these outcomes will be reported for both SJ designated courses.

P-237

The Chemistry Diversity Initiative: A Graduate Student Program for Success

Jean Chmielewski

Purdue University, West Lafayette, USA

The Chemistry Diversity Initiative, CDI is a multi-prong program for the recruitment and retention of graduate students from underserved populations. The goals for the CDI include: **i|** improve recruitment and enrollment of these graduate students, **ii|** successful transition of students from undergraduate programs into our Ph.D. program **iii|** maintain successful retention and graduation of our graduate students, and **iv|** transform the academic environment in Chemistry into one where all graduate students may thrive.

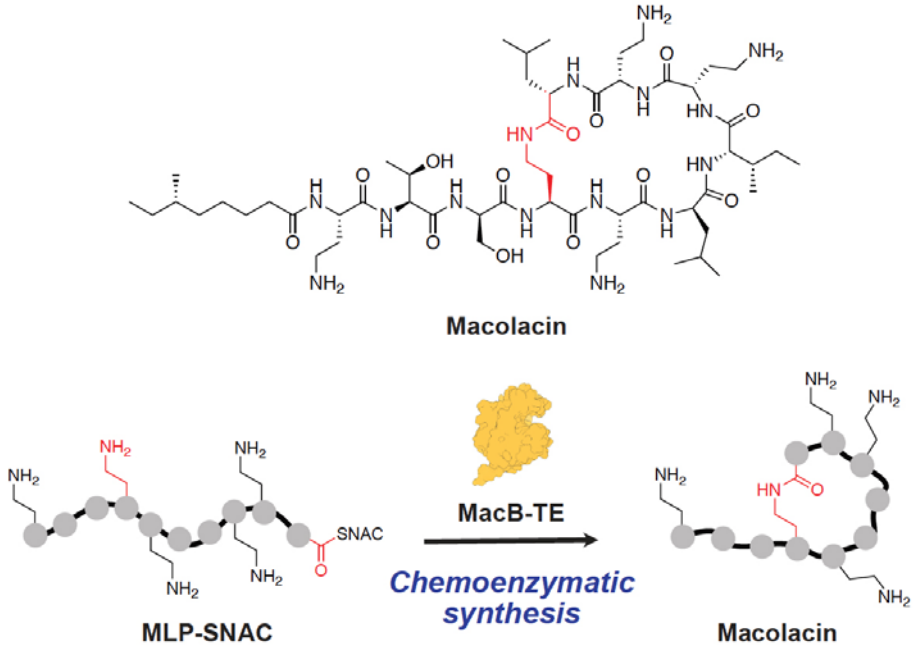
P-238

Exploring MacB Thioesterase Function for Chemoenzymatic Synthesis of Antibiotic Macolacin Analogs

Sho Konno, Miyu Tanaka, Akihiro Taguchi, Atsuhiko Taniguchi, and Yoshio Hayashi

Tokyo University of Pharmacy and Life Sciences, Hachioji, Japan

The rise of multidrug-resistant bacteria presents a serious global threat, while the pace of new antibiotic discovery remains slow. Macolacin, a cyclic lipopeptide natural product, is a promising antibiotic against colistin-resistant Gram-negative bacteria.[1] To enable efficient access to macolacin-derived analogs with improved potency and selectivity, we aim to develop a chemoenzymatic synthesis platform utilizing the thioesterase domain of MacB, which catalyzes the macrocyclization step in macolacin biosynthesis. MacB-TE catalyzes regio- and stereoselective cyclization of the linear peptide precursor.



Here, we characterized the enzymatic activity and substrate specificity of recombinant MacB-TE. A synthetic linear peptide thioester, MLPSNAC, was rapidly and quantitatively cyclized to macolacin by MacBTE. Furthermore, a series of MLP-SNAC analogs with modified amino acid side chains were synthesized to probe substrate tolerance, providing insight into the structural requirements for macrocyclization. Our findings highlight the utility of MacB-TE as a practical biocatalyst for synthesizing new macolacin analogs.

[1]Wang, Z.; Koirala, B.; Hernandez, Y.; Zimmerman, M.; Park, S.; Perlin, D. S.; Brady, S. F. *Nature* **2022**, 601, 606–611.

P-239

Synthesis of Noncanonical Amino Acid-Containing Analogues of Bioactive Peptides

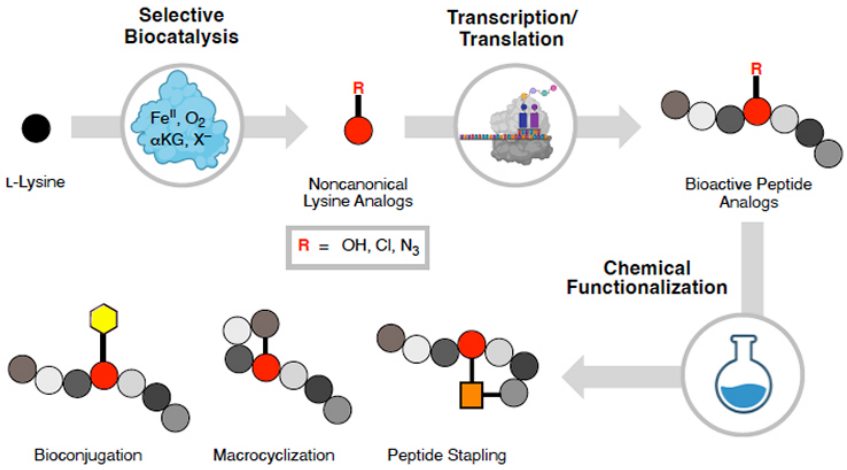
Elizabeth Stone

Fairfield University, Fairfield, USA

The development of improved methods to synthesize molecules of increasing diversity and complexity is necessary to meet the challenges of drug efficacy and safety. Biocatalysis provides an attractive strategy to perform novel chemistry under mild and sustainable reaction conditions. Recently, the Chang Lab has discovered a family of Fe^{II} and α-ketoglutaratedependent, Fe^{II}/αKG halogenases that perform the regio- and stereoselective chlorination of unactivat-

ed C(sp^3)–H bonds using free amino acids substrates, which is a relatively uncommon and challenging transformation.

This work leverages Fe^{II}/αKG enzymes to selectively produce noncanonical amino acids, ncAA, bearing versatile functional group handles, for example, Cl, OH, N_3. Notably, we discovered the first enzyme to catalyze the one-step conversion of free L-lysine into enantiopure (2*S*, 5*R*)-5-hydroxylysine, 5-Hyl, a key component in collagen that contains a β-amino alcohol motif.



Moreover, we have demonstrated that these ncAAs can be residue-specifically incorporated into several biologically active peptides *in vitro* using the native translational machinery of *E. coli*. Using these ncAA-embedded peptides, we are also developing various downstream chemical functionalizations to access structurally and functionally diverse peptides of interest. Overall, this strategy provides a fully biosynthetic method for producing novel analogues of bioactive peptides under mild conditions with the goal of accessing improved peptidic drugs.

Stone, E. A.; Whitten, A. M.; Angelisanti, N. M.; Kissman, E. N; Millar D, Vargas-Figueroa, A.; Chang, M. C. Y. Discovery and Application of a Lysine 5-Hydroxylase for Bioorthogonal Chemistry. *ChemRxiv*. **2024**; doi:10.26434/chemrxiv-2024-xj5bw.

P-240

Physics-Based Modeling Advances Powering the Design of Cyclic Peptide Therapeutics

Goran Krilov[1], Anu Nagarajan[1], Chitrak Gupta2, Jiaye Guo[1], Yan Zhang[1], Nour Saleh[1], Andreas Verras[1], Gary Zhang[1], Shulu Feng[1], Bernandie Jean[1], and Mats Svensson[1]

[1]Schrodinger Inc., New York, USA
[2]Schrodinger Inc., Hyderabad, India

Macrocyclic peptides, CP, have emerged as promising therapeutic agents with distinct biochemical and pharmacokinetic properties. CPs combine high binding affinity, target specificity, and low toxicity of antibodies, with potential oral bioavailability and cell permeability of small molecules. A key challenge in cyclic peptide drug design is optimizing their pharmacokinetic properties, primarily bioavailability and stability, while retaining the favorable on target potency.

Here we describe enhancements to the Schrodinger computational platform, facilitating *in silico* design and high-throughput profiling of CP libraries for hit finding, as well as optimization of potency and permeability for rapid identification of clinically viable orally available agents. In particular, we present an optimized FEP+ protocol for predicting binding potency with accuracy comparable to small molecules. We introduce efficient macrocyclic peptide conformational sampling and docking workflows, and discuss physics-based methods to tackle peptide permeability. We then show how ML approaches can be used for accurate and efficient prediction of structures and properties of macrocyclic peptides. Finally, we introduce several CP specific enhancements to LiveDesign Biologics data integration and design platform.

P-241

Structural Ensemble Predictions of Thioether-Linked Cyclic Peptides Enabled by Molecular Dynamics and Neural Networks

Minh Ho[1], Jiayuan Miao[1], Yi Shan[2], Choi Li[3], James Baleja[2], Hiroaki Suga[3], and Yu-Shan Lin[1]

[1]Tufts University, Medford, USA
[2]Tufts University, Boston, USA
[3]The University of Tokyo,Tokyo, Japan

Cyclic peptides show great potential as a drug modality for disrupting disease-prone protein–protein interactions. Developing cyclic peptide drugs would greatly benefit from characterizing their structural information. However, owing to a cyclic peptide's ability to adopt multiple conformations in solution, it remains challenging to elucidate its structure by using solution NMR. X-ray crystallography reports a single structure, which may not necessarily reflect complete solution structural ensembles.

Alternatively, molecular dynamics, MD, simulations can explore the conformational landscape and quantify the associated population for each conformation. However, MD simulations are computationally expensive and not applicable for large-scale screening. The current **StrEAMM, S**tructural **E**nsembles **A**chieved by **M**olecular **D**ynamics and **M**achine **L**earning, method developed by our group enables rapid and accurate prediction of head-to-tail cyclic pentapeptides and hexapeptides. However, synthesizing head-to-tail cyclized peptides on a large scale can be challenging due to low yield and complicated reaction workup and product isolation.

Inspired by the spontaneous cyclization chemistry of thioether-linked cyclic peptides applied in many mRNA display studies, we expand the **StrEAMM** method to this class of cyclic peptides as a proof of concept by leveraging MD simulations of thioether-linked cyclic peptides and training neural network models. The models identified four thioether-linked cyclic pentapeptides that were well structured, and these peptides were experimentally synthesized for characterization by solution NMR. Ultimately, we envision that **StrEAMM**-thioether models can work synergistically with the current mRNA platform to streamline the resourceintensive drug discovery and design process of cyclic peptides.

P-242

Comprehensive *In Vitro* Pharmacological Characterization of Incretin-Based Obesity Drugs

Nariman Balenga[1], Maria Waldhoer[1], Vanessa Laflamme[2], Billy Breton[2], Madeleine Héroux[2], Michel Bouvier[2], Paul Galatsis[1], and Eric Feyfant[1]

[1]Schrödinger Therapeutics Group, New York, USA
[2]Université de Montréal, Montréal,Canada

A large number of molecules with various mechanisms of action, MoA, are either marketed or in development for obesity and obesity-associated diseases, highlighting the significant unmet medical need in these areas. Preclinical and clinical data show that peptides and small molecules with mono-, dual-, or triple-agonism characteristics on 3 G protein-coupled receptors, GPCRs, glucagon-like peptide-1 receptor, GLP-1R, glucose-dependent insulinotropic polypeptide receptor, GIPR, and glucagon receptor, GCGR lead to significant weight loss in obese patients. This is hypothesized to be driven by the differential engagement of one or multiple receptors in tissues involved in food intake and energy expenditure.

Therefore, drug discovery campaigns can benefit from a comprehensive assessment of the distinct signaling pathways that emanate from these GPCRs in response to these drugs. Using bystander BRET-based proximal assays along with 2nd messenger and internalization assays, here we report the characterization of six obesity drugs, semaglutide, danuglipron, orforglipron, survodutide, tirzepatide, and retatrutide, on multiple pathways.

This study reveals mechanisms by which these receptors respond to drugs of distinct MoA and modality and may pave the way for a more guided approach to designing molecules with desirable efficacy and safety. Moreover, this benchmarking and associated structure-activity relationship studies will allow us to train and improve our modeling capabilities beyond class A GPCRs.

Vögele, M., Zhang, B.W., Kaindl, J., Wang, L. *J Chem Theory Comput*. **2023** 28;19(22):8414-8422

P-243

A Novel Approach for Synthesis of High-Quality Liner-Long Peptides in Peptide Manufacturing

Hossain Saneii[1], Fernando Albericio[2], Rajan Sharma[1], and Fatemeh Karimi Tabar[1]

[1]AAPPTec LLC, Louisville, USA
[2]University of KwaZulu-Natal, Durban, South Africa

Peptides play a crucial role in modern medicine, but their production comes with significant challenges — notably in synthesizing long linear peptides of premium quality at a lower cost. Existing methods struggle to produce crude peptides with 70-90% purity, leading to excessive reagent use, solvent waste, and exposure to harmful chemicals, particularly in Solid-Phase Peptide Synthesis, SPPS, reliant on hazardous solvents like dichloromethane, DCM, and dimethylformamide, DMF.

In response to this issue, scientists have been exploring greener solvent options such as acetonitrile, tetrahydrofuran, *N*-formylmorpholine, and propylene carbonate. However, finding an environmentally friendly solution that maintains SPPS efficiency and reliability remains a challenge.

This study introduces a novel protocol utilizing isopropanol, IPA, as the primary solvent, 80–95%, in peptide synthesis; with the addition of 5–20% co-solvents. We also successfully utilized this approach for synthesizing peptides by incorporating Dipeptides derivatives at specific positions.

This method proves highly effective for liner synthesis of GLP-1 analogs such as Exenatide, Semaglutide, and Tirzepatide. The findings demonstrate that IPA is fully compatible with automated peptide synthesizers, enabling high-yield, high-purity synthesis at a lower cost. Moreover, this approach supports large-scale production, making it a practical and sustainable alternative to industrial peptide manufacturing.

Replacing traditional toxic solvents with IPA in peptide synthesis reduces hazardous waste, enhances safety, and aligns with sustainability goals. This shift lowers costs, maintains scalability, and efficiency compared to DMF-based methods. It ensures eco-friendly, cost-effective large-scale peptide manufacturing, meeting modern sustainability standards.

P-244

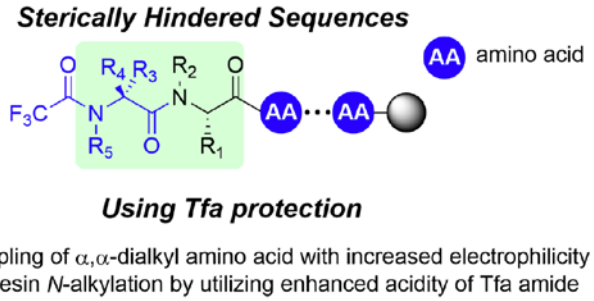
Method for Preparation of Sterically Hindered Peptides Utilizing Trifluoroacetyl Protection

Hidekazu Hochido, Yuya Morita, Satoshi Hashimoto, Hatsuo Kawada, and Kenichi Nomura

Chugai Pharmaceutical Co. Ltd., Kanagawa, Japan

N-alkylated cyclic peptides represent a promising modality for targeting intracellular protein-protein interactions, PPIs in drug discovery. However, their development has been constrained by synthetic limitations, particularly the low reactivity associated with the steric hindrance of *N*-alkyl moieties during conventional solid-phase peptide synthesis.

We present an innovative synthetic methodology specifically designed for sterically hindered sequences containing *N*-alkyl-α,α-dialkyl amino acids adjacent to *N*-alkyl amino acids.[1] Our two-step approach leverages the distinctive



properties of the trifluoroacetyl group for N-protection, which both enhances the electrophilicity of Tfa-protected *N*-H amino acids and increases *N*-H acidity to facilitate site-selective *N*-alkylation. This methodology has successfully enabled the synthesis of previously challenging peptide sequences, thereby expanding the structural diversity accessible for drug development targeting intracellular PPIs.

[1]Nomura, K.; Hochido, H.; Morita, Y.; Hashimoto, S.; Kawada, H. *Chem. Comm.* **2025**, 61, 4856-4859.

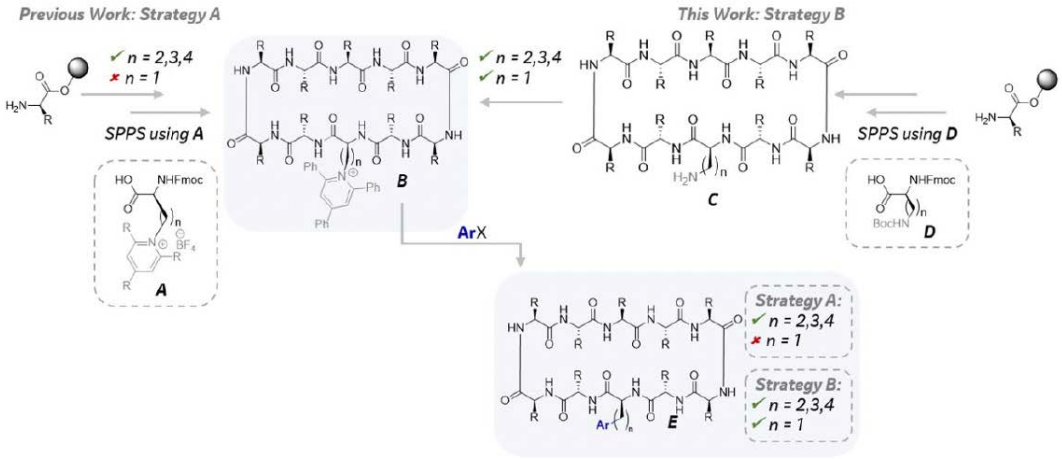
P-245

Unnatural Expansion: Harnessing Late-Stage Functionalization of Macrocyclic Peptides to Accelerate Drug Discovery

Chris Plummer[1], Ahmet Kekec[2], Dipa Kalyani[1], and Lauren Tran[3]

[1]Merck, Rahway, USA
[2]Merck, South San Francisco, USA
[3]University of Wisconsin, Madison, USA

Interest in peptide therapeutics has grown steadily in recent years as demonstrated by the marked increase in peptide-related patent applications and drug approvals. This is largely due to the perception that peptides enable access to previously undruggable targets by providing very high affinity and selectivity comparable to biologics with the potential to achieve small molecule-like biodistribution. Another particularly appealing aspect of this modality is the modular tunability of the amino acid sequence which allows incorporation of non-canonical amino acids, ncAAs, to modulate properties.



Hence, general methods for the efficient incorporation of structurally diverse ncAAs are highly desirable. The most widely used method to incorporate ncAAs into peptides is using Fmoc-protected building blocks via solid phase peptide synthesis, SPPS. Notwithstanding the advantages of SPPS, including amenability with state-of-the-art peptide synthesizers, it is limited by the availability of Fmoc-protected amino acids. A more attractive approach to ncAA-containing peptides would entail the late-stage functionalization, LSF, of peptides from a common intermediate.

This poster will describe the development of a method to readily access diverse aryl and heteroaryl alanine-containing pharmaceutically relevant macrocyclic peptides, MCPs, via a two-step sequence involving late-stage installation of the pyridinium functionality on the peptide followed by reductive couplings with aryl halides. Reaction optimization and definition of substrate scope, with respect to aryl halide and MCP, by leveraging microscale high-throughput experimentation, HTE, will be described to illustrate this method for accessing ncAA-containing MCPs that would otherwise be inaccessible via SPPS using commercially available amino acids. Application of this method in the context of a discovery-phase peptide program will be shown as an example of its utility in a real-world setting.

P-246

Streamlined High-Throughput Screening of GLP-1 Analogues Using Automated Parallel Peptide Synthesis

Tori Angermeier, Colin Simpson, Sandeep Singh, and Jonathan Collins

CEM Corporation, Matthews, USA

Peptides functioning as GLP-1 analogues, such as liraglutide and semaglutide, have gained prominence in recent years for their therapeutic applications, including diabetes management and weight loss. The introduction and combination of these new analogues with new applications has continued to expand the utility for this class of compounds. Automated parallel peptide synthesis has found utility for high-throughput screening of new analogues, but its use has traditionally been limited to shorter sequences, up to 20 amino acid residues.[1]

In this work, we introduce an improved process for investigating a library of GLP-1 analogues using liraglutide, a 31-residue peptide sequence, as the model sequence. This process employs synthesis in 96-well plates on an automated parallel peptide synthesizer, enabling multiple synchronous syntheses under heated conditions to expedite the synthesis method, optimize chemistry, and improve purity. Our methodology incorporates optimizations to ensure high efficiency and quality in sample preparation, synthesis, and workup.

This work serves as a proof of concept for high-throughput screening of GLP-1 analogues and holds promise for other classes of peptides.

[1]Albayrak-Guralp, Saadet; Murgha, Yusuf E.; Rouillard, Jean-Marie; Gulari, Erdogan. From Design to Screening: A New Antimicrobial Peptide Discovery Pipeline. *PLOS ONE*. **2013**, Vol. 8 (3): e59305.

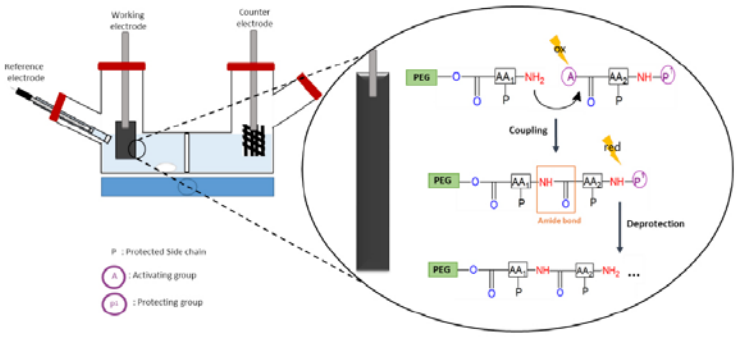
P-247

Electrochemical Oligopeptide Synthesis

Priscila Seveur, and Nicolas Plumeré

Technical University of Munich - Campus Straubing, Straubing, Germany

Given the increasing focus on health and environmental concerns, the "green" aspect of peptide synthesis methods, especially SPPS, is actively investigated, leading to the development of alternative peptide synthesis methods.



We present a novel peptide synthesis method that utilizes electrochemistry to form amide bonds between functionalized α-amino acids. At the C-terminal, the carboxylic acid is esterified with a trimethylhydroquinone compound acting as a carbonyl activator upon electrochemical oxidation, enhancing its electrophilicity to facilitate a nucleophilic attack. At the N-terminal, the amine is protected with a quinone-based group, which can be removed via reduction to prevent unwanted reactions. Through series of electrochemical activation and deprotection steps of the amino acid building-blocks, we synthesized various oligopeptides in solution, starting with dipeptides as proof-of-concept, followed by longer oligopeptides on a polyethylene glycol support. This method proved to be a waste-reducing and atom-economic system where electrons are used as waste-free reagents and the quinone moieties can be recycled for further functionalization.

P-249

Insights Into the Nature of C-H/O and N-H/N Interactions for Structurally Biased Protein Design and Conformationally Dependent Disease Progression of Post-Translationally Modified Proteins

Noah Daniecki, Megh Bhatt, Glenn Yap, Catherine Leimkuhler-Grimes, and Neal Zondlo

University of Delaware, Newark, USA

Proline's lack of an amide hydrogen makes it the ideal model residue for studying interactions within proteins that would otherwise be difficult to study when in competition with hydrogen bonding. Without amide hydrogen bonding, other noncovalent N–H/N and C–H/O interactions become significant driving forces for complex assembly at proline residues in proteins. 4*S*-EWG-proline derivatives were synthesized due to their preferences for *endo* and the *δ* conformations.

With a C-terminal amide hydrogen donor, these derivatives should prefer conformations which facilitate N–H/N interactions. Several 4-hydroxyproline derivatives with *i*+1 amides were synthesized and crystallized for X-ray analysis. An *i*/*i*+1 N–H/N interaction was observed in a crystal structure with a H•••N distance of 2.31 Å and nitrogen pyramidalization of -12°, indicative of lone pair localization about the amide N. This N–H/N interaction stabilized the *δ* conformation in this residue. This conformation is prominent in β-turns and demonstrates use for N–H/N interactions in structural design.

Computational studies further demonstrated altered energetics of *cis*-*trans* isomerism when engaged in an N–H/N interaction. Without amide hydrogen bonding, the formation of C–H/O interactions is also more prominent. The polarized nature of the C–H bonds on proline's pyrrolidine ring introduce a second stabilizing force, forming noncovalent interactions with electron-rich groups within proteins. A search of the Cambridge Crystallographic Database identified 2,238 proline-containing structures exhibiting at least one C–H/O interaction with a distance below the sum of the van der Waals radii for hydrogen and oxygen.

These investigations highlight the ubiquity of C–H/O interactions and their significance in driving assembly. Phosphorylation is a disease relevant post-translational modification in the microtubule-binding protein tau. Upon glycosylation and phosphorylation, the barrier of *cis*-*trans* is altered and the protein structure undergoes dynamic change. These post-translational modifications have opposing effects on the conformational dynamics of tau. C-terminal phosphorylation was found to be related to an autoproteolytic event in tau, potentially contributing to downstream disease progression.

Efforts to study glycosylation in tau have highlighted current limitations for the study of O-linked N-acetylglucosamine transferase, OGT activity. This has led to the development of a novel peptide-based functional assays to probe OGT activity in cellular lysates by utilizing an array of well-characterized peptide substrates, in conjunction with an amplified luminescent proximity homogeneous assay.

P-250

Using 4-Substituted Proline Derivatives to Tune the Catalytic Activity of Polyproline/β-Sheet Based Hydrolases

Yu-Chen Cheng, Yen-Chen Pan, and Jia-Cherng Horng

National Tsing Hua University, Hsinchu, Taiwan

The sensitivity of natural enzymes to environmental conditions limits their practical applications and motivates the development of artificial alternatives. Minimalistic peptide catalysts have emerged as promising tools to replicate the activity and selectivity of natural enzymes. Building on our previous peptide designs,[1,2] we developed a series of peptide-based artificial hydrolases using a scaffold that integrates a polyproline helix with a β-hairpin peptide MAX1.[3]

In this design, a catalytic triad, Asp-His-Ser, was incorporated into the polyproline segment as the active site. Under alkaline conditions, these peptides form β-sheets and selfassemble into fibrils, exhibiting substantial catalytic activities in the hydrolysis of paranitrophenyl acetate, *p*-NPA. In addition, we used 4-substituted proline derivatives to modulate the polyproline structure and investigate their impact on catalytic performance. Our results show that the peptides containing (2*S*,4*S*)-hydroxyproline, hyp, or (2*S*,4*S*)-methoxyproline, mop, exhibit higher catalytic activity compared to those with (2*S*,4*S*)-configured counterparts, Hyp or Mop. Molecular dynamics simulations suggest that hyp- and mop-containing fragments may adopt a distorted polyproline II conformation, enhancing substrate binding and catalytic efficiency. This work highlights an effective scaffold for peptide-based catalyst design and underscores the utility of 4-substituted proline derivatives in tuning catalytic activity.

[1]Hung, P.-Y.; Chen, Y.-H.; Huang, K.-Y.; Yu, C.-C.; Horng, J.-C. Design of Polyproline-Based Catalysts for Ester Hydrolysis. *ACS Omega* **2017**, 2, 5574-5581.
[2]Huang, K.-Y.; Yu, C.-C.; Horng, J.-C. Conjugating Catalytic Polyproline Fragments with a Self-Assembling Peptide Produces Efficient Artificial Hydrolases. *Biomacromolecules* **2020**, 21, 1195-1201.
[3]Schneider, J. P.; Pochan, D. J.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. Responsive Hydrogels from the Intramolecular Folding and Self-Assembly of a Designed Peptide. *J. Am. Chem. Soc.* **2002**, 124, 15030-15037.

P-251

New Technologies Enabling Parallel Purification of Peptide Libraries

Elizabeth Denton

Biotage, Charlotte, USA

Strategies for peptide library construction and follow up screening, incorporating both natural and non-natural amino acids, have improved dramatically in recent years. Perhaps more importantly, the results of those efforts are starting to come to the public forefront.[1,2] As a result, the appetite for pursuing peptide screening and discovery projects has increased across all sectors. From a holistic, practical perspective of the peptide workflow, automated parallel peptide synthesizers enable ready synthesis of hundreds to thousands of compounds simultaneously which then funnel toward optimization and program maturation after the initial hits are identified. However, the limited throughput of conventional purification technologies creates a major bottleneck in producing peptide libraries.

We introduce here two complementary strategies that enable plate-based purification of peptide libraries, delivering a range of purities. These strategies can be performed in parallel and allow researchers to skip costly HPLC purification for compounds evaluated during secondary screening assays. In many of these assays the very high purity achieved with HPLC purification is often not necessary for reliable downstream assay results, making purification very wasteful and costly in terms of time and solvent consumption. Importantly, we will demonstrate that this approach is amenable to automation, further improving library purification consistency and improved laboratory efficiency.

[1]Alleyne, C. et al. *J. Med. Chem.* **2020**, 63, 13796-13824.
[2]Brousseau, M. E. et al. *Cell Chem. Biol.* **2021**, 29, 249-258.

P-252

High-Throughput Screening of Combinatorial Peptide Libraries by Phage Display Reveals Novel GLP-1 and HIV Ligands and Potential Therapeutics

Brian Kay, Jerry Woo, and Emily Orozco

Tango Biosciences, Chicago, USA

The recent commercial success of GLP-1 has sparked increased interest in identifying new peptide agonists and antagonists for drug targets. Phage display offers a high-throughput screening solution to reveal novel peptide ligands for diverse antigens. In this study, we demonstrate the utility of screening large combinatorial libraries, $>10^9$ sequences featuring linear and cyclic peptides against the GLP-1 receptor, GLP-1R, and HIV p24 protein. We show that GLP-1R peptides bind in the phage context, and libraries can be screened for novel peptide ligands that may serve as therapeutic alternatives to current peptides.

The HIV p24 screens revealed a linear twelve amino acid peptide that binds with nanomolar affinity, even in the presence of human serum. We apply sequential truncation and alanine scanning to identify the minimal peptide length and critical amino acid residues contributing to p24 binding. The resulting
peptide can be used to develop diagnostic point-of-care assays to detect HIV and we aim to further evaluate the peptide as a drug candidate. This study underscores the utility of highthroughput phage display screening technology to initiate peptide drug discovery and diagnostic research programs for diverse targets.

P-253

Early-Stage Discovery Using Computationally Designed Helical and Cyclic Peptide Libraries

Maryna Gorelik

ProteinCure, Toronto, Canada

Therapeutic peptides are a rapidly expanding drug class, offering key advantages over antibodies such as enhanced tissue penetration, lower immunogenicity, and cost-effective, scalable synthesis. However, they often suffer from lower binding affinity, limited solubility, and increased susceptibility to degradation—necessitating extensive medicinal chemistry to overcome these liabilities. To address these limitations and generate peptide binders with improved biophysical and binding properties, we developed customized combinatorial peptide libraries.

We designed both helical and cyclic libraries using structure-based approaches to enhance folding propensity, binding potential, and developability. Each library comprises multiple sub-libraries, each incorporating distinct randomization strategies to explore diverse binding interface chemistries. The libraries were displayed on the PIII coat protein of filamentous phage and screened against a broad panel of protein targets.

Interestingly, most antigens showed a strong preference for specific sub-libraries, validating the importance of structural and chemical diversity in library design. Phage-based evaluations revealed a range of target-specific binding, with

most antigens yielding highly selective hits. Detailed characterization of lead binders showed they often engage the same surfaces as natural ligands while achieving superior selectivity across homologous protein families.

Several hits from the helical library were synthesized and tested as free peptides. These candidates demonstrated low nanomolar to micromolar binding affinities, high solubility, and minimal aggregation at elevated concentrations—highlighting their potential as developable leads for therapeutic development.

P-254

Peptide/Peptoid Conjugates for the Water Solubilization of Oligomers of α-Aminoisobutyric Acid

Matthew Kubasik, Kelley Ross, Sarah Breslow, Colin Gorman, and Chinua Aghanwa

Fairifield University, Fairfield, CT, USA

Our group has long been interested in the preparation and characterization of oligomers of the α-aminoisobutyric acid residue, Aib, which is known to form 310 helical structures in solution and the solid phase, at the length of about six residues. In principle, these helices could be used as scaffolds for catalytic groups or as components for peptide assembly. Unfortunately, Aib-based oligomers are hydrophobic, so it is difficult to compare the properties of helical structures generated with Aib to long, water-soluble helices composed of L-amino acids. In this work we have prepared water-soluble Aib helices using a peptide/peptoid conjugate strategy, where peptoid components contain water-solubilizing ionizable groups. We will present data characterizing our water-solubilized Aib oligomers, including FT-NMR, FT-IR, CD and MALDI-ToF data.

P-255

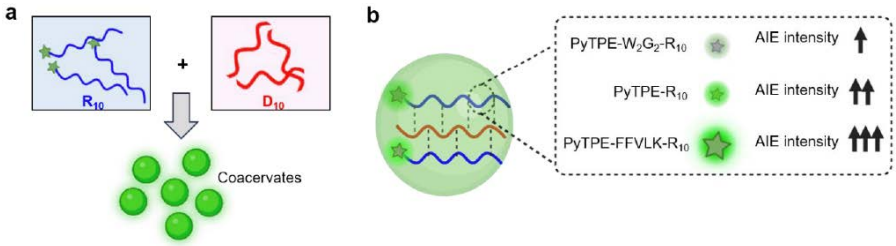
Influence of β-Sheet Spacer in Aggregation Induced Emission of Peptide Based Coacervates

Moumita Halder[1], Zhicheng Jin[1], Ke Li[2], Lubna Amer[1], Tengyu He[1], and Jesse Jokerst[1]

[1]UC San Diego, La Jolla, USA
[2]Institute of Materials Research and Engineering, IMRE, Fusionopolis, Singapore

The liquid-liquid phase separation, LLPS, or coacervation of short peptides is emerging as a significant area of research due to their potential to form membraneless organelles and dynamic compartments. The design of phase-separating peptides, PSPs, holds promise for applications in bioimaging, drug delivery, and disease theranostics. However, the influence of peptide building blocks on the properties of coacervates remains insufficiently explored.

In this study, we investigate how incorporating different β-sheet promoters into peptide sequences affects the fluorescence intensity of coacervates, which could facilitate the development of fluorescent light-up probes. We synthesized oligopeptide-based coacervates comprising an anionic Asp-peptide, D_{10}, and a cationic Arg-peptide, R_{10}, conjugated to PyTPE, an aggregation-induced emission, AIE, generator.



Schematic illustration of coacervation induced emission. **a|** Complex coacervates comprising of cationic Arg-rich domain conjugated with aggregation induced emission generator, PyTPE, and Asp-rich oligo peptides as anionic domain. **b|** Variation in AIE intensity based on β-sheet promoter.

By introducing various β-sheet-promoting self-assembling units between the AIEgen and R10, we examined their impact on the size and AIE intensity of the coacervates.

Our findings demonstrate that the properties of coacervates are influenced by intrinsic factors, peptide sequence, Arg and Asp composition, charge ratio, extrinsic factors, pH, ionic strength, salts, and the β-sheet content of the constituent peptides. Notably, the peptide containing self-assembling unit, FFVLK, present in amyloid β-sheet, exhibited the highest fluorescence turn-on, ~450-fold, and formed self-coacervates in phosphate buffer, whereas the peptide with W_2G_2 as a β-sheet promoter showed minimal enhancement in coacervate-induced emission. Moreover, the coacervates efficiently penetrated murine colon adenocarcinoma cells, MC38, via endocytosis, maintained over 95% cell viability, and retained their structural integrity inside cells. These findings provide valuable insights into the rational design of fluorescent light-up probes based on coacervate-induced emission using AIEgen-conjugated peptides with tunable material properties.

P-256

Coiled-Coil Peptide Nanotubes For Nuclear-Based Gene Delivery

Sneha Dasgupta, and Jean Chmielewski

Purdue University, West Lafayette, USA

Gene therapy, while being a promising therapeutic approach for a myriad of conditions, including neurological, monogenic, and cardiovascular disorders, often faces a significant challenge in identifying an effective and well-tolerated delivery method. This remains a critical bottleneck in achieving successful therapeutic outcomes. Peptide assemblies, in this context, can present a promising solution due to their biocompatibility, biodegradability, and ability to be tailored for enhanced stability and targeted delivery.

Our group has developed nanotubes from coiled-coil peptides via self-assembly, TriNL, and metal-directed coassembly, TriNL-p2L.[1,2] These hollow tubes have a substantial cavity and a positive zeta potential, enabling the successful encapsulation of fluorescein-labelled anionic dextran. Motivated by these results, we have successsfully encapsulated therapeutic oligonucleotides(c-myb) into these nanotubes and our further aim is to deliver them directly to the target cells which can be achieved by attaching a Cell-Adhesion Sequence, CAS, to these nanotubes, thus, enabling them to traverse the cell membrane into the cytoplasm. Long-term studies may also incorporate Nuclear Localization Sequences, NLS, to enable targeted delivery into the nucleus.

[1]Jorgensen, M. D.; Chmielewski, J. *ACS Omega* **2022**, 7, 20945–20951.
[2]Nambiar, M.; Nepal, M.; Chmielewski, J. *ACS Biomater. Sci. Eng.* **2019**, 5, 5082–5087.

P-257

Development of a Cyclic RGDTF-Based Peptide with Enhanced αvβ5 Integrin Binding for Biomedical Applications

Yuji Yamada[1], Seiya Ohki[1], Masumi Matsunuma[1], Masaki Mishima[1], Mitsuhiro Takeda[1], Teppei Ikeya[2], Tomomi Furihata[1], Keisuke Hamada[1], Yamato Kikkawa[1], and Motoyoshi Nomizu[1]

[1]Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan
[2]Tokyo Metropolitan University, Tokyo, Japan

Integrins are key receptors that mediate cell–extracellular matrix interactions and regulate essential processes such as adhesion and migration. The RGD, Arg-Gly-Asp, motif is a well known integrin-binding sequence with broad biomedical applications. We recently identified that two additional residues following RGD significantly enhance binding to integrin αvβ5.[1] In particular, the RGDTF motif promotes strong αvβ5-mediated cell adhesion.[2] In this study, we designed and synthesized cyclic peptides incorporating RGDTF to improve αvβ5-binding affinity. Several cyclic variants were

evaluated by their ability to inhibit cell adhesion to vitronectin, using Cilengitide, cRGDfMeV, as a reference. Among them, cRGDTFI —cyclized via a thioether bond— showed the highest affinity, outperforming Cilengitide. Furthermore, cRGDTFI, when immobilized on a surface, strongly promoted cell adhesion, and its fluorescently labeled form was efficiently internalized in an integrin-dependent manner. These results demonstrate that cRGDTFI is a potent and modifiable peptide with high potential for applications in regenerative medicine, targeted delivery, and molecular imaging.

[1]Yamada Y, Onda T, Hagiuda A, Kan R, Matsunuma M, Hamada K, Kikkawa Y, Nomizu M., RGDX1X2 motif regulates integrin αvβ5 binding for pluripotent stem cell adhesion. *FASEB J*, **2022**, 36, e22389
[2]Yamada Y, Onda T, Wada Y, Hamada K, Kikkawa Y, Nomizu M., Structure-activity relationships of RGD-containing peptides in integrin αvβ5-mediated cell adhesion. *ACS Omega*, **2023**, 8, 4687-4693

P-258

Branched, Multi-component Peptide Nanotherapeutics for the Treatment of Chronic Intestinal Inflammation

Mark R. Karver[1], Matthew I. Bury[2], Tristan D. Clemons[3], Suvendu Biswas[1], Simran M.Sharma[2], Brooke E. Hilderbrant[2], Samuel I. Stupp[1], and Arun K. Sharma[2]

[1]Northwestern University, Chicago, USA
[2]Lurie Childrens Hospital, Chicago, USA
[3]University of Southern Mississippi, Hattiesburg, USA

Inflammatory Bowel Diseases, IBD, such as ulcerative colitis, UC, and Crohn's disease, CD, are debilitating bowel conditions that result in a variety of symptoms including pain and cramping, sores/ulcers, diarrhea, and intestinal bleeding, among others. Current treatment options for patients include biologic and/or pharmacologic anti-inflammatory therapies that are associated with deleterious side effects and some even carry an increased risk of cancer. Other treatments are helpful initially but lose their effectiveness over time as patients grow accustomed to them.

Peptide amphiphiles, PAs, which can self-assemble into nanoscale supramolecular structures that create high density localization of peptide sequences and other bound molecules, provide a unique and versatile platform for developing nanotherapeutics with many potential applications. The Sharma Research Group has demonstrated the effectiveness of using PAs expressing anti-inflammatory, AIF, peptides to regenerate functional urinary bladder tissue while reducing inflammatory processes and to independently reduce lesion size and tissue specific inflammation when directly injected into small intestinal skip lesions in mice with CD-like ileitis that mimics the human condition.

The current project aims to build upon previous work done with AIF PAs to make them applicable to IBDs without invasive surgery involving direct application to the affected areas. Multifunctional PAs were designed and synthesized containing the established AIF peptide as well as a unique intestinal targeting sequence to establish an injectable nanotherapeutic PA system to target and modulate areas of inflamed intestinal tissue with established mice with human-like CD ileitis. Results show significant decreases in key inflammatory events in mice systemically injected with the targeted AIF-PA when compared to controls indicating the promise of our system in treating intestinal inflammation.

P-259

Polysarcosine-Catalytic Peptide Conjugates

Josh Bulski, Isabella Nimm, Alison Harris, and Leah Witus

Macalester College, Saint Paul, USA

Proteins and peptides are important classes of therapeutics. Conjugation to poly(ethylene glycol), PEG, is often used to improve their pharmacokinetic properties. However, allergic responses to PEG necessitates the development of alternative shielding polymers. The polymer of the endogenous amino acid sarcosine, polysarcosine, PSar, is particularly promising as it is biocompatible, hydrophilic, and nonionic. For consideration as a potential shielding polymer for biotherapeutic applications, an understanding of the effects of PSar conjugation on therapeutic peptide and protein activity is important.

We used a two-step bioconjugation scheme to attach PSar to catalytic peptides and enzymes, and studied the activity of the resulting conjugates. We found PSar compared favorably to PEG, although the conjugation chemistry itself affected catalytic activity. These results are promising for the consideration of PSar as an alternative shielding polymer.

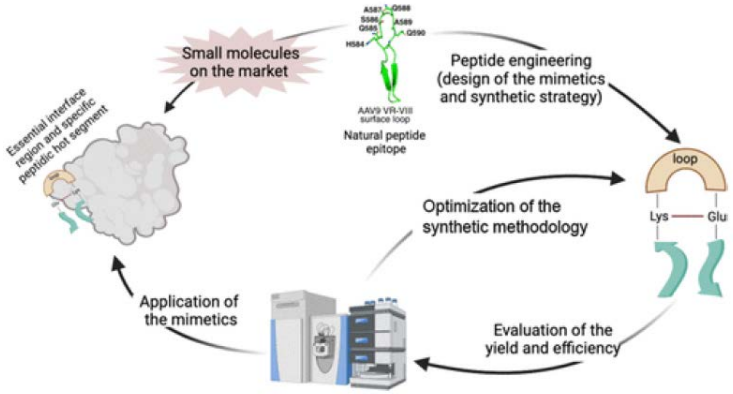
P-260

Advancements in Loop Cyclization Approaches for Enhanced Peptide Therapeutics for Targeting Protein–Protein Interactions

Lucia Lombardi[1], Luke Granger[2], Robin Shattock[2], and Daryl Williams[2]

[1]Queen's University Belfast, Belfast, United Kingdom
[2]Imperial College, London, United Kingdom

Protein–protein interactions, PPIs, are pivotal in regulating cellular functions and life processes, making them promising therapeutic targets in modern medicine. Despite their potential, developing PPI inhibitors poses significant challenges due to their large and shallow interfaces that complicate ligand binding. This study focuses on mimicking peptide loops as a strategy for PPI inhibition, utilizing synthetic peptide loops for replicating critical binding regions. This work explores turn-inducing elements and highlights the importance of proline in promoting favorable conformations for lactamization, yielding high-purity cyclic peptides.[1]



Notably, our one-pot method offers enhanced versatility and represents a robust strategy for efficient and selective macrolactamization, expanding the scope of peptide synthesis methodologies. This approach, validated through the synthesis of AAV capsid-derived loops, offers a robust platform for developing peptide-based therapeutics and highlights the potential of peptide macrocycles in overcoming PPI drug discovery challenges and advancing the development of new therapeutics.

[1]Lombardi, L., Granger, L.A., Shattock, R., Williams, D.R., *J. Org. Chem.* **2025**, 90, 4, 1467–1477

P-261

Utilizing a Hyaluronan-Binding Peptide for Interspecies Neuron-Specific Targeting and Molecule Delivery

Scott Elmore[1], Xiaoqian Ge[1], Joseph Wekselblatt[1], Bo Wang[2], Tongtong Wang[2], Renjinming Dai[1], Harsh Dave[1], Mohammadaref Ghaderi[1], Athul Raj Anilkumar[1], Bill Wang[1], Shashank Sirsi[1], Mikhail Shapiro[2], Yuki Oka[2], Carlos Lois[2], Zhenpeng Qin[1], and Jung-Mo Ahn[1]

[1]University of Texas at Dallas, Richardson, USA
[2]California Institute of Technology, Pasadena, USA

Peptide ligands that have neuron-specificity are consequential in the development of new therapeutic applications in the brain and neuroscience research. This study investigates a 15-mer peptide, named N1, which exhibits substantial *in vivo* neuronal specificity. N1 diffuses throughout the entire neuron including the cytosol, nucleus, axon, and dendritic spines. It also demonstrates neuronal labeling across multiple brain regions – cortex, striatum, hippocampus, cerebellum, and spinal cord – and multiple species – mouse, rat, treeshrew, and zebra finch. The neuronal targeting of N1 can be sustained via intraparenchymal, intrathecal, and intravenous injections after the blood-brain barrier, BBB, is made accessible through focused ultrasound, FUS. Additionally, N1 promotes the delivery of biologically active proteins, such as Cre protein, into neurons which leads to site-specific DNA recombination. N1 is an innovative and adaptable platform for neuron-specific delivery within the central nervous system.

P-262

Inducing Endoplasmic Reticulum Stress as a Therapeutic Strategy for Ovarian Cancer by Using Oligo-Benzamides

Scott Elmore[1], Henry Neal[1], Chia-Yuan Chen[1], Kara Kassees[1], Tae-Kyung Lee[1], Suryavathi Viswanadhapalli[2], Gaurav Sharma[2], Ratna Vadlamudi[2], and Jung-Mo Ahn[1]

[1]University of Texas at Dallas, Richardson, USA
[2]University of Texas Health Science Center at San Antonio, San Antonio, USA

Ovarian cancer, OCa, remains one of the deadliest malignancies, often developing resistance to standard chemotherapy, leading to metastatic progression. This study investigated the potential of targeting the high level of endoplasmic reticulum stress, ERS, in OCa cells to trigger apoptosis by utilizing oligo-benzamides,[1] focusing on LIPA as a critical therapeutic target.[2] Oligo-benzamides are a scaffold comprising of 4-aminobenzoic acids which can be readily functionalized at position 3 for building chemical libraries.

A small library of over 200 oligo-benzamides was designed and synthesized to identify candidates that inhibit LIPA and modulate ERS signaling. Among these, ERX-208 emerged as a potent compound, exhibiting significant growth inhibition across multiple OCa cell lines. To elucidate binding interactions of ERX-208 with LIPA, *in silico* molecular docking simulation was conducted. Further biological examination of ERX-208 determined that it specifically induced ERS, unfolded protein response, UPR, activation, and apoptosis, as confirmed by RNA sequencing and mechanistic assays. ERX-208 also demonstrated efficacy in OCa patient-derived tumors. These results suggest that ERX-208 targets LIPA to enhance ERS and promote apoptosis, offering a promising therapeutic strategy for overcoming tumor heterogeneity in ovarian cancer.

[1]Chen, C.-Y.; Elmore, S.; Lalami, I.; Neal, H.; Vadlamudi, R. K.; Raj, G. V.; Ahn, J.-M. *Method Enzymol*. **2024**, 698, 221-245.
[2]Liu, X.; Viswanadhapalli, S.; Kumar, S.; Lee, T.-K.; Moore, A.; Ma, S.; Chen, L.; Hsieh, M.; Li, M.; Sareddy, G. R.; Parra, K.; Blatt, E. B.; Reese, T. C.; Zhao, Y.; Chang, A.; Yan, H.; Xu, Z.; Pratap, U. P.; Liu, Z.; Roggero, C. M.; Tan, Z.; Weintraub, S. T.; Peng, Y.; Tekmal, R. R.; Arteaga, C. L.; Lippincott-Schwartz, J.; Vadlamudi, R. K.; Ahn, J.-M.; Raj, G. V. *Nat. Cancer*. **2022**, 3, 866-884.

P-263

Oligo-Benzamide-Based Helix Mimetics to Inhibit Protein-Protein Interactions and Treat Hard-to-Kill Cancers

Jung-Mo Ahn

University of Texas at Dallas, Richardson, USA

We have designed an oligo-benzamide scaffold[1] as a rigid template to emulate protein helices, and it presents 2-3 side chains found at the *i*, *i*+4, and *i*+7 positions in a helix in addition to 2 functional groups at its N- and C-termini for achieving higher affinity, selectivity, and improved physicochemical properties. It has demonstrated outstanding mimicry of LXXLL motifs that is critical to facilitate protein-protein interactions between nuclear receptors and their coactivators. Bisbenzamide D2 was found to disrupt androgen receptor and its coactivators effectively in prostate cancer models,[2] whereas trisbenzamide ERX-11 showed potent inhibition of estrogen receptor in breast cancer models.[3,4]

Recently, our efforts to further improve these leads identified a new therapeutic target, lyososomal acid lipase A, LIPA, for treating hard-to-kill cancers like triple-negative breast cancer.[5] Tris-benzamide-based ERX-41 was found to induce endoplasmic reticulum, ER, stress, resulting in cell death. Mechanistically, ERX-41 binding to LIPA decreases expression of multiple ER-resident proteins involved in protein folding. This targeted vulnerability has a large therapeutic window with no adverse effects either on normal mammary epithelial cells or in mice. It is also found to be metabolically stable and orally available. We are currently evaluating this new targeted strategy in phase 1 clinical trial for solid tumors like triple-negative breast cancer.

[1]Chen, C.-Y. et al. *Methods Enzymol*. **2024**, 698, 221-245.
[2]Ravindranathan, P. et al. *Nature Communications* **2013**, 4, 1923.
[3]Raj, G. V. et al. *eLife* **2017**, 6, e26857.
[4]Lee, T.-K. et al. *ACS Pharmacol. Transl. Sci*. **2024**, 7, 2023-2043.
[5]Liu, X. et al. *Nature Cancer*, **2022**, 3, 866-884.

P-264

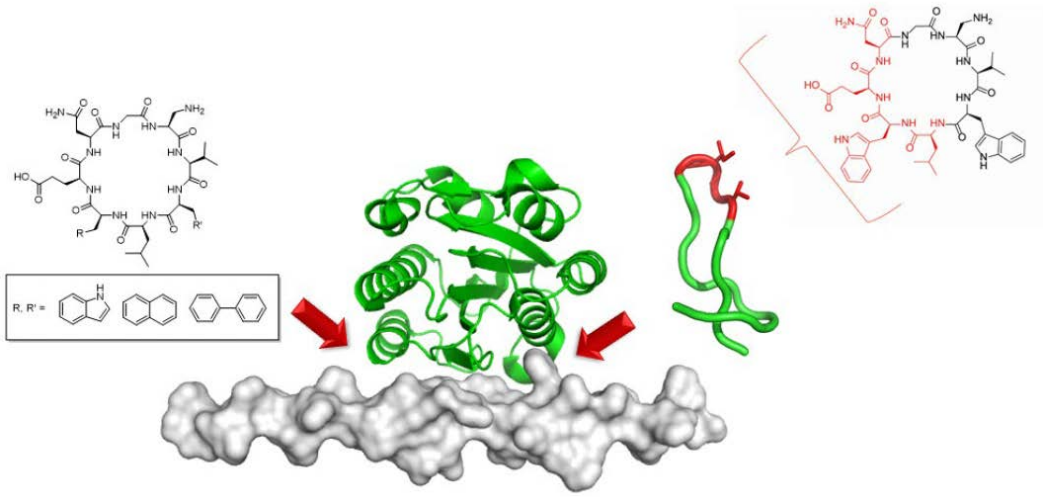
Novel Peptide Inhibitors to the vWF-Collagen Protein-Protein Interaction Involved in Thrombosis Initiation: Comparing Synthetic and Biologically Expressed Peptide Scaffolds

Danielle Guarracino[1], A. James Link[2], Drew Carson[2], Christina Tonks[1], Ryley David[1], Breanna Wixted[1], and Austin Lisojo[1]

[1]The College of New Jersey, Ewing, USA
[2]Princeton University, Princeton, USA

The interaction between blood protein von Willebrand Factor, vWF, and collagen initiates thrombosis, to which cardiovascular events such as heart attacks and strokes attribute their devastating effects. This provides a potential target for peptide drug design. Macrocyclic peptides have great potential as safe and potent therapeutics. In our pursuit of highly proficient peptides, we have followed two paths. To enhance target-binding and stability we synthesize head-to-tail cyclized peptides substituting several unnatural amino acids in key locations. In parallel, working in collaboration, we express lasso peptides with amino acid mutations, grafting a sequence of interest on the scaffold. Lasso peptides are natural products with an isopeptide bond between the N-terminus and an acidic side chain providing the threaded, macrocyclic structure.

Using site-directed mutagenesis, we exploited the loop region of a well-known lasso peptide. By testing both synthetic and biologically expressed cyclic peptides, we make comparisons between both paths to inhibition, assessing efficacy and protease stability. We use an *in vitro* fluorescence-linked immunosorbent assay with anti-vWF antibodies to detect the quantity of vWF remaining bound to collagen-coated plates in the presence of our peptides. Additionally, we evaluate the degradation of our peptides when treated by a panel of proteases, emulating cellular conditions.



Currently, we are examining rationally designed, vWF-inspired, sequences of peptides in both paths. The link between amino acid sequence, structure, and inhibitory potency is paramount to developing new peptide-based therapeutics. Our efforts have the potential to provide ground-breaking information to the field as no peptide-based anti-thrombosis medications are in circulation.

P-265

Discovery of Peptidomimetic Ligands for UBR1 E3 Ligase for Targeted Protein Degradation via the N-Degron Pathway

Hee Myeong Wang, Dongmin Shin, and Hyun-Suk Lim

POSTECH, POHANG, South Korea

Proteolysis-targeting chimeras, PROTACs are an emerging therapeutic modality for targeted protein degradation. PROTACs are heterobifunctional molecules that hijack E3 ligases and ubiquitin-proteasome system, thereby leading to selective degradation of target proteins. However, despite the great utility of this system, only a limited number of E3 ligases, for example, CRBN, VHL, have been leveraged for targeted protein degradation. Consequently, many disease-related proteins cannot be effectively degraded by PROTACs, in part due to the low expression levels or functional activity of the target E3 ligases in the relevant cells or tissues. Additionally, the usage of conventional PROTACs can be constrained by potential risks such as drug resistance and on-target, off-tumor toxicity, which may negatively influence drug efficacy.

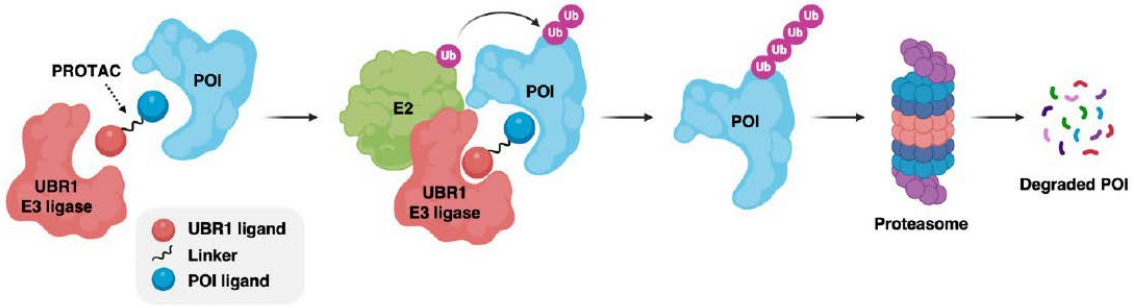


Fig. 1. Schematic illustration of the N-degron pathway-based targeted protein degradation.

To expand the therapeutic window of conventional PROTACs, we developed a new class of PROTACs based on the N-degron pathway.[1] The N-degron pathway is a proteolytic system that utilizes N-recognins, for example, UBR1 E3 ligase, to recognize N-terminal residues of proteins. Since components of this pathway are ubiquitously expressed, hijacking the N-degron pathway for targeted protein degradation would have great potential to degrade a protein of interest, POI, regardless of cell types.

Herein, we report the structure-based design, synthesis, and biological investigations of the highly potent, small-molecule UBR1 ligands. To demonstrate the utility of UBR1 for targeted protein degradation, proof-of-concept PROTACs were generated by linking UBR1 ligands to a BRD4 inhibitor, JQ1. Notably, the developed PROTACs showed significant degradation of BRD4, accompanied by pronounced therapeutic efficacy in the treatment of both cancer and metabolic disorders, including obesity and metabolic dysfunction-associated steatohepatitis, MASH. This type of PROTACs can be applied for targeting a wide range of diseases previously considered challenging with conventional techniques.

[1]Lee, Y.; Heo, J.; Jeong, H.; Hong, K. T.; Kwon, D. H.; Shin, M. H.; Oh, M.; Sable, G. A.; Ahn, G.; Lee, J. S.; Lim, H. S. "Targeted Degradation of Transcription Co-activator SRC-1 through the N-Degron Pathway." *Angew. Chem. Int. Ed.* **2020**, 59, 17548-17555.

P-266

Potential of Anticancer Peptide Derived from Green Algae in Non-Small Cell Lung Cancer

Jei Ha Lee, Heabin Kim, Seonmi Jo, Seung-Hyun Jung, and Grace Choi

National Marine Biodiversity Institute of Korea, Seocheon, South Korea

Developing new anticancer drugs that are highly effective yet have minimal side effects poses a significant challenge for drug development research on non-small cell lung cancer. Anticancer peptides, whether derived from natural or synthesized sources, present a promising avenue in the pursuit of next-generation anticancer agents characterized by selectivity and specificity.

Herein, we aimed to investigate the potential role of inhibiting lung cancer cell growth using candidates. We focused on a 27-mer peptide, termed MP28, derived from a sequence in the green sea algae, *Bryopsis plumose*. We found that MP28 exhibited more suppression of proliferative effects in adenocarcinoma types compared to the previously reported MP06, which may function as an anti-cancer and -angiogenesis.[1,2]

Interestingly, we confirmed that MP28 suppressed cellular migration and invasion, and inhibited the markers of epithelial-mesenchymal transition, such as Zeb1 and vimentin. As well, we confirmed that decreased cell growth rate and increased cell apoptosis upon MP28 treatment. Consistently, MP28 effectively reduced the tumor size of the subcutaneous xenograft tumor model. These findings highlight the potential of MP28 as a therapeutic agent against adenocarcinoma, offering promise in combating the aggressive metastatic behavior characteristic of this form of lung cancer.

This research was supported by a grant, 2025M00500, from the Ministry of Oceans and Fisheries in 2025.

[1]Kim, H., Kim, H. T., Jung, S.-H., et al. A Novel Anticancer Peptide Derived from Bryopsis plumosa Regulates Proliferation and Invasion in Non-Small Cell Lung Cancer Cells. *Marine Drugs*, **2023**, 21(12), 607.

[2]Kim, H., Jung, S.-H., Jo, S., et al. Anti-angiogenic effect of Bryopsis plumosa-derived peptide via aquaporin 3 in non-small cell lung cancer. International Journal of Oncology, 2024, 66(1), 5.

P-267

Octopus-Derived Peptide for Copper Detox: A New Approach to Heavy Metal Protection

Seung-Hyun Jung, Jeiha Lee, Haebin Kim, Seonmi Jo, Hye Ri Park, and Grace Choi

Marine Bioindustrial Research Division, Seocheon, South Korea

Copper, Cu, is an essential trace element involved in various metabolic and physiological functions. However, excessive copper exposure can lead to cytotoxicity, organ damage, and diseases such as Wilson's disease. Understanding copper homeostasis and toxicity mechanisms is crucial for developing preventive and therapeutic strategies.

In this study, we identified a novel copper-binding peptide from the genome of *Octopus minor*, an organism with highly specialized copper metabolism. Using AlphaFold-based structural predictions and *in vitro* assays, we evaluated the peptide's copper-binding affinity. Furthermore, the biological effects of copper exposure and peptide-mediated detoxification were assessed using Zebrafish, *Danio rerio*, a widely used vertebrate model for human disease and toxicology studies. We examined survival rates, growth, and tissue-specific effects, including neuroprotective and dermal responses. RNA-seq, NGS, was performed to elucidate molecular pathways associated with recovery. Additionally, stability and toxicity evaluations of the peptide were conducted to assess its feasibility for biopharmaceutical applications. This study highlights the potential of marine-derived peptides as novel biomaterials for copper detoxification and therapeutic interventions in heavy metal toxicity-related disorders.

This research was supported by a grant, 2025M00500, from the Ministry of Oceans and Fisheries in 2025.

[1]Kim, B.-M.; Kang, S.; Ahn, D.-H.; Jung, S.-H., et al. The genome of common long-arm octopus *Octopus minor. GigaScience*, **2018**. 7 (11), giy119.

P-268

Local Cationic Charge Density Enhancement via Polyamine Incorporation Improves the Selectivity of Antimicrobial Peptoids

Jinyoung Oh, and Jiwon Seo

Gwangju Institute of Science and Technology, Gwangju, South Korea

Antimicrobial resistance is a critical global health threat, underscoring the need for new therapeutic strategies. Peptoids—synthetic peptide analogues with an N-substituted glycine backbone—are attractive candidates for antimicrobial development because they resist proteolysis and offer tunable side-chain architectures. In this study, we investigated peptoids functionalized with polyamine side chains, harnessing the cationic nature of polyamines to enhance interactions with bacterial membranes.

Structure–activity relationship analyses revealed the influence of polyamine chain length and density on antimicrobial potency and selectivity. Optimized peptoids exhibited potent activity against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains, while maintaining low cytotoxicity toward mammalian cells. Mechanistic studies showed that peptoids kill through multiple pathways, including membrane disruption, oxidative damage, and intracellular aggregation of proteins and nucleic acids. Overall, this work highlights the potential of polyamine-functionalized peptoids as next-generation antimicrobial agents and offers design principles for improving their efficacy and safety.

P-269

Mechanistic Insights Into Novel Indole- and Imidazole-Containing Antimicrobial Peptoids

Minsang Kim, Jieun Choi, and Jiwon Seo

Gwangju Institute of Science and Technology, Gwangju, South Korea

Antimicrobial peptides, AMPs, and their synthetic analogues represent a promising class of therapeutic agents for combating the pressing issue of multidrug resistant, MDR, bacterial infection. As peptide mimetics, peptoids oligo *N*-substituted glycine offer a robust platform due to their proteolytic stability, structural versatility, and tunable bioactivity .

In this study , we designed and synthesized a series of heterocycle functionalized peptoids , incorporating indole and imidazole moieties, inspired by the side chain of tryptophan and histidine. The series of peptoids were evaluated for their antimicrobial potency, hemolytic toxicity, and cytotoxicity against mammalian cells . Circular dichroism, CD, spectroscopy revealed distinct secondary structural features under varying conditions, providing insight into structure

activity relationship, SAR, study. Mechanistic investigations using *in vitro* membrane disruption assays suggested that both outer and inner bacterial membrane disruption contribute to the bactericidal activity of peptoids. Further investigations are underway to elucidate additional antibacterial mechanisms.

Collectively, our findings highlight the potential of the novel heterocycle-containing peptoids as promising compounds for the development of multitarget antimicrobial therapeutics.

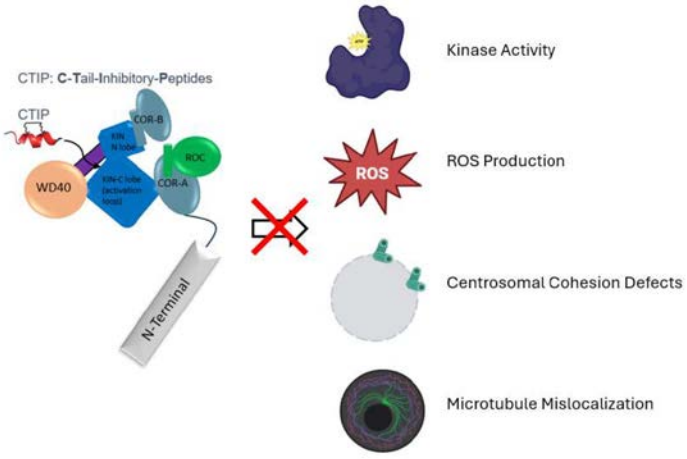
P-270

C-tail Mimics of LRRK2 Downregulate Kinase Activity in Parkinson's Disease

Tiancheng Chen, and Eileen Kennedy

University of Georgia, Athens, USA

Leucine-rich repeat kinase 2, LRRK2, is the most commonly mutated gene in Parkinson's disease, PD, a neurodegenerative disorder affecting over 10 million people worldwide. Pathogenic mutations in LRRK2 often result in hyperactivation, leading to detrimental effects such as centrosomal cohesion defects. Despite the recognized contribution of hyperactive LRRK2 in PD, the mechanisms by which multiple domains in LRRK2 regulate its activation and activity have not been well understood.



Here, we designed a library of constrained peptides, termed C-Tail-Inhibitory-Peptides, CTIPs, to mimic the C-terminal tail of LRRK2 to investigate its role in regulating LRRK2 activity. We demonstrate that these cell-permeable peptides bind to LRRK2, downregulate its kinase activity, and suppress downstream effects, including substrate phosphorylation, reactive oxygen species, ROS, production, and centrosomal splitting. Unlike many ATP-competitive type I LRRK2 kinase inhibitors that induce toxicity and mislocalization of LRRK2 within cells, these constrained peptides do not disrupt LRRK2 localization. Our findings suggest that the C-tail of LRRK2 plays a critical role in its regulation and presents a promising alternative approach for targeted LRRK2 inhibition.

P-271

Peptide Discovery Platform for Advancing Peptide-Oligonucleotide Conjugation

Yukiko Ishii

FUJIFILM, Ashigarakamigun, Kanagawa, Japan

Cyclic peptides are gaining attention as a novel modality for drug discovery and drug conjugation due to their exceptional characteristics, including target specificity, tissue permeability, and synthetic suitability. In particular, peptide-oligonucleotide conjugation has emerged as a widely utilized approach to overcome challenges associated with oligonu-

cleotide-based therapeutics. This strategy enhances delivery, cellular uptake, and bioavailability, significantly boosting overall therapeutic efficiency.

FUJIFILM offers a variety of contract services to accelerate peptide drug discovery. We have developed a rapid drug discovery platform that enables high-affinity peptide identification and diverse hit evaluation through mRNA display technology. This platform utilizes uniquely designed large peptide libraries incorporating unnatural amino acids, coupled with a rapid activity-based optimization system employing biosynthetic peptides. Using our platform, we successfully generated a high-affinity binding peptide targeting a surface receptor, which was subsequently conjugated with an oligonucleotide. In this presentation, we will show data about its activity as a drug conjugate.

P-272

Cost, Solvent, and Time Savings using Super Critical Fluid Chromatography for Peptide Purification Compared to Traditional HPLC Methods

Dylan Cavey

Teledyne Labs, Lincoln, USA

Reverse-phase chromatography is the most common method used when purifying peptides. However, these processes can be long and generate large amounts waste. This poses a challenge for peptide production sustainability. Super Critical Fluid Chromatography, SFC, is an alternative method for peptide purification.

SFC uses super-critical CO_2 as the weak solvent which lowers the viscosity and increases the diffusivity of the solvent system. This allows SFC to be significantly faster than HPLC methods. The increase in separation speed allows user to save on cost, time, and waste, creating a more sustainable future for peptide production.

P-273

Strategies to Mitigate Aspartimide Formation in Peptide Synthesis: Temperature and Base Strength Considerations

C.J Mitchell, and J. Bello

Gyros Protein Technologies, Tucson, USA

Aspartimide formation is a common side reaction during peptide synthesis that can complicate purification and negatively impact downstream assays. This phenomenon occurs when the nucleophilic nitrogen from the peptide backbone attacks the carbonyl in the aspartic acid, resulting in cyclization through a condensation reaction.[1]

Three factors can affect the extent of aspartimide formation, including **1|** Sequence[2], **2|** Temperature[3,4], and **3|** Base strength.[5] As sequence is often not an adjustable variable, here we explore the effects of temperature and base strength on aspartimide formation.

This work demonstrates that while heating during deprotection steps can improve the Fmoc removal efficiency, it significantly contributes to the formation of aspartimide. However, the application of heat during the coupling steps did not have the same impact. To reduce the formation of the aspartimide, we added formic acid and HOBt to the 20% piperidine solution. Overall, a reduction of the base strength with either additive resulted in decreased aspartimide formation at all temperatures tested, but it did not inhibit aspartimide formation entirely. To achieve zero aspartimide formation in a sequence devoid of Asp-Gly, we used the monomer Fmoc-Asp(Obno)-OH.

This study provides valuable insights for chemists seeking to minimize aspartimide formation in their crude peptide samples, emphasizing the importance of adjusting reaction conditions based on peptide sequences.

[1]Yang, Y., Sweeney, W. V, Schneider, K., Thornqvist, S., Chait, B. T., and Tam, J. P. Aspartimide Formation in Base-driven 9-Fluorenylmethoxycarbonyl Chemistry. *Tetrahedron Lett* **1994** 35,9689–9692.
[2] Behrendt, R., White, P., and Offer, J. Advances in Fmoc solid-phase peptide synthesis. *Journal of Peptide Science* **2016** 22, 4–27.
[3]Pham, T. L., Zilke, J., Müller, C. C., and Thomas, F. The CSY-protecting group in the microwave-assisted synthesis of aggregation-prone peptides. *RSC Chem Biol* **2022** 3.
[4]Personne, H., Siriwardena, T. N., Javor, S., and Reymond, J. L. Dipropylamine for 9-Fluorenylmethyloxycarbonyl (Fmoc) Deprotection with Reduced Aspartimide Formation in Solid-Phase Peptide Synthesis. *ACS Omega 8* **2023**.
[5]Wade, J. D., Mathieu, M. N., Macris, M., and Tregear, G. W. Base-induced side reactions in Fmoc-solid phase peptide synthesis: Minimization by use of piperazine as N α-deprotection reagent. *Letters in Peptide Science* **2000** 7, 107–112.

P-274

GMP Peptide API Isolation via Precipitation at Manufacturing Scale

Nadiia Kovalenko, and Jiang Ziqing

CordenPharma Colorado, Boulder, USA

Over recent years peptides have emerged as an important class of therapeutics with around 120 peptide drugs on the market and many more in clinical trials and development.[1] As such, the demand for peptide active pharmaceutical ingredient, API, continues to grow. Further, the indications for these peptidic therapeutics are quite broad, requiring the development of efficient, scalable and reliable methods for isolation at scale.

The peptide API is typically isolated as a dried powder utilizing one of the three most commonly used strategies: lyophilization, spray drying or precipitation. This poster will give an overview of the three strategies discussing their advantages and limitations. All three techniques of API isolation are offered by Corden Pharma for GMP manufacturing at any scale.

[1]Al Musaimi, O.; Al Shaer, D.; De La Torre, B. G. and Albericio, F. 2024 FDA TIDES - Peptides and Oligonucleotides- Harvest. *Pharmaceuticals* **2025**, 18 (3), 291.

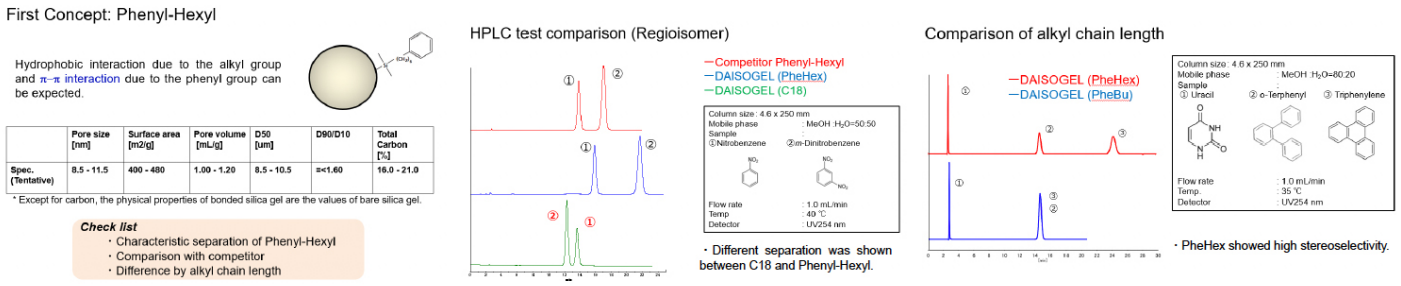
P-275

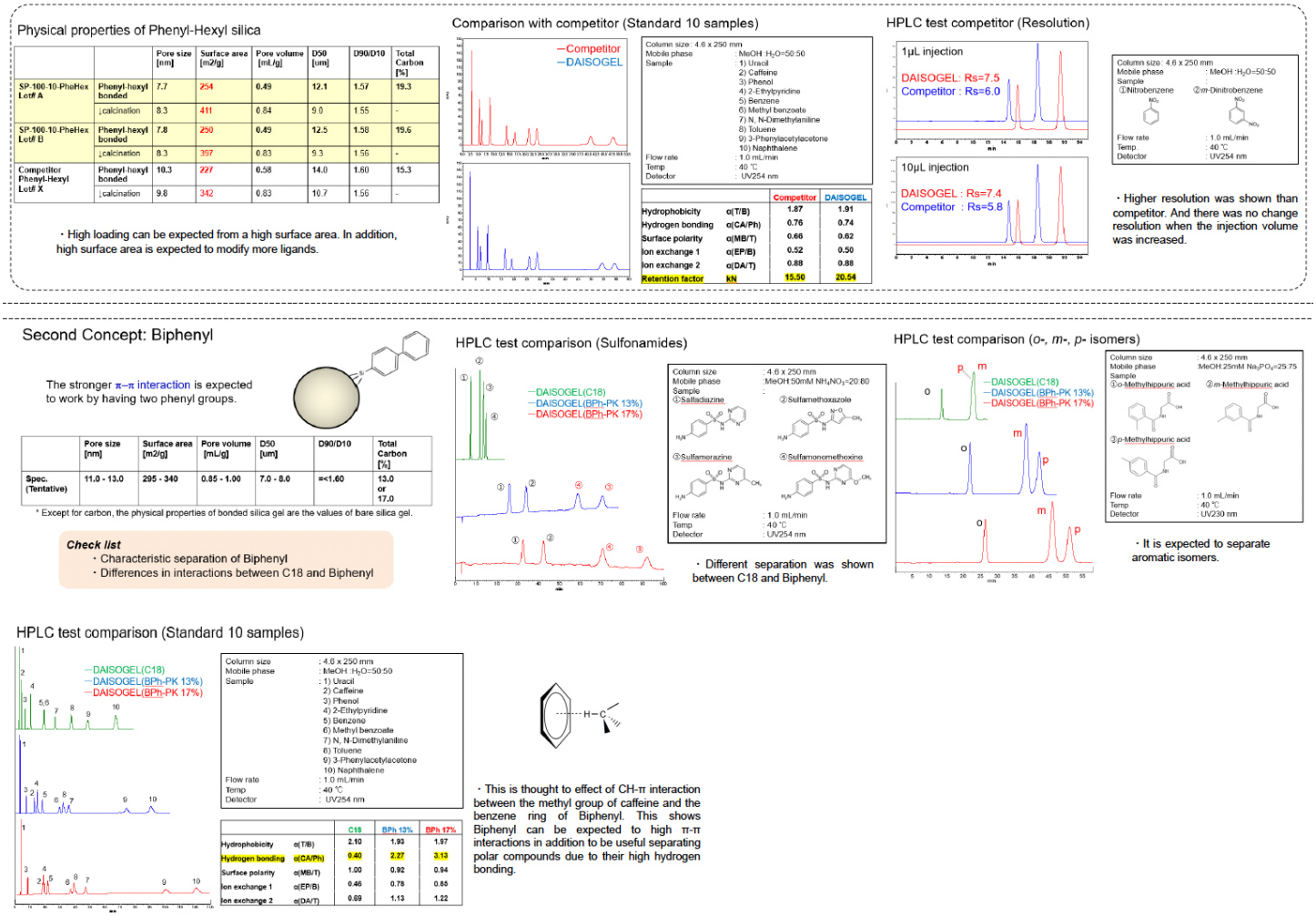
Alternative Peptide Separation Modes in Action for Large-Scale Purification

Jonathan Edelman

DAISO Inc, Torrance, USA

With the explosive increase in the number of new large-scale peptide purification processes, more and better tools are needed. Distinguishing between correctly formed target peptide molecule and those mishappened can be a real challenge, as often they only differ by a single amino acid. The separation power of the conventional alkyl chain-bonded reversed phase silica types, C18, C8 bonded, mostly utilize hydrogen bond interactions as the means of separation. For stronger, better, or ***alternative*** separation, secondary interactions can be employed, or if available, different separation modes should be explored. A rather powerful alternative separation is the **π-π** interaction.





Regiostereomers and diastereomers can be separated with Phenyl or biphenyl-bonded stationary phases. The poster compares the different separation patterns achieved by Phenyl, attached via C6 chains, and biphenyl, in two different bonded ligand densities, with the conventional C18 separation. The new, different separation patterns open a dazzling variety of ways to achieve separation in your large-scale API purification processes. Alternative separation modes may be the answer to many tough peptide purification challenges! The "PIE IN THE SKY" has been made a reality!

P-276

Accelerated Preclinical Discovery and Optimization of PD-L1 and Other Receptors using 48HD Technology

Rima Mistry, Andreas Dorian, Manjot Kaur, Zoe O'Gara, Vincent Albert, Adam Brown, Martin Truksa, and Ratmir Derda

48Hour Discovery, Edmonton, Canada

48Hour Discovery, 48HD, accelerates peptide drug discovery through a genetically encoded phage display platform, enabling screening on both proteins and live cells. Our platform explores a unique macrocyclic peptide space, integrating modified libraries with linkers and chelators for radiopharmaceutical applications. We further refine hits to single-digit nM affinity through positional scans, affinity maturation, and AI/ML-driven optimization. As a case study, we highlight our PD-L1 peptide program and demonstrate in-house validation across multiple other oncology targets.

P-277

Improving Peptide Separation and Purification Using an Asymmetric Bonding Strategy

Jerry Wang

Unified Separation Technologies, Wilmington, USA

The recent surge in GLP-1-related drug development has heightened interest in enhancing peptide separation techniques. GLP-1-targeted peptides typically consist of over 30 amino acids. Whether they are produced through biosynthesis or chemical synthesis, these peptides present a complex mixture with numerous structurally similar impurities, making purification particularly challenging.

In high-performance liquid chromatography, HPLC, the separation of molecules is governed by the difference in their distribution coefficients, ΔK, between the mobile and stationary phases, which in turn depends on the difference in Gibbs free energy, ΔG.

Traditionally, most industrial reverse-phase HPLC packing materials are created by bonding isotropic molecules to the silica surface. This approach offers limited ΔG variation, making it difficult to separate diastereomers effectively. In this presentation, we will share our research on employing an asymmetric bonding strategy, replacing isotropic molecules with anisotropic ones. This modification has significantly improved separating peptides and various other organic compounds. We will present our preliminary findings and discuss the underlying principles, providing insights and guidelines for further enhancing the HPLC separation of diastereomers.

P-278

Development of a Two-Step Chromatographic Process for thePurification of a Dual GIP/GLP-1 Receptor Agonist

Marc Jacob, and J Preston

YMC AMERICA, Devens, USA

GLP-1 agonists are a class of medications that mimic the natural hormone GLP-1. These medications help regulate blood sugar and promote feelings of fullness. They are primarily used to treat type 2 diabetes and can aid weight management. A dual GIP and GLP-1 receptor agonist has gained significant attention in the pharmaceutical industry as a breakthrough therapy for type 2 diabetes and obesity. The structural complexity of this peptide necessitates an advanced purification strategy to achieve optimal purity and yield. To address this, we developed a two-step chromatographic process utilizing two different YMC Triart media in preparative formats.

The first purification step employes Triart Prep Bio200 C8, which effectively removed the majority of the impurities present in the initial crude, improving purity from 20% to 93%. A secondary polishing step using Triart Prep C4-S, further refined the purification, ultimately achieving 99.6% purity to meet the desired pharmaceutical-grade criteria.

This study describes column screening and method development for a two-step chromatographic purification process for a dual GIP and GLP-1 receptor agonist. The combination of a YMC Triart Prep Bio200 C8 and a Triart Prep C4-S were chosen for this purification process. The applicability of this two-step process is demonstrated by starting with low purity crude and producing material that is greater than 99.6% pure. The recovery of the first step was 58.3% and is considered good when the initial material only contained 20% of the desired component. The recovery of the second step was 71.3%. These findings reinforce the importance of method development, including the stationary phase screening and selection, for preparative chromatography of complex biopharmaceutical molecules.

P-279

High Performance Flash Purification for a Greener and More Scalable Peptide Purification

Austin Schlirf, Elizabeth Denton, and Symone Carty

Biotage, LLC, Charlotte, USA

Traditionally, peptide purification has relied on preparative high-performance liquid chromatography, prep-HPLC, known for its excellent resolution and impurity removal. However, this method is often hampered by low column loading capacity and extended purification times making it inefficient and difficult to scale. In response to these challenges, Biotage has developed strategies for sustainable flash chromatography as an alternative to prep-HPLC for the purification of synthetic peptides.

Automated reversed-phase flash purification offers a fast, flexible, and cost-effective alternative to traditional methods, enabling researchers to purify synthetic peptides efficiently and redirect their efforts toward more important tasks. This approach addresses issues such as molecule-specific optimization, scale-up difficulties, and the inefficiencies of current processes, providing a greener and more efficient solution.

Herein we evaluated the effectiveness of flash purification for 3 cell penetrating peptides of varied length, charge, and polarity to demonstrate method design for high-performance flash chromatography, HPFC. We assess traditional purification solvents against alternative solvent compositions that offer a greener safer process. These strategies significantly reduce solvent consumption and purification time compared to prep-HPLC, enabling faster peptide development for downstream assays and analysis.

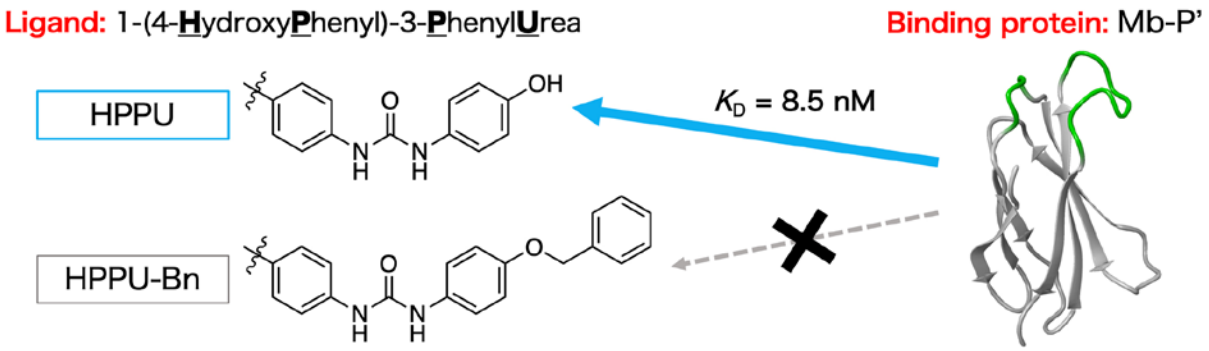
P-280

TRAP Display-Enabled *De Novo* Discovery of a Novel Ligand–Protein Binding Pair

Shun Umemoto[1], Tomoki Miyazaki[2], Koushiro Endo[3], Nariaki Tsuzuki[1], Nguyen Kim Chung[1], Natsumi Fukaya[2], Tatsuyuki Yoshii[2], Tomoshige Fujino[1], Gosuke Hayashi[1], Tomoya Hino[3], Shinya Tsukiji[2], and Hiroshi Murakami[1]

[1]Graduate School of Engineering, Nagoya University, Nagoya, Japan
[2]Graduate School of Engineering, Nagoya Institute of Technology, Nagoya, Japan
[3]Graduate School of Engineering, Tottori University, Tottori, Japan

Small-molecule ligand–protein binding pairs have been powerful tools for studying and controlling biological functions in living cells and organisms. However, discovering such binding pairs from natural products remains challenging. Here, we report the development of a novel ligand–protein pair using a *de novo* approach.



We first designed 1-(4-hydroxyphenyl)-3-phenylurea, HPPU, as the target ligand and used TRAP display[1,2] to select an HPPU-binding monobody. Through an initial selection and affinity maturation, we obtained a monobody, Mb-P', with a single-nanomolar dissociation constant against HPPU, K_D = 8.5 nM. The HPPU–monobody system demonstrat-

ed such high specificity that Mb-P' did not bind to the benzyl-protected HPPU ligand, HPPU-Bn. By derivatizing the HPPU ligand, we also modulated the affinity of the HPPU–monobody system across a wide range of binding affinities, from a few nM to a few μM KD. X-ray crystal structure analysis revealed that HPPU ligand penetrated into the cave-like binding site structure of Mb-P'. Based on this structure, we introduced five mutations to the Mb-P' and optimized the HPPU–monobody system for live-cell application. Finally, we demonstrated imaging of several organelles in living cells by using the HPPU–monobody system.

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[2]Kondo, T., Iwatani, Y., Matsuoka, K., Fujino, T., Umemoto, S., Yokomaku, Y., Ishizaki, K., Kito, S., Sezaki, T., Hayashi, G., and Murakami, H., *Sci. Adv.*, **2020** 6(42), eabd3916.

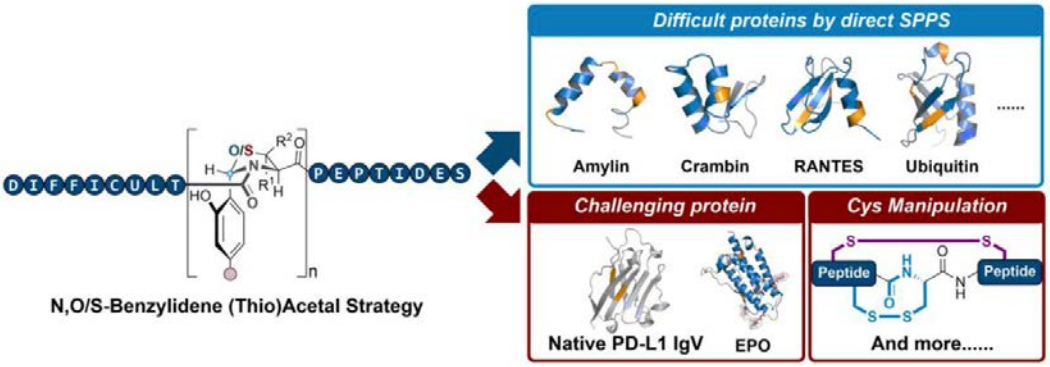
P-281

Chemical Synthesis of Difficult Peptide and Protein by *N,O/S*-Benzylidene (thio)Acetals, NBA, Strategy

Zhenquan Sun[1], Hongxiang Wu[2], and Xuechen Li[2]

[1]University of Chicago, Chicago, USA
[2]University of Hong Kong, Hong Kong, Hong Kong

The development of solid phase peptide synthesis, SPPS, and chemical ligation strategies enable the chemical synthesis of peptide or protein at atomic level precision.[1] However, difficult peptide/protein that are highly prone to aggregate, are still challenging to be synthesized due to their serious residue deletion in SPPS, poor reactivity in ligation and miserable separation in purification.



Herein we present a novel and general strategy based on the *N,O/S*-benzylidene (thio)acetal, NBA, to address above obstacles. During the peptide synthesis, the twisted NBA structure from the Ser/Thr ligation serves as an efficient aggregation disruptor. Also, both *O*- and *S*- NBA scaffolds can be readily introduced at different stages of synthesis, either by the SPPS coupling of easily prepared building blocks[2,5] or via late-stage chemical ligation[3]. Meanwhile, the unnatural Cys/Pen residue in the S-NBA can be converted into Ala/Val by desulfurization,[4] covering broader substrate scope of NBA insertion. The utility of NBA strategy was further demonstrated by successful syntheses of various challenging peptides and proteins, including amylin, crambin, RANTES, ubiquitin, histones, erythropoietin and human programmed death ligand 1 (PD-L1 IgV). To sum up, the NBA strategy provides a flexible and robust platform to synthesize difficult peptides and proteins in an efficient way.

[1]Sun, Z.; Liu, H.; Li, X. *Chem*, **2024**, 10, 767–799.
[2]Wu, H.; Sun, Z.; Li, X. *Angew. Chem. Int. Ed.* **2023**, e202310624.
[3]Wu, H.; Sun, Z.; Li, X. *Angew. Chem. Int. Ed.* **2024**, e202403396.
[4]Sun, Z. et al. *Chem*, **2022**, 8, 2543-2557.
[5]Sun, Z.; Wu, H.; Zhang, Y.; Li, X. *in preparation*.

P-282

Characterizing the Peptide-Based Quorum Sensing Systems in *Streptococcus gordonii* and *Lactiplantibacillus plantarum*

Michael Bertucci

Lafayette College, Easton, USA

Quorum sensing, QS, is a density-dependent process of chemical communication in bacteria that regulates colony-wide gene expression. In the commensal bacteria *Streptococcus gordonii* and *Lactiplantibacillus plantarum*, QS has been demonstrated to control key phenotypes that impact both intraspecies and interspecies behavior with downstream implications in human health. The competence regulon in *Streptococcus gordonii* is controlled by a competence-stimulating peptide, CSP.

We discovered several proliferative phenotypes associated with uncharacterized strains of *S. gordonii* and conducted initial structure-activity studies to determine which portions of the CSP are necessary for QS control. In *Lactiplantibacillus plantarum*, a pH-dependent S-to-N acyl transfer has confused characterization of the active form of the cyclic QS peptide, LamD558. To this end, we have synthesized both possible isomers of the peptide and are developing a reporter strain and subsequent bioassay in which to screen these isomers and related synthetic LamD derivatives. Controlling QS in *S. gordonii* and *L. plantarum* could reduce infectivity of pathogens in the oral cavity and mitigate inflammation in gastrointestinal tract, respectively.

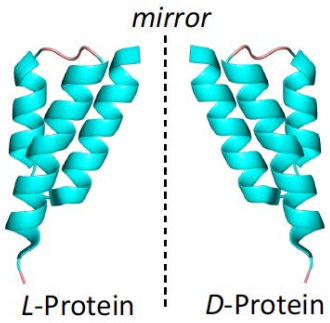
P-283

Chemical Peptide Engineering and Mirror-Image Biological Display to Inhibit Red Blood Cell Invasion by Malaria Parasites

Kalyaneswar Mandal

Tata Institute of Fundamental Research Hyderabad, Hyderabad, India

Malaria parasites claim over half a million lives annually worldwide. The interaction between two crucial parasite proteins, apical membrane antigen 1, AMA1, and rhoptry neck protein 2, RON2, plays a pivotal role in the formation of the moving junction – a critical step that initiates malaria parasite entry into human erythrocytes. Identifying a suitable peptide or a small protein to inhibit the interaction between AMA1 and the extracellular domain of RON2 would represent an ideal strategy for disrupting the junction formation, and consequently, halting the invasion process.



We utilize chemistry tools to engineer peptides that inhibit AMA1-RON2 interactions.[1-3] Additionally, we combine chemical protein synthesis[4] and biological display techniques to systematically identify mirror-image protein, D-protein inhibitors that block erythrocyte invasion by malaria parasites. D-proteins are resistant to proteolysis and less immunogenic. Therefore, a suitably engineered D-protein molecule, composed entirely of D-amino acids and glycine, would stand as a superior option for antimalarial therapeutic use compared to conventional natural peptides or proteins.

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[2]Mannuthodikayil, J.; Sinha, S.; Singh, S.; Biswas, A.; Ali, I.; Mashurabad, P. C.; Tabassum, W.; Vydyam, P.; Bhattacharyya, M. K.; Mandal, K. *ChemBioChem*, **2023**, 24, e202200533.
[3]Biswas, A.; Narayan, A.; Sinha, S.; Mandal, K. *bioRxiv*, **2023**.06. 23.546305.
[4]Kar, A.; Mannuthodikayil, J.; Singh, S.; Biswas, A.; Dubey, P.; Das, A.; Mandal, K. *Angew. Chem. Int. Ed.*, **2020**, 59, 14796-14801.

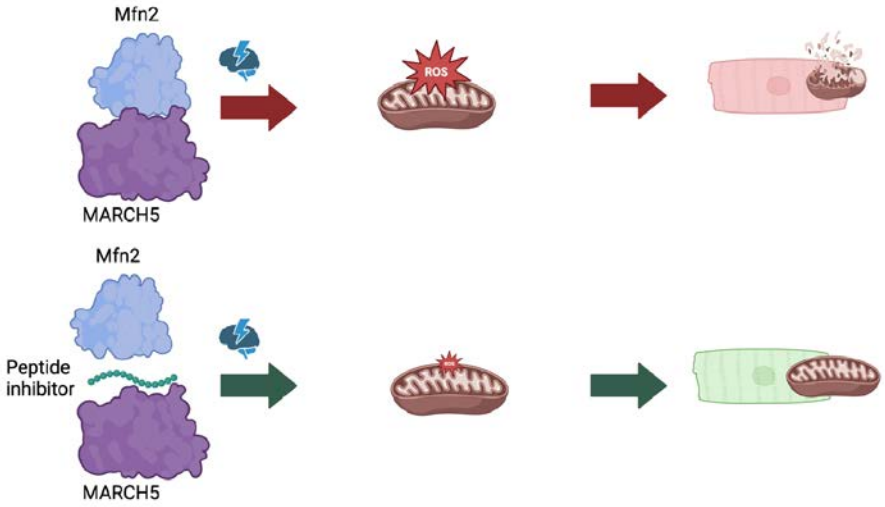
P-284

MARCH5/Mfn2 Peptide Inhibitors: A Mitochondrial Therapeutic for CVDs

Nir Qvit

Safed, Safed, Israel

Cardiovascular diseases, CVDs, remain a leading cause of mortality and morbidity worldwide, emphasizing the need for innovative therapeutic strategies. Mitochondria play a pivotal role in cardiac health, acting as the primary energy producers and regulators of key cellular functions. Mitochondrial dynamics, including fusion, fission, and mitophagy, are essential for maintaining mitochondrial quality and function. Dysregulation of these processes has been implicated in the pathogenesis of CVDs, yet the molecular mechanisms underlying mitochondrial dysfunction in cardiac diseases remain incompletely understood.[1]



Mitochondrial protein-protein interactions, PPIs, are promising therapeutic targets for modulating mitochondrial function. The mitochondrial ubiquitin ligase MARCH5, Membrane-Associated Ring-CH-Type Finger 5, through its interaction with Mitofusin2, Mfn2, plays a critical role in regulating mitochondrial fusion and overall mitochondrial homeostasis.[2] To explore the therapeutic potential of targeting this interaction, a peptide inhibitor was rationally designed to disrupt the MARCH5/Mfn2 PPI.[3-4]

This peptide demonstrated high specificity, effectively inhibiting the interaction without affecting other MARCH5 PPIs. Functional studies in cardiomyocytes revealed its cardioprotective effects, with significant improvements in mitochondrial function and quality. Importantly, the inhibitor exhibited no observable toxicity in preclinical rat models, underscoring its potential as a lead compound for further development.

These findings highlight the potential of targeting mitochondrial PPIs, such as MARCH5/Mfn2, as a novel approach to mitigating mitochondrial dysfunction in CVDs. Further research is needed to elucidate the mechanisms and advance these inhibitors toward clinical application, offering a promising avenue for improving CVD management.

[1]Tsao, C. W., A. W. Aday, Z. I. Almarzooq, C. A. Anderson, P. Arora, C. L. Avery, C. M. Baker-Smith, A. Z. Beaton, A. K. Boehme and A. E. Buxton. "Heart disease and stroke statistics—2023 update: a report from the American Heart Association." *Circulation* **2023** 147(8): e93-e621.
[2]Park, Y. Y., S. Lee, M. Karbowski, A. Neutzner, R. J. Youle and H. Cho. "Loss of MARCH5 mitochondrial E3 ubiquitin ligase induces cellular senescence through dynamin-related protein 1 and mitofusin 1." *J Cell Sci* **2010** 123(Pt 4): 619-626.
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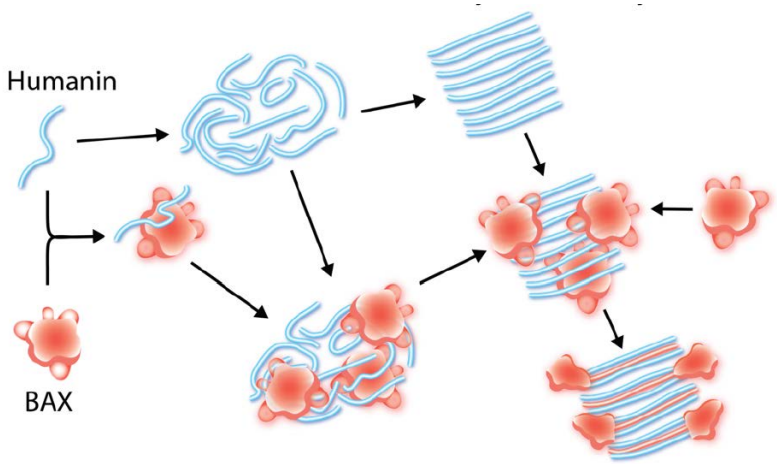
P-285

Mutation of Mitochondrial-Derived Humanin: Variant fibril Morphology and Interaction With the Apoptotic Protein BAX

Daniel Morris[1], James Gruschus[1], David Nyenhuis[1], Rashmi Puja[1], Sarah Nyenhuis[2], Jenny Hinshaw[2], and Nico Tjandra[1]

[1]NHLBI/NIH, Bethesda, USA
[2]NIDDK/NIH, Bethesda, USA

Humanin is human peptide encoded by mitochondrial DNA but with signaling activity outside mitochondria. Exercise increases its production, and its cytoprotective effects, including inhibition of apoptosis via interaction with BCL-2 proteins such as BAX, are welldocumented. Because of this, the administration of humanin and related endogenous human peptides for treatment of disease, aging, and enhancement of athletic performance is becoming more widespread. Bioinformatic analysis of humanin sequences in vertebrates has shown it has undergone natural selection, underscoring its importance. However, very little is known about the actual molecular structure of the humanin and how it interacts with its protein partners.



Here we present the amyloid-like β-sheet fibrillization of humanin. Its secondary structure and morphological properties are characterized via transmission electron microscopy and other biophysical techniques, both for wild type and for mutant versions of humanin. Mutants that display inhibited β-sheet fibrillization are associated with those previously identified to be secretion deficient in vitro, suggesting β-sheet structure helps enable transport across membranes. Successful β-sheet structural transitions are also required for fold-switching interactions with BAX and other BCL-2 family proteins, suggesting an important role for β-sheet structural transitions in apoptosis inhibition. Finally, the results show that humanin as currently administered is likely in fibrillar form, which could affect its bioavailability and activity.

P-286

Optimization and Application of the Endosomal Escape Vehicle, EEV™, Platform for Enhanced Delivery of Oligonucleotides to Skeletal and Cardiac Muscle

Patrick Dougherty, Xiang Li,Anushree Pathak,Kimberli Kamer, Vyoma Patel, Matthew Streeter, Mahboubeh Kheirabadi, Ashweta Sahni, Mohanraj Dhanabal, Natarajan Sethuraman, and Ziqing Qian

Entrada Therapeutics, Boston, USA

Intracellular delivery of oligonucleotide therapeutics is challenging because of poor cell entry and limited escape from the endosome in the target cell. These limitations often necessitate high therapeutic doses and can be associated with less-than-optimal therapeutic activity. One strategy to overcome these limitations is conjugation of oligonu-

cleotides to cell-penetrating peptides, CPPs. Compared with linear CPPs, cyclic CPPs have been shown to efficiently penetrate the cell membrane and are more resistant to proteolytic degradation.

We have designed a family of proprietary cyclic CPPs that form the core of our Endosomal Escape Vehicle, EEV™ technology, which are capable of delivering covalently conjugated cargo to several tissue types. First generation EEV cyclic CPPs underwent several specific chemical modifications to optimize their ability to functionally deliver antisense oligonucleotides to skeletal and cardiac muscle. Notably, the overall charge and conformational flexibility of lead EEVs was modulated to improve efficacy and tolerability. These modified EEVs were extensively characterized through in vitro, in vivo, and preclinical disease model studies, demonstrating that the medicinal chemistry underlying the structure of cyclic CPPs are integral in their ability to efficiently deliver therapeutic cargo and advancing our understanding of CPP-based therapeutics.

The therapeutic potential of EEV-oligonucleotide conjugates has been confirmed in several preclinical models of Duchenne muscular dystrophy (DMD), as well as in a clinical study of healthy human volunteers. Together, these findings support further study and optimization of EEVs to enable intracellular delivery of cell-impermeable therapeutics for the treatment of diseases with significant unmet need.

P-287

Engineering Reversible Macrocyclic Peptides for Affinity Selection Mass Spectrometry

Michael Wuo

Entrada Therapeutics, Boston, USA

The identification of low molecular weight macrocyclic peptides with high affinity and selectivity to their targets remains a challenge. While mRNA display provides a powerful means of assessing large, 10^{13} combinatorial libraries for peptidic binders, the technique lacks the synthetic control afforded by traditional solid phase peptide synthesis for hit maturation.

We discovered an mRNA display hit with molecular weight around 1.2 kDa and modest affinity to KRAS G12D. To accelerate a lead identification campaign using affinity selection mass spectrometry, we designed and synthesized a disulfide reversible mimic of the encoded thioether bond. We found using molecular dynamics simulations and biophysical characterization that disulfide mimics did not perturb the overall structure of the hit molecule. We expect this work to lay the groundwork for developing reversible, macrocyclic peptide libraries for hit maturation using affinity selection mass spectrometry.

P-288

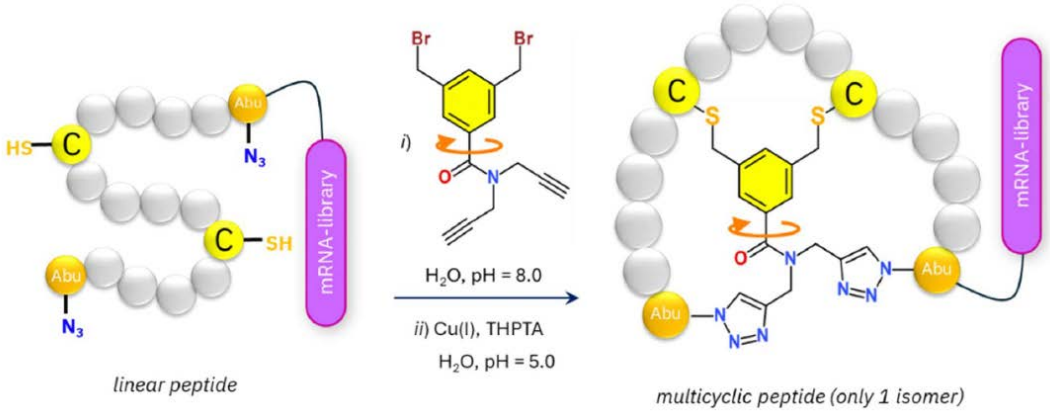
A High-Diversity mRNA-Platform for the Discovery of Multicyclic Peptides

Minglong Liu[1], Seino Jongkees[1], Michael Goldflam[2], Sanne Verhoork[2], Vito Thijssen[2], and Peter Timmerman[2]

[1]Vrije Universiteit, Amsterdam, Netherlands
[2]Biosynth B.V., Lelystad, Netherlands

The discovery of selective and high-affinity ligands to membrane receptors and other protein targets is an essential first step in the development of novel therapeutic drugs.[1] For small molecule drugs, high throughput screening, HTS, is a popular approach where of 105-106 different compounds per day can be screened with the help of robots. For peptides, proteins and nucleic acids, biological selection methods, like phage, mRNA, ribosome, or yeast display offer an attractive format, with throughputs of 10^{9} to 10^{13} different variants being realistic these days. By developing smart de-selection strategies, selective binders with affinities in the single and double-digit nanomolar range can sometimes be identified.

Sometime ago we developed a synthetic methodology for multicyclic peptides,[2] which we now have combined with mRNA-library screening for identifying bioactive multicyclic peptides. A decent number of naturally occurring drugs are multicyclic, for example, vancomycin, nisin, cyclotides, and lately tricyclic inhibitor of PCSK9, MK-0616 was reported as a next-generation orally-available cholesterol-lowering drug.[3]



In this lecture, we present first data on some very potent, double-digit nM, and selective binders to Frizzled-5, FZD-5, and an anti-CCR7 monoclonal antibody. Using the proper controls, we can also illustrate that participation of each individual peptide loop is crucial to the binding. Moreover, we observe a strongly increased proteolytic stability for the multicyclic leads as compared to the single-loop or linear variants. These data prove that this novel multicyclic peptide-platiorm is now ready to be applied for the discovery of novel peptide therapeutics.

[1]Heinis et al., "Phage-encoded combinatorial chemical libraries based on bicyclic peptides", *Nat. Chem. Biol.* **2009**, 5(7), 502-7
[2]Richelle et al., "General and Facile Route to Isomerically Pure Tricyclic Peptides Based on Templated Tandem CLIPS/CuAAC Cyclizations", *Angew. Chem. Int. Ed.* **2018**, 57, 501-5
[3]Iskandar & Bowers, "mRNA Reaches for the Clinic with New PCSK9 Inhibitor", *ACS Med. Chem. Lett.* **2022**, 13, 1379-83

P-289

Vancomycin-Teixobactin Conjugates

Maria S.T. Lee Padilla

University of California, Irvine, Irvine, USA

Vancomycin continues to be a widely used antibiotic of last resort in treating drug-resistant pathogens, despite the emergence of vancomycin-resistant strains such as vancomycin-resistant *Enterococci,* VRE. This paper reports that conjugation of vancomycin to a second antibiotic that targets a different region of lipid II enhances and rescues its antibiotic activity.

Conjugation of vancomycin to a minimal teixobactin pharmacophore in which residues 1–6 are replaced with an aromatic amide results in substantial enhancement in activity over the individual components or mixtures thereof. Three conjugates with minimum inhibitory concentrations, MICs of 0.5 μg/mL against methicillin-resistant *Staphylococcus aureus,* MRSA, and 0.063–0.125 μg/mL against methicillin-susceptible *Staphylococcus aureus,* MSSA, were identified.

Each of these conjugates is also active against VRE, even though the individual components are inactive, with the most active conjugate, Cbp-Lys10-teixo7–11-vanco, having an MIC of 2–4 μg/mL. These findings demonstrate that conjugation of vancomycin to a minimal teixobactin pharmacophore is an effective strategy for enhancing the activity of vancomycin against important Gram-positive pathogens.

P-291

Design, Synthesis, and Study of Opioid Cyclic Tetrapeptides

Alejandra Cordova[1], Yuanming Song[1], Diana Chu[1], Daniel Plata[1], and Michael Ferracane[2]

[1]University of Redlands, Redlands, USA
[2]California State University, Fullerton, Fullerton,USA

Over 500,000 Americans have died from an overdose involving an opioid over the past twenty years. New medications that better treat pain and addiction are urgently needed to help stem the opioid overdose epidemic. The cyclic tetrapeptide *cyclo*[Pro-Sar-Phe-D-Phe] demonstrates utility in treating pain with reduced liabilities, and it attenuates the rewarding effects of cocaine and morphine in mouse model systems. Notably, *cyclo*[Pro-Sar-Phe-D-Phe] is both orally bioavailable and blood-brain barrier permeable, properties that are rare for a peptide.

Herein, we describe our effort to design, synthesize, and study this peptide as well as a series of initial analogs, with a focus on connecting NMR, X-ray, and computed structures to PAMPA permeability and opioid activity. The combined efforts will improve our understanding of this molecular scaffold and its therapeutic potential.

P-292

Design, Synthesis, and Conformational Analysis of Constrained Melanocortin Peptides

Paolo Grieco[1], Francesco Merlino[1], Ida Boccino[1], Alfonso Carotenuto[1], Nicola Grasso[1], and Philip Thompson[2]

[1]University of Naples Federico II, Dept Pharmacy, Naples, Italy
[2]2Monash University,Parkville VIC, Australia

The melanocortin receptors are involved in many physiological functions, including pigmentation, sexual function, feeding behavior, and energy homeostasis, making them potential targets to treat obesity, sexual dysfunction, et cetera.[1] Understanding the basis of the ligand-receptor interactions is crucial for the design of potent and selective ligands for these receptors.

In this study we investigated the impact on biological activity of specific conformational constraints performed on peptide analogues related to MT-II, SHU9119, Pro6-MT-II and PG-901. These analogues feature a modified structure in which the traditional side chain bridge is replaced by shorter bonds, which connect a C-terminal amino acid side chain to the essential N-terminal residue. The lactam bridge was designed as either a head-to-side or head-to-tail chain and in some cases including further conformational restriction. These structural modifications are based on previous studies of similar molecules, which demonstrated the importance of these features in determining receptor selectivity and potency.[2] Conformational properties of selected novel analogues were studied and compared with those of the parent compounds.

[1]R. Cone, Ed. The Melanocortin Receptors; *Humana Press: Totowa*, NJ, **2000**.
[2]P. Grieco, D. Brancaccio, E. Novellino, V. J. Hruby, A. Carotenuto, *Eur. J. Med. Chem.*, **2011**, 46, 3721–3733.

P-293

Development of GABARAP-Specific Stapled and *N*-Methylated Peptide Modulators of Autophagy

Imani McDonald and Joshua Kritzer

Tufts University, Medford, USA

Autophagy is an essential recycling pathway where cytosolic material is shuttled to the lysosome for degradation. In advanced-stage cancers, autophagy can aid tumor progression and can promote resistance to DNA-damaging chemotherapy. Nonspecific autophagy inhibitors like chloroquine can resensitize chemoresistant cancer cells, but there are few highly selective small-molecule autophagy inhibitors.

Protein-protein interactions involving autophagy proteins LC3 and GABARAP are promising targets for autophagy inhibition due to their vital roles at every stage of autophagy. Binding partners of LC3 and GABARAP interact via the LC3-interaction region, LIR, which docks into the LIR domain of LC3 and GABARAP, forming an intermolecular β-sheet. Using structure-based design, we developed minimized LIR-derived stapled peptides and *N*-methylated peptides with sub-micromolar affinity for GABARAP. These peptides are promising as potential combination therapies with DNA-damaging agents. In addition, these peptides can be used as building blocks for autophagy-mediated targeted protein degraders.

P-294

Investigation into the Relationship Between Cyclic Tetrapeptide Structure and Therapeutic Activity

Jian Raines and Michael Ferracane

California State University, Fullerton, Fullerton, USA

The United States has been suffering from an epidemic of opioid overdose deaths, including over 80,000 fatalities in 2023 alone. Alternative medications are urgently needed to help stem the opioid epidemic. The novel cyclic tetrapeptide *cyclo*[Pro-Sar-Phe-D-Phe] demonstrates multifunctional opioid receptor agonism/antagonism and has shown the potential to elicit potent analgesia with reduced potential for overdose and addiction. In addition, this peptide is orally bioavailable and blood-brain barrier permeable.

Our research group is interested in investigating the relationship between the peptide structure and therapeutic activity by studying conformation, NMR, X-ray crystallography, molecular modeling, membrane permeability, PAMPA, and pharmacological activity (*in vitro* and *in vivo* assays). Herein, we share preliminary work with a series of analogs related to *cyclo*[Pro-Sar-Phe-D-Phe]. Otur research may provide useful information for chemists and scientists interested in developing medications for treating pain and substance abuse.

P-295

Greening Peptide Purification Through Replacement of Acetonitrile with Ethanol

Colin Simpson, Tucker Payne, Drew Cesta, Sandeep Singh, and Jonathan Collins

CEM Corporation, Matthews, USA

Increased global demand for peptide therapeutics has put a renewed emphasis on improving the efficiency and sustainability of peptide production, and have included efforts to identify greener alternatives to acetonitrile for purification. Ethanol has been identified as an ideal alternative, based on both a low UV-cutoff and similar separation characteristics, but its higher viscosity has hindered routine use.[1]

This work describes the use of an optimized approach, using a novel integrated heating system, for elevated temperature HPLC purification that enables routine access to ethanol even with 5µm particle size columns. The use of this approach, using 60 °C conditions, for the purification of a range of peptides resulted in approximately a 50% average increase in recovery as compared to ambient temperature conditions using acetonitrile. Additionally, an assessment of the potential for esterification side-reactions known to occur with methanol were undertaken with ethanol to establish the robustness of the approach. Use of this new methodology is believed to offer a meaningful improvement toward greener peptide manufacturing, given the significant contribution of purification to overall PMI.[2]

[1] *J. Sep. Sci.* **2024**, 47, e202400554.
[2] *J. Org. Chem.* **2024**, 89, 4261-4282.

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