# APS2022 WHISTLER

*Co-Chairs*

Mark D. Distefano, PhD, Les Miranda, PhD

## Peptide Science at the Summit

Whistler, BC  June 11 – 16, 2022

27th American Peptide Symposium

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27th American Peptide Symposium

# WELCOME!

Welcome to Whistler and the 27th American Peptide Symposium, APS2022! Despite a one-year delay, we are delighted to be holding this meeting in person. After a dearth of face-to-face interactions due to the COVID-19 pandemic. We hope you, your loved ones, and colleagues are doing well, and we are excited to see all of you for a week of cutting-edge science and networking.

APS2022 will present new developments in the synthesis and application of peptides in a range of areas. Peptides have historically served as leads for drug design but are now increasingly accepted as drug candidates. Peptides are also attractive as materials and when coupled with bioconjugation methods enable preparation of a plethora of novel architectures. New technologies are also a major driving force in peptide science and the program features sessions on new structural methods, green synthesis and data science. In assembling the list of speakers for this meeting, we sought to showcase exciting recent developments and emphasize new directions in the field. By providing an in-person forum and diverse group of scientists, we hope to catalyze new ideas and collaborations. The APS has always served as a forum for bringing together an engaging and supportive group of participants; we hope you will find this year to be no different. This year, we will be using a mobile phone app, Whova, in lieu of a printed program for environmental sustainability, and as a tool to facilitate information transfer, networking and communication.

The 2022 APS program will kick-off with a preview session called Young Investigator & Industry Showcase featuring a selection of 8 presentations from young investigators and industrial scientists. After the official welcome by APS President Ved Srivastava, the opening keynote lecture "mRNA as Medicine" will be delivered by Dr. Melissa Moore, Chief Scientific Officer of Moderna, at 6 PM on Saturday, June 11 followed by an hour of student flash talks and a reception in the Grand Foyer, Sea to Sky Ballroom of the Whistler Conference Center. Exhibits will be open during this social event. The closing lecture on Thursday, June 16, will be presented by Professor David Craik from the University of Queensland on "Discovery and Applications of Cyclotides: Nature's Ultra-Stable Peptide Scaffolds." The closing banquet will start that evening at 7:00 PM in Sea to Sky Ballroom.

The symposium will feature talks by scientists at the leading edge of peptide science and at a variety of career stages including several presentations by graduate students and postdoctoral fellows and winners of American Peptide Society Awards. The Merrifield Award lecture will be presented on Sunday, June 12 by Professor Padmanabhan Balaram of the Indian Institute of Science. On Monday, June 13, Professor Jean Chmielewski of Purdue University will deliver the Goodman Lecture. Professor Bradley Pentelute from the Massachusetts Institute of Technology will give the Makineni Award lecture on Tuesday, June 14. The two du Vigneaud Award lectures will be presented on Wednesday, June 15, by Professor Joel Schneider of the National Cancer Institute and on Thursday, June 15, by Alanna Schepartz from the University of California-Berkeley. We will also feature two Early Career Award Lectureships presented by Professor Yftah Tal-Gan from the University of Nevada-Reno on Monday, June 13 and Professor Caroline Proulx from North Carolina State University on Wednesday, June 15. Finally, a key part of the scientific program are the two poster sessions, which will include remarkable young scientists who are part of the Young Investigator Poster Competition. The poster presentations will be preceded by several sessions featuring 3 minute "flash talks" by a subset of poster presenters.

In addition to the scientific program, we have included a number of additional activities to broaden the spectrum and value of the meeting. A panel discussion on Diversity and Social Justice, led by Professor Monika Raj will be held on Sunday, June 12. A Career Workshop featuring panelists who have pursued a range of different career paths will be held on Tuesday, June 14. That will be followed by a workshop on Entrepreneurship featuring several scientists who have started their own companies. Opportunities for networking include the opening reception on Saturday night, a student mixer on Sunday night and the Dr. Elizabeth Schram Young Scientists' Lunch & Mixer on Tuesday as well as the closing banquet on Thursday night.

We greatly appreciate the generous support of our sponsors and exhibitors who make this conference possible. We strongly encourage you to interact with them and learn about the exciting products, services and equipment they have available. To facilitate those interactions a number of them will present 3 min "flash talks" before the coffee breaks in the morning. Many of them have also uploaded presentations and/or information available in the Whova app. Without their support, the exciting scientific program as well as the social events would not be possible.

Thank you for joining and participating in the symposium! We hope that you will have a wonderful time and enjoy the experience of an in-person meeting set in the spectacular mountains of Whistler.

**Mark Distefano**, Co-Chair

**Les Miranda**, Co-Chair

# A MESSAGE FROM THE APS PRESIDENT

On behalf of the American Peptide Society, it is my great honor and pleasure to welcome you to the 27th American Peptide Symposium in Whistler, British Columbia. The last couple of years were full of pandemic challenges and difficulties in having in-person scientific meetings. This led us to postpone the 2021 American Peptide Symposium to June 2022.

However, last year, we have introduced a monthly 'e-seminar series' over a virtual platform to promote scientific and social interactions within the peptide community. It was delightful to listen to the exciting science, given the lab presence many of us experienced during the COVID-19 pandemic. Despite these unprecedented times, the co-chairs of the Symposium, Prof. Mark Distefano of the University of Minnesota and Dr. Les Miranda of Amgen, have been working very hard with the Society's Executive Board to bring the Symposium back on track. I admire the patience and resilience of the co-chairs for bringing innovative topics and outstanding speakers to this meeting. Kudos to Wendy Hartsock, and co chairs and the rest of the Sponsorship Committee for their significant contribution to fundraising efforts. We could not have this conference without their tireless work. Congratulations and my best wishes for a successful symposium.

Peptide Science, including peptide-based therapeutics, continues to be an innovative strategy for developing biopharmaceutical pipelines. The Symposium, with a theme of 'Peptide Science at the Summit', will cover the advancement of new technologies essential for next-generation discovery platforms such as genetically encoded libraries of macrocyclic, mirror-Image CLIPS Phage Display, development of peptide-based tools to study cell-cell signaling, the discovery of targeted affinity peptide binders using machine learning and artificial intelligence, molecular transport systems for delivery to the central nervous system and technologies for oral peptide delivery. The talks will also cover engineering enzymes for green manufacturing of peptide drug products and new peptide synthetic technologies.

My heartiest congratulations to Professor Padmanabhan Balaram of India for receiving our highest honor, the Merrifield Award, for his outstanding lifetime contributions to peptide science. I also want to congratulate other Awardees - Alanna Schepartz and Joel Schneider (du Vigneaud Award), Jean Chmielewski (Goodman Award), Bradley Pentelute (Makineni Lectureship), and Caroline Proulx and Yftah Tal-Gan (Early Career Lectureship) for their excellence in peptide science.
The symposium program is well balanced with a diversity of investigators to address the cutting-edge peptide science. Symposium co-chairs, Mark and Les, have identified experts in the subject matter from Academia and Industry. We are delighted to have Dr. Melissa Moore of Moderna and David Craik of the University of Queensland giving plenary keynote lectures. In addition to the traditional schedule from the previous years, our co-chairs have introduced poster flash talks, exhibitor flash talks, an entrepreneurship workshop, and a panel discussion on diversity and social justice- my favorite! At this meeting, we have also implemented a "Whova App" to improve your experience at the conferences for ease of navigation of the program and for planning personal schedules and accessing documents and slides shared by organizers or speakers.

I wish you all the best. Enjoy the scientific talks and networking event. I look forward to seeing all of you in person!

Ved Srivastava

# APS SYMPOSIA CHRONOLOGY

| Symposium | Year | Chair(s) | Location |
|---|---|---|---|
| First | 1968 | **Saul Landa**<br>Yale University,<br>**Boris Weinstein**<br>University of Washington-Seattle | Yale University<br>New Haven, CT |
| Second | 1970 | **F. Merlin Bumpus**<br>Cleveland Clinic | Cleveland Clinic<br>Cleveland, OH |
| Third | 1972 | **Johannes Meinhofer**<br>Harvard Medical School | Children's Cancer Research Foundation<br>Boston, MA |
| Fourth | 1975 | **Roderich Walter**<br>University of Illinois Medical Center-Chicago | The Rockfeller University and Barbizon Plaza Hotel,<br>New York, NY |
| Fifth | 1977 | **Murray Goodman**<br>University of California-San Diego | University of California-San Diego<br>San Diego, CA |
| Sixth | 1979 | **Erhard Gross**<br>National Institutes of Health | Georgetown University<br>Washington, DC |
| Seventh | 1981 | **Daniel H. Rich**<br>University of Wisconsin-Madison | University of Wisconsin-Madison<br>Madison, WI |
| Eighth | 1983 | **Victor J. Hruby**<br>University of Arizona | University of Arizona<br>Tucson, AZ |
| Ninth | 1985 | **Kenneth D. Kopple**<br>Illinois Institute of Technology<br>**Charles M. Deber**<br>University of Toronto | University of Toronto<br>Toronto, Ontario, Canada |
| Tenth | 1987 | **Garland R. Marshall**<br>Washington University School of Medicine, St. Louis | Washington University<br>St. Louis, MO |
| Eleventh | 1989 | **Jean E. Rivier**<br>The Salk Institute of Biological Studies, LaJolla | University of California-San Diego<br>San Diego, CA |
| Twelfth | 1991 | **John A. Smith**<br>Massachusetts General Hospital | Massachusetts Institute of Technology<br>Cambridge, MA |
| Thirteenth | 1993 | **Robert S. Hodges**<br>University of Alberta-Edmonton | Edmonton Convention Center<br>Edmonton, Alberta, Canada |
| Fourteenth | 1995 | **Pravin T.P. Kaumaya**<br>The Ohio State University | The Ohio State University<br>Columbus, OH |
| Fifteenth | 1997 | James P. Tam<br>Vanderbilt University | Nashville Convention Center<br>Nashville, TN |
| Sixteenth | 1999 | **George Barany**<br>University of Minnesota-Minneapolis<br>**Gregg B. Fields**<br>Florida Atlantic University | Minneapolis Convention Center<br>Minneapolis, MN |

| Symposium | Year | Chair(s) | Location |
|---|---|---|---|
| Seventeenth | 2001 | **Richard A. Houghten**<br>Torrey Pines Institute for Molecular Studies, CA<br>**Michal Lebl**<br>Illumina, Inc., CA | Town and Country Resort Hotel<br>San Diego, CA |
| Eighteenth | 2003 | **Michael Chorev**<br>Beth Israel Deaconess Medical &<br>Harvard Medical School, MA<br>**Tomi K. Sawyer**<br>ARIAD Pharmaceuticals Inc., MA | Boston Marriott Copley Place<br>Boston, MA |
| Nineteenth | 2005 | **Jeffery W. Kelly**<br>The Scripps Research Institute, CA<br>**Tom W. Muir**<br>Rockefeller University, NY | Town and Country Resort Hotel<br>San Diego, CA |
| Twentieth | 2007 | **William D. Lubell**<br>University of Montreal<br>**Emanuel H. F. Escher**<br>University of Sherbrooke | Palais des Congres<br>Montreal, Canada |
| Twenty-first | 2009 | **Richard DiMarchi**<br>Indiana University<br>**Hank Mosberg**<br>University of Michigan | Indiana University<br>Bloomington, Indiana |
| Twenty-second | 2011 | **Philip Dawson**<br>The Scripps Research Institute<br>**Joel Schneider**<br>National Cancer Institute | Sheraton San Diego<br>San Diego, CA |
| Twenty-third | 2013 | **David Lawrence**<br>UNC Chapel Hill<br>**Marcey Waters**<br>UNC Chapel Hill | Hilton Waikoloa Village<br>Waikoloa, Hawai'i |
| Twenty-fourth | 2015 | **Ved Srivastava**<br>GlaxoSmithKline<br>**Andrei Yudin**<br>University of Toronto | Hyatt Regency Grand Cypress<br>Orlando, FL |
| Twenty-fifth | 2017 | **Jonathan Lai**<br>Albert Einstein College of Medicine<br>**John Vederas**<br>University of Alberta | Whistler Conference Centre<br>Whistler, BC, Canada |
| Twenty-sixth | 2019 | **Paramjit Arora**<br>New York University<br>**Anna Mapp**<br>University of Michigan | Portola Hotel and Monterey<br>Conference Center<br>Monterey, CA |
| Twenty-seventh | 2022 | **Mark D. Distefano**<br>University of Minnesota<br>**Les Miranda**<br>Amgen, Inc. | Whistler Conference Centre<br>Whistler, BC, Canada |

# SPONSOR LIST

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27th American Peptide Symposium

# SYMPOSIUM-AT-A-GLANCE

## Saturday, June 11, 2022

| | |
|---|---|
| 1:00 PM – 6:00 PM | Registration ..... Grand Foyer |
| 1:00 PM – 4:30 PM | APS Council Meeting ..... Wedgemount A/B |
| 3:30 PM – 5:30 PM | **YOUNG INVESTIGATOR & INDUSTRY SHOWCASE** ..... Fitzsimmons |
| 6:00 PM – 6:10 PM | **PRESIDENT'S WELCOME**. ..... Sea to Sky Ballroom A |
| 6:10 PM – 7:15 PM | **OPENING PLENARY KEYNOTE**. ..... Sea to Sky Ballroom A |
| 7:15 PM – 8:00 PM | **POSTER FLASH TALKS** ..... Sea to Sky Ballroom A |
| 8:00 PM – 10:00 PM | Opening Reception with Exhibitors ..... Sea to Sky Ballroom B & C |

## Sunday, June 12, 2022

| | |
|---|---|
| 7:30 AM – 4:30 PM | Registration ..... Grand Foyer |
| 8:00 AM | Poster Set up ..... Sea to Sky Ballroom B & C<br>*Prefer that both poster groups set up at this time but Group One must set up at this time.* |
| 8:00 AM – 8:05 AM | OPENING REMARKS: APS 2022 CO-CHAIRS..... Sea to Sky Ballroom A |
| 8:05 AM – 10:00 AM | **SESSION 1: POST-TRANSLATIONAL MODIFICATIONS** ..... Sea to Sky Ballroom A |
| 10:00 AM – 10:10 AM | **EXHIBITOR FLASH TALKS**..... Sea to Sky Ballroom A |
| 10:10 AM – 10:35 AM | *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C |
| 10:35 AM – 12:05 PM | **SESSION 2: NEW TOOLS FOR PEPTIDE AND PROTEIN CHEMISTRY** ..... Sea to Sky Ballroom A |
| 12:05 PM – 1:45 PM | *Lunch with Exhibitors (provided)*..... Sea to Sky Ballroom B & C |
| 12:10 PM – 1:40 PM | International Liaison Meeting. ..... Wedgemount A/B |
| 1:45 PM – 3:35 PM | **SESSION 3: PEPTIDES IN THE CLINIC**..... Sea to Sky Ballroom A |
| 3:35 PM – 3:55 PM | *Break* |
| 3:55 PM – 5:10 PM | **MERRIFIELD AWARD LECTURE**..... Sea to Sky Ballroom A |
| 5:10 PM – 6:00 PM | **PANEL DISCUSSION ON DIVERSITY AND SOCIAL JUSTICE**. ..... Fitzsimmons |
| 7:00 PM – 9:00 PM | Student Mixer..... Garibaldi A/B |

## Monday, June 13, 2022

| | |
|---|---|
| 7:30 AM – 4:30 PM | Registration ..... Grand Foyer |
| 8:00 AM – 9:40 AM | **SESSION 4: SYNTHETIC METHODOLOGY FOR PEPTIDE AND PROTEIN SYNTHESIS** .... Sea to Sky Ballroom A |
| 9:40 AM – 9:50 AM | **EXHIBITOR FLASH TALKS**..... Sea to Sky Ballroom A |
| 9:50 AM – 10:15 AM | *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C |
| 10:15 AM – 10:45 AM | **EARLY CAREER LECTURESHIP AWARD I**..... Sea to Sky Ballroom A |
| 10:45 AM – 11:55 AM | **SESSION 5: PEPTIDE AND PROTEIN CONJUGATES** ..... Sea to Sky Ballroom A |
| 11:55 AM – 1:35 PM | *Lunch with Exhibitors (provided)*..... Sea to Sky Ballroom B & C |
| 1:35 PM – 3:45 PM | **SESSION 6: PEPTIDE LIBRARIES, ARRAYS AND PROTEOMICS** ..... Sea to Sky Ballroom A |
| 3:45 PM – 4:05 PM | *Break* ..... |
| 4:05 PM – 5:30 PM | **SESSION 7: PEPTIDES IN DELIVERY** ..... Sea to Sky Ballroom A |
| 5:30 PM – 6:00 PM | **GOODMAN LECTURE** ..... Sea to Sky Ballroom |
| 6:00 PM – 6:30 PM | **POSTER FLASH TALKS** ..... Sea to Sky Ballroom A |
| 6:30 PM – 8:30PM | **GROUP ONE POSTER SESSION & RECEPTION**..... Sea to Sky Ballroom B & C |

## Tuesday, June 14, 2022

| | |
|---|---|
| 7:30 AM – 12:30 PM | Registration ..... Grand Foyer |
| 8:00 AM | Group Two Poster Set up ..... Sea to Sky Ballroom B & C<br>*If presenters from Poster Group Two have not already set up, they must set up at this time.* |
| 8:00 AM – 9:45 AM | **SESSION 8: PEPTIDE- AND PROTEIN-BASED THERAPEUTICS** ..... Sea to Sky Ballroom A |
| 9:40 AM – 9:50 AM | **EXHIBITOR FLASH TALKS** ..... Sea to Sky Ballroom A |
| 9:50 AM – 10:15 AM | *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C |
| 10:15 AM – 10:45 AM | **MAKINENI LECTURE** ..... Sea to Sky Ballroom |
| 10:45 AM – 12:40 PM | **SESSION 9: INNOVATIONS IN PEPTIDE AND PROTEIN CHEMISTRY** ..... Sea to Sky Ballroom A |
| 12:45 PM – 2:45 PM | Dr. Elizabeth Schram Young Scientists' Lunch & Mixer ..... Garibaldi A/B<br>*Graduate students & post-docs only* |
| 2:45 PM – 4:00 PM | **WORKSHOP ON CAREER DEVELOPMENT** ..... Fitzsimmons |
| 5:00 PM – 7:00 PM | **ENTREPRENEURSHIP WORKSHOP** ..... Sea to Sky Ballroom A |

## Wednesday, June 15, 2022

| | |
|---|---|
| 7:30 AM – 4:00 PM | Registration ..... Grand Foyer |
| 8:00 AM – 9:50 AM | **SESSION 10: NEW HORIZONS IN PEPTIDE SCIENCE: GREEN METHODS AND DATA SCIENCE** ..... Sea to Sky Ballroom A |
| 10:15 AM – 10:40 AM | *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C |
| 10:40 PM – 12:15 PM | **SESSION 11: SCAFFOLDS AND PEPTIDOMIMETICS** ..... Sea to Sky Ballroom A |
| 12:15 PM – 1:50 PM | *Lunch (On your own)* |
| 12:15 PM – 1:45 PM | **EXHIBITOR WORKSHOP** ..... Sea to Sky Ballroom A |
| 1:50 PM – 2:20 PM | Announcement of 28th APS and American Peptide Society General Assembly ..... Sea to Sky Ballroom A |
| 2:20 PM – 4:10 PM | **SESSION 12: PEPTIDE DESIGN AND FUNCTION** ..... Sea to Sky Ballroom A |
| 4:10 PM – 4:30 PM | *Break* |
| 4:30 PM – 5:00 PM | **EARLY CAREER LECTURESHIP AWARD II** ..... Sea to Sky Ballroom A |
| 5:00 PM – 5:30 PM | **DU VIGNEAUD LECTURE I** ..... Sea to Sky Ballroom A |
| 5:30 PM – 6:30 PM | **POSTER FLASH TALKS** ..... Sea to Sky Ballroom A |
| 6:30 PM – 8:30 PM | **GROUP TWO POSTER SESSION RECEPTION** ..... Sea to Sky Ballroom B & C |

## Thursday, June 16, 2022

| | |
|---|---|
| 7:30 AM – 10:30 AM | Registration ..... Grand Foyer |
| 8:00 AM – 9:55 AM | **SESSION 14: PEPTIDE INSPIRED MATERIALS** ..... Sea to Sky Ballroom A |
| 9:55 AM – 10:20 AM | *Coffee break* ..... Grand Foyer |
| 10:20 AM – 12:15 PM | **SESSION 15: STRUCTURAL METHODS IN PEPTIDE SCIENCE** ..... Sea to Sky Ballroom A |
| 12:15 PM – 1:45 PM | *Lunch Break (On your own)* |
| 1:45 PM – 2:15 PM | **DU VIGNEAUD LECTURE II** ..... Sea to Sky Ballroom A |
| 2:15 PM – 4:10 PM | **SESSION 16: PEPTIDE NATURAL PRODUCTS AND BIOLOGICAL METHODS** ..... Sea to Sky Ballroom A |
| 4:10 PM – 4:55 PM | **CLOSING PLENARY KEYNOTE LECTURE** ..... Sea to Sky Ballroom A |
| 4:55 PM – 5:00 PM | **CLOSING REMARKS** ..... Sea to Sky Ballroom A |
| 7:00 PM – 10:00 PM | Closing Banquet ..... Sea to Sky Ballroom A |

# SCHEDULE OF EVENTS

## Saturday, June 11, 2022

1:00 PM – 6:00 PM — Registration ..... Grand Foyer

1:00 PM – 4:30 PM — APS Council Meeting. ..... Wedgemount A/B

3:30 PM – 5:30 PM — **YOUNG INVESTIGATOR & INDUSTRY SHOWCASE** ..... Fitzsimmons

Seoyeon (Cece) Hong, *Polypeptide Group*
Mirat Sojitra, *University of Alberta*
Alexander Lund Nielsen, *Ecole Polytechnique Fédérale de Lausanne*
Guilherme Meira Lima, *University of Alberta*
Alexis Verwoert, *Philadelphia College of Osteopathic Medicine*
Kejia Yan, *University of Alberta*
Luísa Aguiar, *Gyros Protein Technologies*

6:00 PM – 6:10 PM — **PRESIDENT'S WELCOME** ..... Sea to Sky Ballroom A

Ved Srivastava, *Aktis Oncology, Inc.*

6:10 PM – 7:15 PM — **OPENING REMARKS AND OPENING** ..... Sea to Sky Ballroom A
**PLENARY KEYNOTE**

L01 Melissa Moore, *Moderna*
"mRNA as Medicine"

7:15 PM – 8:00 PM — **POSTER FLASH TALKS** ..... Sea to Sky Ballroom A

8:00 PM – 10:00 PM — Opening Reception with Exhibitors. .. Grand Foyer & Sea to Sky Ballroom B & C

## Sunday, June 12, 2022

7:30 AM – 4:30 PM — Registration ..... Grand Foyer

8:00 AM — Poster Set up ..... Sea to Sky Ballroom B & C
*Both Groups may set up but Group One must set up at this time*

8:00 AM – 8:05 AM — **OPENING REMARKS: APS 2022 CO-CHAIRS** ..... Sea to Sky Ballroom A

Les Miranda, *Amgen*, and Mark Distefano, *University of Minnesota*

8:05 AM – 10:00 AM — **SESSION 1: POST-TRANSLATIONAL MODIFICATIONS** ..... Sea to Sky Ballroom A

8:05 AM – 8:30 AM — L02 Tom Muir, *Princeton University*
"Illuminating Chromatin: Chemical Biology Tools Light the Way"

8:30 AM – 8:55 AM — L03 Andrew Jamieson, *University of Glasgow*
"A Hydroxamic Acid-Modified Peptide Library Provides Insight into the Molecular Basis for Substrate Selectivity of HDAC Corepressor Complexes"

8:55 AM – 9:10 AM — L04-YI1 Garrett Schey, *University of Minnesota*
"Expanding the Scope of Extended Farnesyltransferase Substrates with a MALDI/MS Screening Approach"

| | | |
|---|---|---|
| 9:10 AM – 9:35 AM | L05 | Laurie Parker, *University of Minnesota*<br>"Real-Time Kinase Assays in Vitro and in Cells Using Fluorophore-Labeled Peptides" |
| 9:35 AM – 10:00 AM | L06 | Rebecca Scheck, *Tufts University*<br>"Learning the Rules for Selective Protein Glycation" |
| 10:00 AM – 10:10 AM | **EXHIBITOR FLASH TALKS** ..... Sea to Sky Ballroom A | |
| 10:10 AM – 10:35 AM | *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C | |
| 10:35 AM – 12:05 PM | **SESSION 2: NEW TOOLS FOR PEPTIDE AND PROTEIN CHEMISTRY** ..... Sea to Sky Ballroom A | |
| 10:35 AM – 11:00 AM | L07 | Philip Dawson, *Scripps Research Institute*<br>"Peptide Conformational Engineering Using Rigid, Low Molecular Weight Linkers" |
| 11:00 AM – 11:20 AM | L08 | Rongsheng (Ross) Wang, *Temple University*<br>"Fluorine-Displacement Based Probes to Interrogate Novel Protein-Protein Interactions" |
| 11:20 AM – 11:45 AM | L09 | James Checco, *University of Nebraska-Lincoln*<br>"Chemical Approaches to Identify Cell-Cell Signaling Peptide Receptors" |
| 11:45 AM – 12:05 PM | L10 | Monika Raj, *Emory University*<br>"Chemical Tools for Selective Detection of Methyl Lysine PTMs" |
| 12:05 PM – 1:45 PM | *Lunch with Exhibitors (provided)* ..... Sea to Sky Ballroom B & C | |
| 12:10 PM – 1:40 PM | International Liaison Meeting ..... Wedgemount A/B | |
| 1:45 PM – 3:35 PM | **SESSION 3: PEPTIDES IN THE CLINIC** ..... Sea to Sky Ballroom A | |
| 1:45 PM – 2:10 PM | L11 | Yuhua Huang, *Merck & Co., Inc.*<br>"Squeezing through the Junctions: From mRNA Display to an Oral Cyclic Peptide Pcsk9 Inhibitor" |
| 2:10 PM – 2:35 PM | L12 | Rami Hannoush, *Genentech*<br>"Next Generation Peptide Therapeutics for Cellular Re-programming" |
| 2:35 PM – 2:50 PM | L13-YI2 | Marcelo Muñoz Figueroa, *University of Ottawa - Heart Institute*<br>"Nano-spray Gold-peptide Therapy for Heart Attack Treatment" |
| 2:50 PM – 3:15 PM | L14 | Brian Finan, *Novo Nordisk Research Center Seattle*<br>"Semaglutide; From GLP-1 to Patient" |
| 3:15 PM – 3:35 PM | L15 | Kamaljit Kaur, *Chapman University*<br>"Targeting Triple-negative Breast Cancer (TNBC) and Melanoma with Small Peptide Ligands" |
| 3:35 PM – 3:55 PM | *Break* | |
| 3:55 PM – 5:10 PM | **MERRIFIELD AWARD LECTURE** ..... Sea to Sky Ballroom A | |
| | L16 | Padmanabhan Balaram, *Indian Institute of Science*<br>"Constraining Peptide Conformations: Finding Aib and Beyond" |
| 5:10 PM – 6:00 PM | **PANEL DISCUSSION ON DIVERSITY AND SOCIAL JUSTICE** ..... Fitzsimmons | |
| 7:00 PM – 9:00 PM | Student Mixer ..... Garibaldi A/B | |

## Monday, June 13, 2022

| | | |
|---|---|---|
| 7:30 AM – 4:30 PM | Registration ..... Grand Foyer | |
| 8:00 AM – 9:40 AM | **SESSION 4: SYNTHETIC METHODOLOGY FOR PEPTIDE AND PROTEIN SYNTHESIS** .... Sea to Sky Ballroom A | |
| 8:00 AM – 8:25 AM | L17 | Qiang Zhang, *State University of New York, Albany*<br>"Studies toward Protein Conjugation via Reversible Covalent Sigma Bonds" |
| 8:25 AM – 8:45 AM | L18 | Norman Metanis, *The Hebrew University of Jerusalem*<br>"Chemoselective Modification of Cysteine and Selenocysteine in Peptides and Proteins" |
| 8:45 AM – 9:00 AM | L19-YI3 | Juan Esteban, *University of Western Ontario*<br>"A Survey of Stapling Methods to Increase Affinity, Activity, and Stability of Ghrelin Analogues" |
| 9:00 AM – 9:20 AM | L20 | Paramjit Arora, *New York University*<br>"Rational Design of an Organocatalyst for Peptide Bond Formation" |
| 9:20 AM – 9:40 AM | L21 | Wenshe Liu, *Texas A&M University*<br>"Expressed Protein Ligation without Intein" |
| 9:40 AM – 9:50 AM | **EXHIBITOR FLASH TALKS** . ..... Sea to Sky Ballroom A | |
| 9:50 AM – 10:15 AM | *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C | |
| 10:15 AM – 10:45 AM | **EARLY CAREER LECTURESHIP AWARD I** ..... Sea to Sky Ballroom A | |
| | L22 | Yftah Tal-Gan, *University of Nevada at Reno*<br>"Development of Peptide-Based Tools to Study Cell-Cell Signaling in Bacteria" |
| 10:45 AM – 11:55 AM | **SESSION 5: PEPTIDE AND PROTEIN CONJUGATES** ..... Sea to Sky Ballroom A | |
| 10:45 AM – 11:10 AM | L23 | Christian Hackenberger, *FMP Berlin*<br>"Coming Full Circle: Peptide-conjugates for Intracellular Protein and Antibody Delivery" |
| 11:10 AM – 11:35 AM | L24 | Jessica Kramer, *University of Utah*<br>"Synthetic Mucins by NCA Polymerization" |
| 11:35 AM – 11:55 AM | L25 | Ziqing "Leo" Qian, *The Ohio State University*<br>"Development of Endosomal Escape Vehicles to Enhance the Intracellular Delivery of Oligonucleotides" |
| 11:55 AM – 1:35 PM | *Lunch with Exhibitors (provided)* ..... Sea to Sky Ballroom B & C | |
| 1:35 PM – 3:45 PM | **SESSION 6: PEPTIDE LIBRARIES, ARRAYS AND PROTEOMICS** ..... Sea to Sky Ballroom A | |
| 1:35 PM – 2:00 PM | L26 | Kathlynn Brown, *SRI International*<br>"DiaCyt: A Platform Technology for the Discovery of Molecular Transport Systems for Delivery to the Central Nervous System" |

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|---|---|---|
| 2:00 PM – 2:20 PM | L27 | Casey Krusemark, *Purdue University*<br>"In Vitro Selection of DNA-encoded, Synthetic Peptide Libraries for Development of Selective Protein Ligands and Substrates" |
| 2:20 PM – 2:45 PM | L28 | Scott Lokey, *University of California, Santa Cruz*<br>"Passively Permeable Cyclic Peptide Scaffolds: An Abundance of Diversity" |
| 2:45 PM – 3:00 PM | L29-YI4 | Stepan Denisov, *Maastricht University*<br>"Chemokine-binding Peptides Development using Mirror-Image Clips Phage Display" |
| 3:00 PM – 3:25 PM | L30 | Jumi Shin, *University of Toronto*<br>"Rational Design & Continuous Evolution of Franken-Proteins: Potential Drugs & Synthetic Biology Tools" |
| 3:25 PM – 3:45 PM | L31 | Jody Mason, *University of Bath*<br>"Transcription Block Survival: An Intracellular Peptide Library Screening Platform to Derive Functional PPI Antagonists" |
| 3:45 PM – 4:05 PM | *Break* | |
| 4:05 PM – 5:30 PM | **SESSION 7: PEPTIDES IN DELIVERY** ..... Sea to Sky Ballroom A | |
| 4:05 PM – 4:30 PM | L32 | Meritxell Teixidó, *Gate2Brain*<br>"Blood-Brain Barrier Shuttle Peptides, From Discovery to Applications" |
| 4:30 PM – 4:50 PM | L33 | Dehua Pei, *The Ohio State University*<br>"Design, Mechanism, and Applications of Cell-Penetrating Proteins" |
| 4:50 PM – 5:10 PM | L34 | David M Perrin, *University of British Columbia*<br>"Improving on alpha-Amanitin, One of Nature's Most Toxic Peptides - Synthesis of More Cytotoxic Analogs, Bioconjugation, and Use in Peptide-directed Targeted Therapy" |
| 5:10 PM – 5:30 PM | L35 | Krishna Kumar, *Tufts University*<br>"Molecular Design of Peptide Therapeutics for the "Other Pandemics" |
| 5:30 PM – 6:00 PM | **GOODMAN LECTURE** ..... Sea to Sky Ballroom A | |
| | L36 | Jean Chmielewski, *Purdue University*<br>"Stalking Elusive Pathogenic Bacteria: How to Dive into Cells to Treat Infections" |
| 6:00 PM – 6:30 PM | **POSTER FLASH TALKS** ..... Sea to Sky Ballroom A | |
| 6:30 PM – 8:30PM | **GROUP ONE POSTER SESSION & RECEPTION** ..... Sea to Sky Ballroom B & C | |

## Tuesday, June 14, 2022

| | | |
|---|---|---|
| 7:30 AM – 12:30 PM | Registration ..... Grand Foyer | |
| 8:00 AM | Poster Set up ..... Sea to Sky Ballroom B & C<br>*Group Two must be set up at this time* | |
| 8:00 AM – 9:45 AM | **SESSION 8: PEPTIDE- AND PROTEIN-BASED THERAPEUTICS** ..... Sea to Sky Ballroom A | |
| 8:00 AM – 8:25 AM | L37 | Michael Bertucci, *Lafayette College*<br>"Designing Peptide-based Quorum Sensing Modulators for Bacterial Pathogens" |
| 8:25 AM – 8:40 AM | L38-YI5 | Arunika Ekanayake, *University of Alberta*<br>"Genetically Encoded Fragment-Based Discovery (Ge-Fbd) from Covalent and Non-Covalent Pharmacophores" |
| 8:40 AM – 9:00 AM | L39 | Martin Münzel, *Novo Nordisk*<br>"Molecular Engineering of Safe and Efficacious Oral Basal Insulin" |
| 9:00 AM – 9:25 AM | L40 | Carston R. Wagner, *University of Minnesota*<br>"Macromolecular Chemical Biology: Engineering Cell-Cell Interactions and Communication with Chemically Self-Assembled Nanorings" |
| 9:25 AM – 9:40 AM | L41-YI6 | Laia Miret Casals, *Ghent University*<br>"Furan-oxidation Mediated Cross-link Technology: From in vitro Analysis of Protein-protein Interactions to Covalent GPCR-ligand interactions on Live Cells" |
| 9:40 AM – 9:50 AM | **EXHIBITOR FLASH TALKS** . ..... Sea to Sky Ballroom A | |
| 9:50 AM – 10:15 AM | *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C | |
| 10:15 AM – 10:45 AM | **MAKINENI LECTURE** ..... Sea to Sky Ballroom A | |
| | L42 | Bradley Pentelute, *Massachusetts Institute of Technology*<br>"Chemistry Matched with Mechanical and Computational Machines for Rapid Synthesis, Discovery, and Delivery of Proteins" |
| 10:45 AM – 12:40 PM | **SESSION 9: INNOVATIONS IN PEPTIDE AND PROTEIN CHEMISTRY** ..... Sea to Sky Ballroom A | |
| 10:45 AM – 11:10 AM | L43 | Gong Chen, *Nankai University*<br>"New Methods for Construction of Complex Peptide Macrocycles" |
| 11:10 AM – 11:35 AM | L44 | Ben Davis, *Oxford University*<br>"Sugars & Proteins: Towards a Synthetic Biology" |
| 11:35 AM – 11:55 AM | L45 | Isaiah Gober, *Bachem*<br>"An Underreported Side Reaction in Maleimide Bioconjugation: Thiazine Rearrangement of Maleimido N-Terminal Cys Peptides" |

| | | |
|---|---|---|
| 11:55 AM – 12:20 PM | L46 | Itaru Hamachi, *Kyoto University*<br>"Chemical Labeling/Imaging of Neurotransmitter Receptors in Live Cell and Brain" |
| 12:20 PM – 12:40 PM | L47 | Michael Taylor, *University of Wyoming*<br>"Optically Controlled Protein Modification Chemistry" |

12:45 PM – 2:45 PM — Dr. Elizabeth Schram Young Scientists' Lunch & Mixer ..... Garibaldi A/B
*Graduate students & post-docs only*

2:45 PM – 4:00 PM — **WORKSHOP ON CAREER DEVELOPMENT** ..... Fitzsimmons

5:00 PM – 7:00 PM — **ENTREPRENEURSHIP WORKSHOP** ..... Sea to Sky Ballroom A
Panelists: Janis Naeve, Rami Hannoush, Richard DiMarchi

## Wednesday, June 15, 2022

7:30 AM – 4:00 PM — Registration ..... Grand Foyer

8:00 AM – 9:50 AM — **SESSION 10: NEW HORIZONS IN PEPTIDE SCIENCE: GREEN METHODS AND DATA SCIENCE** ..... Sea to Sky Ballroom A

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|---|---|---|
| 8:00 AM – 8:25 AM | L48 | Wendy Hartsock, *CEM Corporation*<br>"Expanded Molecular Access with Enhanced Efficiency in Microwave-Assisted Solid-Phase Peptide Synthesis" |
| 8:25 AM – 8:55 AM | L49 | David Baker, *University of Washington*<br>"Design of Proteins and Macrocycles" |
| 8:55 AM – 9:15 AM | L50 | Beatriz de la Torre, *University of KwaZulu-Natal*<br>"Cleaving Protected Peptides from 2-Chlorotrityl Chloride Resin. Moving away from Dichloromethane" |
| 9:15 AM – 9:35 AM | L51 | Ewa Lis, *Koliber Biosciences Inc.*<br>"Peptide Hit Identification and Lead Optimization using Artificial Intelligence Approaches" |
| 9:35 AM – 9:50 AM | L52-YI7 | Joseph Brown, *Massachusetts Institute of Technology*<br>"Targeted Affinity Selection of Peptide Binders Using Machine Learning" |
| 9:50 AM – 10:15 AM | L53 | Fernando Albericio, *University of KwaZulu-Natal*<br>"New Developments for the Solid-Phase Peptide Synthesis (SPPS). Greening the Process" |

10:15 AM – 10:40 AM — *Coffee with Exhibitors & Posters* ..... Sea to Sky Ballroom B & C

10:40 PM – 12:15 PM — **SESSION 11: SCAFFOLDS AND PEPTIDOMIMETICS** ..... Sea to Sky Ballroom A

| | | |
|---|---|---|
| 10:40 AM – 11:00 AM | L54 | Jane Aldrich, *University of Florida*<br>"Macrocyclic Tetrapeptides Prevent Reinstatement of Opioid- seeking Behavior" |
| 11:00 AM – 11:20 AM | L55 | William Lubell, *Université de Montréal*<br>"Peptide Mimicry using N-Aminoimidazole-2-ones" |
| 11:20 AM – 11:35 AM | L56-YI8 | Chloe Mitchell, *The Hospital for Sick Children*<br>"Peptide-Based Disruption of Membrane-Embedded Protein-Protein Interactions in Bacterial Efflux Pumps" |

| | | |
|---|---|---|
| 11:35 AM – 11:55 AM | L57 | Craig Hutton, *University of Melbourne*<br>"Exploiting Thioamide Reactivity in Peptide Synthesis" |
| 11:55 AM – 12:15 PM | L58 | Christian Schafmeister, *Temple University*<br>"Automated Synthesis of Spiroligomers: Programmable, Shape- defined, and Cell-permeable Peptidomimetics" |
| 12:15 PM – 1:50 PM | *Lunch (on your own)* | |
| 12:15 PM – 1:45 PM | Vincent du Vigneaud Award Lunch - *by invitation* .....Garibaldi A/B | |
| 12:15 PM – 1:45 PM | **EXHIBITOR WORKSHOP**, *hosted by CEM*..... Fitzsimmons<br>"Towards an Optimized Approach for Peptide Purification at Elevated Temperature" | |
| 1:50 PM – 2:20 PM | Announcement of 28th APS and American Peptide Society General Assembly ..... Sea to Sky Ballroom A | |
| 2:20 PM – 4:10 PM | **SESSION 12: PEPTIDE DESIGN AND FUNCTION** . .....Sea to Sky Ballroom A | |
| 2:20 PM – 2:40 PM | L59 | Steve Bourgault, *Université du Québec à Montréal*<br>"Manipulating Peptide Self-assembly into Cross-ß-sheet Supramolecular Structures to Design Synthetic Nanovaccines" |
| 2:40 PM – 3:00 PM | L60 | Emel Adaligil, *Genentech*<br>"Towards Next Generation Therapeutics: Cell-permeable Macrocyclic Peptides" |
| 3:00 PM – 3:25 PM | L61 | Lynne Regan, *University of Edinburgh*<br>"Peptide Interactions *in vivo* and *in vitro*" |
| 3:25 PM – 3:50 PM | L62 | Marcey Waters, *University of North Carolina at Chapel Hill*<br>"Determining What Else Trimethyllysine Reader Proteins Can Read" |
| 3:50 PM – 4:10 PM | L63 | Ivan Korendovych, *Syracuse University*<br>"Catalytic Amyloids Promote Carbon Dioxide Hydration with Efficiencies that Rival those of Native Carbonic Anhydrases" |
| 4:10 PM – 4:30 PM | Break | |
| 4:30 PM – 5:00 PM | **EARLY CAREER LECTURESHIP AWARD II** . .....Sea to Sky Ballroom A | |
| | L64 | Caroline Proulx, *North Carolina State University*<br>"New Tools for Peptide Mimicry and Functionalization" |
| 5:00 PM – 5:30 PM | **DU VIGNEAUD LECTURE I** .....Sea to Sky Ballroom A | |
| | L65 | Joel Schneider, *National Cancer Institute – Center for Cancer Research*<br>"Peptides in Materials Science" |
| 5:30 PM – 6:30 PM | **POSTER FLASH TALKS** .....Sea to Sky Ballroom A | |
| 6:30 PM – 8:30 PM | **GROUP TWO POSTER SESSION RECEPTION** .....Sea to Sky Ballroom B & C | |

## Thursday, June 16, 2022

| | | |
|---|---|---|
| 7:30 AM – 10:30 AM | Registration ..... Grand Foyer | |
| 8:00 AM – 9:55 AM | **SESSION 14: PEPTIDE INSPIRED MATERIALS** .....Sea to Sky Ballroom A | |
| 8:00 AM – 8:25 AM | L66 | Dek Woolfsen, *University of Bristol*<br>"Designing New Peptide Assemblies for Fun and for In-Cell Applications" |
| 8:25 AM – 8:50 AM | L67 | Ting Xu, *University of California, Berkeley*<br>"Peptides: Valuable Motifs for Material Design" |
| 8:50 AM – 9:10 AM | L68 | Motoyoshi Nomizu, *Tokyo University of Pharmacy and Life Sciences*<br>"Development of Cell Adhesive Peptide-Agarose Matrices for Cell Culture" |
| 9:10 AM – 9:35 AM | L69 | Julie Champion, *Georgia Institute of Technology*<br>"Self-Assembled Protein Vesicles for Drug Delivery and Biocatalysis" |
| 9:35 AM – 9:55 AM | L70 | David Sabatino, *Carleton University*<br>"Cancer-Targeting Immunostimulatory Peptides as Synthetic Antibody Mimics for Tumor Immunotherapy Applications" |
| 9:55 AM – 10:20 AM | *Coffee break*..... Grand Foyer | |
| 10:20 AM – 12:15 PM | **SESSION 15: STRUCTURAL METHODS IN PEPTIDE SCIENCE**. .....Sea to Sky Ballroom A | |
| 10:20 AM – 10:45 AM | L71 | Tamir Gonen, *University of California, Los Angeles*<br>"MicroED Conception and Current Practices" |
| 10:45 AM – 11:10 AM | L72 | Michael Powner, *University College London*<br>"Nitriles, Peptides, Cofactors and the Origins of Life" |
| 11:10 AM – 11:30 AM | L73 | Juan Del Valle, *University of Notre Dame*<br>"Beyond N-methylation: Synthesis, Structure, and Function of N-heteroatom-substituted Peptides" |
| 11:30 AM – 11:50 AM | L74 | Alethea Tabor, *University College London*<br>"A Chemical Biology And Biophysics Approach to Understanding the Molecular Recognition of Lipid II by the Lantibiotic Nisin" |
| 11:50 AM – 12:15 PM | L75 | Patrick Sexton, *Monash University*<br>"Structure and Dynamics of Class B Peptide Hormone G Protein-Coupled Receptors' |
| 12:15 PM – 1:45 PM | *Lunch Break (On your own)* | |
| 1:45 PM – 2:15 PM | **DU VIGNEAUD LECTURE II** . .....Sea to Sky Ballroom A | |
| | L76 | Alanna Schepartz, *The University of California, Berkeley*<br>"Protein Delivery to Cells and Animals" |

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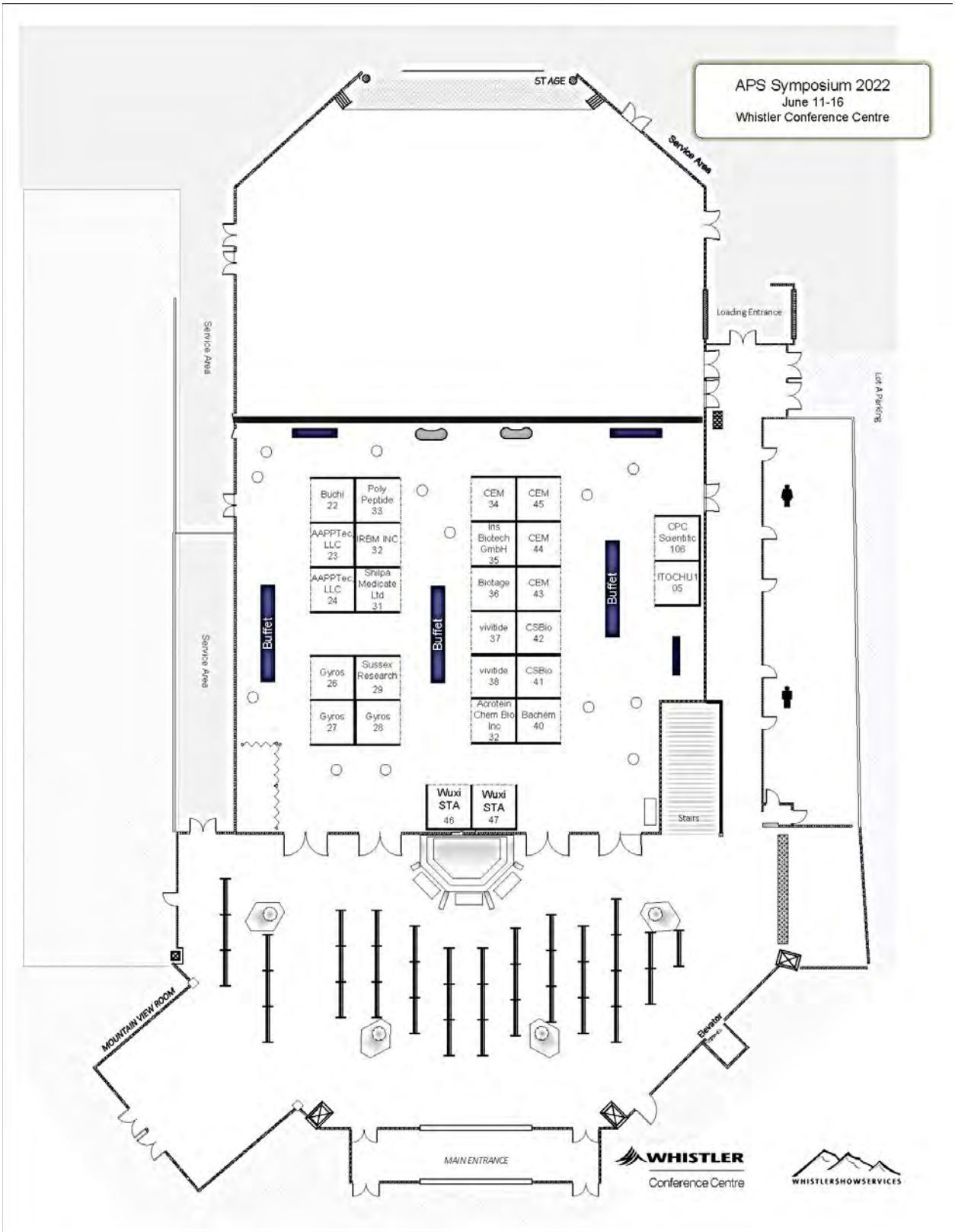
|---|---|---|
| 2:15 PM – 4:10 PM | **SESSION 16: PEPTIDE NATURAL PRODUCTS AND BIOLOGICAL METHODS** ..... Sea to Sky Ballroom A | |
| 2:15 PM – 2:40 PM | L77 | Mandë Holford, *Hunter College*<br>"Killer Snails: Agents of Change and Innovation" |
| 2:40 PM – 3:00 PM | L78 | Anna Mapp, *University of Michigan*<br>"Lipopeptides are a Modular Scaffold for Tunable Targeting of Transcriptional Coactivators" |
| 3:00 PM – 3:20 PM | L79 | Danny Chou, *Stanford University*<br>"A Natural Product-inspired Insulin Analog Induces New Insulin Receptor Conformations Upon Activation" |
| 3:20 PM – 3:45 PM | L80 | Hiroaki Suga, *University of Tokyo*<br>"The Next Generation RaPID System" |
| 3:45 PM – 4:10 PM | L81 | Christina Boville, *Aralez Bio*<br>"Engineering Enzymes for Green Manufacturing of Noncanonical Amino Acids" |
| 4:10 PM – 4:55 PM | **CLOSING PLENARY KEYNOTE LECTURE** ..... Sea to Sky Ballroom A | |
| | L82 | David Craik, *University of Queensland*<br>"Discovery and Applications of Cyclotides: Nature's Ultra-stable Peptide Scaffolds" |
| 4:55 PM – 5:00 PM | **CLOSING REMARKS** ..... Sea to Sky Ballroom A | |
| 7:00 PM – 10:00 PM | Closing Banquet . ..... Sea to Sky Ballroom A | |

# EXHIBITOR LISTING

| Exhibitor | Booth |
|---|---|
| AAPPTec, LLC | 23, 24 |
| Acrotein ChemBio Inc. | 39 |
| Bachem | 40 |
| Biotage | 36 |
| Buchi | 22 |
| CEM | 34, 43, 44, 45 |
| CPC Scientific Inc. | 18 |
| CSBio | 41, 42 |
| Gyros Protein Technologies | 26, 27, 28 |
| IRBM, Inc. | 32 |
| Iris Biotech GmbH | 35 |
| ITOCHU Chemicals America Inc. | 19 |
| PolyPeptide | 33 |
| Shilpa Medicare Limited | 31 |
| Sussex Research | 29 |
| vivitide | 37, 38 |
| Wuxi STA | 46, 47 |
| | |

# EXHIBITOR LAYOUT



# THE 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.

| | |
|---|---|
| 2021 | **Padmanabhan Balaram**, Indian Institute of Science |
| 2019 | **Lila Gierasch**, University of Massachusetts, Amherst |
| 2017 | **Charles M. Deber**, University of Toronto |
| | **Robert Hodges**, University of Colorado, School of Medicine |
| 2015 | **Horst Kessler**, TU München Institute for Advanced Study |
| 2013 | **James P. Tam**, Nanyang Technological University |
| 2011 | **Richard DiMarchi**, Indiana University |
| 2009 | **Stephen B.H. Kent**, University of Chicago |
| 2007 | **Isabella L. Karle**, Naval Research Laboratory |
| 2005 | **Richard A. Houghten**, Torrey Pines Institute for Molecular Studies |
| 2003 | **William F. DeGrado**, University of Pennsylvania, School of Medicine |
| 2001 | **Garland R. Marshall**, Washington University Medical School |
| 1999 | **Daniel H. Rich**, University of Wisconsin – Madison |
| 1997 | **Shumpei Sakakibara**, Peptide Institute, Inc. |
| 1995 | **John M. Stewart**, University of Colorado – Denver |
| 1993 | **Victor J. Hruby**, University of Arizona – Tucson |
| 1991 | **Daniel F. Veber**, Merck Sharp & Dohme, Inc. |
| 1989 | **Murray Goodman**, University of California, San Diego |
| 1987 | **Choh Hao Li**, University of California, San Francisco |
| 1985 | **Robert Schwyzer**, Swiss Federal Institute of Technology |
| 1983 | **Ralph F. Hirschmann**, Merck Sharp & Dohme, Inc. |
| 1981 | **Klaus Hofmann**, University of Pittsburgh, School of Medicine |
| 1979 | **Bruce Merrifield**, The Rockfeller University |
| 1977 | **Miklos Bodansky**, Case Western Reserve University |

* Previously, the Alan E. Pierce Award sponsored by the Pierce Chemical Company (1977-1995). The Merrifield Award was established in 1997 by an endowment from Rao Makineni.

# THE 2021 R. BRUCE MERRIFIELD AWARD

## Padmanabhan Balaram

Padmanabhan Balaram was on the faculty of the Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India for most of his scientific career from 1973-2014. He is currently a Chair Professor at the National Centre for Biological Sciences, Bangalore. He was educated at the Fergusson College, Pune (BSc), Indian Institute of Technology Kanpur, and Carnegie-Mellon University Pittsburgh (Ph.D.). After a post-doctoral year with R. B. Woodward, at Harvard, he returned to Bangalore to join a department newly created by G. N. Ramachandran, a pioneer in the study of polypeptide conformations.

Balaram's early research focused on the membrane active peptide alamethicin, leading to a long-standing interest in the conformational restrictions on backbone folding promoted by the unusual residue alpha aminoisobutyric acid (Aib). The facile formation of 310-helical structures in Aib peptides was established by his studies in the late 1970s. His research on the use of conformational constraints to direct peptide backbone folding led to the characterization in solution and in crystals of designed synthetic sequences that mimic secondary structures found in proteins. The use of D-Pro residues in nucleating beta-hairpin formation, the design of multistranded sheets, and the use of beta and gamma amino acid residues in generating hybrid helices were reported from his laboratory in the 1990s.

His laboratory has contributed to studies of the enzyme triosephosphate isomerase from plasmodium falciparum and more recently in the mass spectrometric and transcriptome based characterization of disulfide-rich peptides from marine cone snail venom. He has mentored and trained a large number of graduate students and post-doctoral fellows.

**L16 Constraining Peptide Conformations: Finding Aib and Beyond**

Padmanabhan Balaram
*Molecular Biophysics Unit, Indian Institute of Science and National Centre for Biological Sciences Bangalore, India*

The report by Mueller and Rudin (1969) that alamethicin, a cyclic peptide, induces voltage-dependent ion channel formation in artificial lipid bilayers, sparked interest in peptides of fungal origin, rich in alpha aminioisobutyric acid (Aib, 2-methylalanine). The structure of alamethicin was corrected in 1976 to reveal an acyclic sequence, rich in Aib, blocked at both N and C-termini. An endeavour to produce alamethicin by chemical synthesis, soon unveiled the unusual and attractive properties of peptides containing Aib.

The alamethicin N-terminal tetrapeptide was quickly reached by solution phase chemistry. Surprisingly, crystals appeared almost instantly after purification. Diffraction photographs, then painstakingly measured, seemed promising enough to move even further forward towards structure determination. Direct methods of phase determination, still in their infancy, revealed a folded structure with two internal 10-atom CO—HN hydrogen bonds, launching the peptide $3_{10}$-helix on its way

The backbone conformational constraints imposed by $C^{\alpha}$ alkylation provided an easy route to helix design and to restrict conformational excursions in biologically active peptides. The growing understanding of polypeptide chain folding in proteins suggested that D-Pro induced prime turns would nucleate ß-hairpins in designed peptides Attempts to probe helix stability by insertion of the backbone expanded ß and γ residues uncovered the wealth of hydrogen bonded folded structures in hybrid sequences, even as the field of ß and γ peptides was established by Seebach and Gellman. This lecture traces the journey taken at Bangalore form the mid-1970s on promoting the use of non-canonical amino acids in peptide design.

# THE VINCENT DU VIGNEAUD AWARD

Vincent du Vigneaud, May 18, 1901 – December 11, 1978, was an American biochemist. He won 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 cyclic peptide oxytocin. The Vincent du Vigneaud Awards recognize outstanding achievement in peptide research at mid-career.

| | |
|---|---|
| 2021 | **Alanna Schepartz**, The University of California, Berkeley<br>**Joel P. Schneider**, National Cancer Institute |
| 2019 | **Annette Beck-Sickinger**, Leipzig University<br>**Hiroaki Suga**, The University of Tokyo |
| 2017 | **Paul Alewood**, University of Queenland<br>**Roland T. Raines**, University of Wisconsin-Madison |
| 2015 | **Jean Chmielewski**, Purdue University<br>**David Craik**, University of Queensland |
| 2013 | **Michael Chorev**, Harvard Medical School<br>**Kit S. Lam**, University of California Davis Cancer Center |
| 2011 | **Fernando Albericio**, University of Barcelona<br>**Morten P. Meldal**, Carlsberg Laboratory, Copenhagen |
| 2010 | **Philip Dawson**, The Scripps Research Institute<br>**Reza Ghadiri**, The Scripps Research Institute |
| 2008 | **Tom W. Muir**, Rockefeller University<br>**Jeffery W. Kelly**, The Scripps Research Institute |
| 2006 | **Samuel H. Gellman**, University of Wisconsin-Madison<br>**Barbara Imperiali**, Massachusetts Institute of Technology |
| 2004 | **Steven B. H. Kent**, University of Chicago<br>**Dieter Seebach**, ETH Zurich |
| 2002 | **Robert S. Hodges**, University of Colorado-Denver<br>**Horst Kessler**, Technical University, Munich |

2000 **Charles M. Deber**, University of Toronto
**Richard A. Houghten**, Torrey Pines Institute for Molecular Studies

1998 **Peter W. Schiller**, Clinical Research Institute of Montreal
**James A. Wells**, Genentech, Inc.

1996 **Arthur M. Felix**, Hoffmann-La Roche, Inc.
**Richard G. Hiskey**, University of North Carolina

1994 **George Barany**, University of Minnesota-Minneapolis
**Garland R. Marshall**, Washington University-St. Louis

1992 **Isabella L. Karle**, Naval Research Laboratory
**Wylie W. Vale**, The Salk Institute for Biological Studies

1990 **Daniel H. Rich**, University of Wisconsin-Madison
**Jean E. Rivier**, The Salk Institute for Biological Studies

1988 **William F. De Grado**, DuPont Central Research
**Tomi K. Sawyer**, The Upjohn Company

1986 **Roger M. Freidinger**, Merck Sharpe Dohme
**Michael Rosenblatt**, Massachusetts General Hospital
**James P. Tam**, The Rockefeller University

1984 **Betty Sue Eipper**, The Johns Hopkins University
**Lila M. Gierasch**, University of Delaware
**Richard E. Mains**, The Johns Hopkins University

* Sponsored by BACHEM Inc.

# THE 2021 VINCENT DU VIGNEAUD AWARD

## Alanna Schepartz

Alanna Schepartz obtained her undergraduate education in chemistry at the State University of New York, Albany. She earned a Ph.D. from Columbia University under the direction of Ronald Breslow, and spent two years as an NIH Postdoctoral Fellow at Caltech working with Peter Dervan. In 1988, she joined the faculty at Yale University. She was promoted to the rank of Professor in 1995, named the Milton Harris '29 Ph.D. Professor in 2000, and in 2017 was named a Sterling Professor, Yale's highest faculty honor. In 2019 Professor Schepartz and her laboratory moved to the University of California, Berkeley where she is currently the C.Z. and Irmgard Chu Distinguished Chair of Chemistry and Professor of Molecular and Cell Biology.

Alanna Schepart'z research group is broadly interested in understanding the chemistry that governs how complex cellular machines function, devising tools to probe that function, and applying that knowledge to design or discover new molecules–both small and large–with unique or useful properties. She is particularly well known for pioneering and creative development and application of ▫-and ▫-peptides to explore and expand the chemistry in biology. Her honors include a Packard Fellowship in Science and Engineering, an NSF Presidential Young Investigator Award, the Eli Lilly Award in Biological Chemistry, an ACS Cope Scholar Award, the ACS Chemical Biology Prize, the Ralph F. Hirschmann Award in Peptide Chemistry, the Ronald Breslow Award for Achievement in Biomimetic Chemistry, the Frank H. Westheimer Prize, and the Wheland Medal. She is a member of the American Academy of Arts and Sciences and the National Academy of Sciences.

**L76 Protein Delivery to Cells and Animals**
Alanna Schepartz
*The University of California, Berkeley*

I will describe the discovery of a miniature protein that guides proteins and enzymes into the cell interior by promoting endosomal escape, a single-molecule tool that quantifies this trafficking event in live cells with accuracy and precision, experiments to probe how endosomal escape occurs, and how this knowledge can be applied for (cytosolic) enzyme replacement therapy.

# THE 2021 VINCENT DU VIGNEAUD AWARD

## Joel P. Schneider

Dr. Joel Schneider received his B.Sc. in chemistry at the University of Akron in 1991 before moving to Texas A&M University for a Ph.D. in chemistry developing beta-sheet peptide mimetics to study the mechanisms leading to amyloid formation. He then pursued post-doctoral studies in the laboratory of William DeGrado at the University of Pennsylvania where he applied the principles of protein de novo design to study helical ion channels, coiled coils and bundles. He started his independent career at the University of Delaware in 1999 in the Department of Chemistry and Biochemistry and later took a secondary appointment in the Materials Science and Engineering Department. He rose through the ranks to full professor and was then recruited in 2009 to the National Cancer Institute (NCI) to build their newly formed Chemical Biology Laboratory. Currently, he serves as the Chief of Chemical Biology and Deputy Director of Basic Science in the Center for Cancer Research, NCI

Dr. Schneider's lab develops injectable hydrogel materials, bioadhesives, and cellular delivery vehicles capable of administering therapeutics locally to tissue. He is particularly interested in peptide and protein-based hydrogel materials formed by self-assembly mechanisms. His work spans molecular design, materials synthesis, nano- and bulk mechanical materials characterization, cell-material interactions, biocompatibility, and assessment of performance efficacy. In addition to developing materials towards clinical applications, his group studies the mechanisms by which materials form, their structures at all length-scales and determines how their molecule structure influences their mechanical and biological properties. His basic research establishes how material composition and structure influences material function.

**L65 Peptides in Materials Science**

Joel P. Schneider
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute*

Peptides have, and continue, to impact materials development. They can be used to functionalize the surfaces of existing materials to impart function or serve as building blocks to construct new materials through their polymerization and/or self-assembly. Self-assembling peptides can form a broad array of supramolecule structures including sheets, disks, spheres, barrels, tubes, and fibrils. Fibrils are privileged structures capable of higher-order assembly leading to the formation of networks that constitute the formation of macroscopic gels. The physical, mechanical and biological properties of peptide gels can be finely tuned through molecular design to enable a wide range of biomedical applications. Further, understanding the assembly mechanisms by which these materials are formed and their structures to molecular detail catalyze their development towards targeted applications. We designed a class of peptide hydrogels that allows for the direct encapsulation of therapeutics and their subsequent local delivery to tissue. Peptide assembly leading to fibrillar gels can be triggered in the presence of small molecules, proteins, nucleic acids, cells, and nanoparticles resulting in their direct encapsulation. Resultant gels display shear-thin/recovery mechanical properties, allowing their direct application to targeted tissue by syringe or spray, where they deliver their payload locally. Through the design of over 200 sequences, we have developed a deep mechanistic and structural understanding of this class of material. This has allowed the development of gels that facilitate a broad range of applications including microanastomosis (the suturing of ultrasmall blood vessels), gels that limit tissue rejection after vascularized composite allotransplantation, and as treatments for mesothelioma.

# THE MURRAY GOODMAN AWARD

Professor Murray Goodman passed away in Germany on June 1, 2004 at the age of 75 after a very brief illness. Murray, as he was known to thousands of peptide chemists world wide, was the immediate past president of our society. He was an incisive and influential force in the field of peptide chemistry and remained active, dedicated and enthusiastic until the end. 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.

| | |
|---|---|
| 2021 | **Jean Chmielewski**, Purdue University |
| 2019 | **Fernando Albericio**, University of KwaZulu-Natal |
| 2017 | **Paul Alewood**, University of Queensland |
| 2015 | **George Barany**, University of Minnesota |
| 2015 | **George Barany**, University of Minnesota |
| 2013 | **Robert S. Hodges**, University of Colorado-Denver |
| 2011 | **Victor J. Hruby**, University of Arizona |
| 2009 | **Charles M. Deber**, University of Toronto |

* Endowed by Zelda Goodman (2007)

# THE 2021 MURRAY GOODMAN AWARD

## Jean Chmielewski

Jean Chmielewski completed her Ph.D. in Bioorganic Chemistry with Ronald Breslow at Columbia University. She joined the labs of E. T. Kaiser of Rockefeller University and, subsequently, Peter Schultz of the University of California, Berkeley for NIH postdoctoral fellowships in Chemical Biology. After postdoctoral appointments, she was recruited to the faculty in the Chemistry Department of Purdue University in 1990 where she is now the AW Kramer Distinguished Professor of Chemistry and a faculty member in the Weldon School of Biomedical Engineering.

Chmielewski has won numerous awards for her research, including the Arthur C. Cope Scholar Award and the Edward Leete Award, both from the American Chemical Society, the Agnes Fay Morgan Research Award from Iota Sigma Pi, and the Vincent du Vigneaud Award from the American Peptide Society. She has also been honored many times for her teaching, including the Charles R. Murphy Award the highest teaching award of Purdue University, and for her efforts in diversity, including the Stanley C. Israel Award for Advancing Diversity from the American Chemical Society. She is a fellow of the American Association for the Advancement of Science and a member of the Teaching Academy of Purdue University.

She was a pioneer in the development of peptide-based agents to disrupt protein-protein interactions, and her current research interests include the design of novel peptide biomaterials for regenerative medicine, the design of cationic polyproline antibiotics that target intracellular pathogenic bacteria, and the development of agents that modulate drug efflux transporters.

**L36 Stalking Elusive Pathogenic Bacteria: How to Dive into Cells to Treat Infections**

Jean Chmielewski
*Department of Chemistry, Purdue University, West Lafayette, IN 47907 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.

# 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.

| | |
|---|---|
| 2021 | **Bradley Pentelute**, Massachusetts Institute of Technology |
| 2019 | **Xuechen Li**, The University of Hong Kong |
| 2017 | **Thomas Kodadek**, The Scripps Research Institute |
| 2015 | **Paramjit Arora**, New York University |
| 2013 | S**amuel H. Gellman**, University of Wisconsin |
| 2011 | **Jeffery W. Kelly**, Scripps Research Institute |
| 2009 | **William F. DeGrado**, University of Pennsylvania |
| 2007 | **Ronald T. Raines**, University of Wisconsin - Madison |
| 2005 | **Robin E. Offord**, Centre Medical Universitaire, Switzerland |
| 2003 | **James P. Tam**, Vanderbilt University |

*Endowed by PolyPeptide Laboratories and Murray and Zelda Goodman (2003)

# THE 2021 MAKINENI LECTURESHIP

## Bradley Pentelute

Bradley L. Pentelute is an Associate Professor of Chemistry at Massachusetts Institute of Technology. He is also an Associate Member, Broad Institute of Harvard and MIT, an Extramural Member of the MIT Koch Cancer Institute, and Member, Center for Environmental Health Sciences MIT. He received his undergraduate degree in Psychology and Chemistry from the University of Southern California, and his M.S and Ph.D. in Organic Chemistry from the University of Chicago with Prof. Steve Kent. He was a postdoctoral fellow in the laboratory of Dr. R. John Collier at Harvard Medical School, Microbiology.

Professor Pentelute's research program lies at the intersection of chemistry and biology. The main research directions in his lab entail building fast flow technologies for the rapid production of proteins and cell penetrating biopolymers while employing computational design, combinatorial peptide library affinity selection methods, and high-throughput mass sequencing to identify functional peptide-based binders to protein targets.

**L42 Chemistry Matched with Mechanical and Computational Machines for Rapid Synthesis, Discovery, and Delivery of Proteins**

Bradley Lether Pentelute
*Massachusetts Institute of Technology, Department of Chemistry, 77 Massachusetts Avenue, Cambridge, MA 02139, USA*
*Department of Chemistry and Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States; The Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, United States; Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, United States*

Methods for the automated high-fidelity chemical production of proteins are needed. Proteins manufactured with mechanical machines, as opposed to bio-based systems, can bulletproof production circumventing endotoxin contamination and cell-line variability. Further, chemical synthesis enables the ability to rewire the covalent framework with non-natural amino acids, drugs, and carbohydrates. I will discuss the fully automated single-shot chemical synthesis of protein chains up to 220 amino acids in hours. After purification and folding, the synthetic proteins functioned analogously to ribosomally produced material. In addition, rapid protein synthesis enables accelerated drug discovery when combined with our single-shot affinity-selection mass spectrometry platform. I will discuss our results toward the discovery of high-affinity peptidomimetics that target oncoproteins or SARS-CoV-2.

# THE EARLY CAREER LECTURESHIP

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.

| | |
|---|---|
| 2021 | **Caroline Proulx**, North Carolina State University at Raleigh |
| 2020 | **Yftah Tal-Gan**, University of Nevada at Reno |
| 2019 | **Monika Raj,** Auburn University |
| | **Jevgenij A. Raskatov,** University of California Santa Cruz |

# THE 2020 EARLY CAREER LECTURESHIP

## Yftah Tal-Gan

Professor Yftah Tal-Gan received his B.S. from the Hebrew University of Jerusalem. He then worked on his M.S. and Ph.D. in Organic Chemistry at the Hebrew University of Jerusalem, in the groups of Professor Chaim Gilon and Professor Alexander Levitzki. His doctoral dissertation was on the development of new peptide-based inhibitors of Protein Kinase B (PKB/Akt) as potential drugs for Cancer. Upon completion of his Ph.D. work, Yftah joined the laboratory of Professor Helen Blackwell at University of Wisconsin-Madison as a Postdoctoral Research Associate working on the development of peptide-based tools and materials to study quorum sensing in Staphylococcus aureus. In 2014 Yftah joined the Chemistry Department at the University of Nevada, Reno as an Assistant Professor in Chemical Biology and as of July 1, 2020 he was promoted to the Associate Professor rank.

Since joining the University of Nevada, Reno, Yftah has established a chemical biology research program with the overarching goal of developing and utilizing peptide-based probes to study bacterial communication pathways and their role in bacterial pathogenesis and inter-species competition. This research program is multidisciplinary and spans from organic synthesis and analytical characterization, to biological screening, structural determination of biomacromolecules, and molecular microbiology. Current projects in the lab are funded by two National Institutes of Health (NIH) and one National Science Foundation (NSF) grants, and resulted in 20 papers from the lab.

### L22 Development of Peptide-Based Tools to Study Cell-Cell Signaling in Bacteria

Yftah Tal-Gan
Department of Chemistry, University of Nevada, Reno, 1664 N. Virginia Street, Reno, NV, 89557, USA

Quorum sensing (QS) is a cell density-based communication mechanism that bacteria utilize to regulate a variety of symbiotic and pathogenic phenotypes. As such, QS has attracted significant attention as a potential anti-virulence therapeutic target. Gram-positive bacterial species utilize peptides, termed autoinducers, as the signaling molecules for QS. We aim to develop autoinducer-based QS modulators that would be applied to study different Gram-positive bacterial species, with an emphasis on streptococci, investigate the molecular mechanisms that drive signal peptide-receptor binding and lead to QS activation, and interrogate the role of QS in bacterial virulence and inter-species competition.

# THE 2021 EARLY CAREER LECTURESHIP

## Caroline Proulx

Dr. Caroline Proulx is an assistant professor in the Department of Chemistry at North Carolina State University. She received her Hon. B.Sc. in Biopharmaceutical Sciences (Medicinal Chemistry) from the University of Ottawa in 2007. In 2012, she obtained her Ph.D. in Chemistry from the University of Montreal under the guidance of Prof. William D. Lubell, where she developed new methods for azapeptide synthesis. From 2012-2016, she was an NSERC postdoctoral fellow at the Molecular Foundry, Lawrence Berkeley National Laboratory, working with Dr. Ronald N. Zuckermann on peptoid synthesis and self-assembly. She joined the faculty at North Carolina State University as an assistant professor in July 2016.

Dr. Proulx's current research interests lie primarily in the development of synthetic methods to access new classes of conformationally constrained peptides and peptidomimetics, with applications in medicinal chemistry and chemical biology. In 2018, the Proulx lab disclosed a new strategy for oxime ligations under mild conditions, featuring the chemoselective oxidation of tunable N-aryl peptide precursors to give reactive α-imino amide intermediates. They later demonstrated that Cα-substituted N-aryl peptides could also undergo oxidative couplings with α-nucleophiles, significantly expanding the scope and utility of this methodology. In addition, the Proulx lab has a continued interest in the design, synthesis, and conformational analysis of backbone-modified peptidomimetics, including N-substituted glycines (peptoids) and azapeptides. Dr. Proulx received the LeRoy and Elva Martin Teaching Award from NCSU in 2020, and more recently an NSF CAREER award in 2021.

### L64 New Tools for Peptide Mimicry and Functionalization

Caroline Proulx
*Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695-8204, United States*

Peptides modulate a variety of biological responses, including as hormones and neurotransmitters. Occupying the space between small molecules and biologics, they have also been pursued to target protein-protein interaction surfaces. However, strategies are needed to convert them into more stable molecules while retaining the bioactivity of the native peptides, ideally employing scaffolds that can adopt precise secondary structures while being amenable to rapid derivatization for library synthesis and screening. Towards that goal, we develop new methods for late-stage peptide functionalizations, and devise new strategies for the synthesis and conformational control of highly substituted peptidomimetics. This includes 1) the synthesis and study of *N*-aryl peptides as tunable precursors for kethydrazone and ketoxime ligations,[1] 2) the development of new methods for chemoselective functionalizations of azapeptides,[2] and 3) the design and application of new conformationally-constrained *N*-substituted glycine (peptoid) monomers.[3] This presentation will provide an overview of the new broadly applicable tools for peptide mimicry developed in the Proulx lab, with emphasis on synthetic method development.

# THE YOUNG INVESTIGATOR POSTER COMPETITION

**Zoe Adams,** *The Scripps Research Institute*
**Dorien Aerssens,** *University of Ghent*
**Rafat Ali,** *Indian Institute of Technology Kanpur*
**Caleb Anderson,** *National Cancer Institute, NIH*
**Mónica Aróstica,** *USM/PUCV*
**Jon Babi,** *University of Toronto*
**Taysir Bader,** *University of Minnesota*
**Krista Barbour,** *University of Georgia*
**Alec Brennan,** *University of Nevada, Reno*
**Andrew Brennan,** *University of Bath*
**Joseph Brown,** *Massachusetts Institute of Technology*
**Christopher Cain,** *University of Notre Dame*
**Li Cao,** *Princeton University*
**Arif Celik,** *Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP Berlin)*
**Francois Charih,** *Carleton University*
**Ramesh Chingle,** *National Cancer Institute (NCI), National Institutes of Health (NIH)*
**Sorina Chiorean,** *Scripps Research*
**Brian Choi,** *Princeton University*
**Abigail Clapperton,** *University of Toronto*
**Gregory Cole,** *University of Toronto*
**Caroline Corrêa-Almeida,** *Federal University of Rio de Janeiro*
**Carolynn Davern,** *North Carolina State University*
**Naysilla Dayanara,** *University of British Columbia*
**Rahul Deb,** *Masaryk University, Czech Republic*
**Stepan Denisov,** *Maastricht University*
**Steven Draper,** *University of Utah*
**Arunika Ekanayake,** *University of Alberta*
**Andrew Encinas,** *Purdue University*
**Juan Esteban,** *University of Western Ontario*
**Judah Evangelista,** *Kay Lab, University of Utah*
**Daniel Ferrer Vinals,** *University of Alberta*
**Luise Franz,** *Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) Berlin*
**Georgina Girt,** *Rosalind Franklin Institute*
**Shanal Gunasekera,** *University of British Columbia*
**Shu Hui Hiew,** *Nanyang Technological University*
**Shu Hui Hiew,** *Nanyang Technological University*
**Olamilekan Ibukun,** *Indian Institute of Science Education and Research - Kolkata*
**Corey Johnson,** *Purdue University*
**Chelsea Jones,** *University of California, Irvine*
**Michael Jorgensen,** *Purdue University*
**Vladimir Khayenko,** *University of Wuerzburg*
**Anton Kliuchynskyi,** *University of Victoria*
**Grant Koch,** *University of California, Santa Cruz*
**Bimal Koirala,** *The Rockefeller University*
**Jonathan Kwok,** *New York University*
**Tess Lamer,** *University of Alberta*
**Alexander Lander,** *Cardiff University*
**Muralikrishna Lella,** *University of Nevada Reno*
**Moritz List,** *University of Leipzig*
**Lucia Lombardi,** *Imperial College London*
**Tania Lopez Silva,** *National Cancer Institute*
**Valentina Lukinovic,** *Carleton University*
**Ryan Malonis,** *Albert Einstein College of Medicine*
**Guilherme Meira Lima,** *University of Alberta*
**Laia Miret Casals,** *Ghent University*
**Chloe Mitchell,** *The Hospital for Sick Children*
**Cristobal Morfin,** *UCSC*
**Danielle Morgan,** *University of Glasgow*
**Priti Mudgil,** *United Arab Emirates University*
**Marcelo Muñoz,** *University of Ottawa Heart Institute*
**Monessha Nambiar,** *National Cancer Institute*
**Pankhuri Narula,** *Indian Institute of Technology Delhi India*
**Neelakshi,** *Indian Institute of Technology Kanpur*
**Alexander Lund Nielsen,** *Ecole Polytechnique Fédérale de Lausanne (EPFL)*
**Mark Nolan,** *Trinity College Dublin*
**Radoslaw Piast,** *Warsaw University*
**Hector Pineda-Castañeda,** *Universidad Nacional de Colombia*
**Ryan Qiu,** *University of Alberta*
**Benjamin Rathman,** *University of Notre Dame*
**Maria Rios,** *University of Edinburgh*
**Roberto Rojas,** *Universidad de Las Américas*
**Ganesh Sable,** *University of Alberta*
**Tuan Samdin,** *Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health*
**Srijani Sarkar,** *National Cancer Institute*
**George Saunders,** *University of Toronto*
**Sunit Singh,** *Philadelphia College of Osteopathic Medicine*
**Mirat Sojitra,** *University of Alberta*
**Paul Spaltenstein,** *University of Utah*
**Christian Stieger,** *FMP Berlin*
**Sven Ullrich,** *Australian National University*
**Alexis Verwoert,** *Philadelphia College of Osteopathic Medicine*
**Xiaozheng Wei,** *University of Montreal*
**Hope Weyrick,** *Dordt University*
**Matthew Whittaker,** *University of Georgia*
**Kejia Yan,** *University of Alberta*
**Hailey Young,** *North Carolina State University*

27th American Peptide Symposium

# TRAVEL AWARD RECIPIENTS

## APS Travel Awards

Zoe Adams ..... Scripps Research- CA, USA
Dorien Aerssens ..... Universiteit Gent, Belgium
Mónica Aróstica Paez ..... PUCV and USM, Chile
Taysir Bader ..... University of Minnesota USA
Krista Barbour ..... University of Georgia, USA
Maxwell Bowles..... North Carolina State University, USA
Christopher Cain ..... University of Notre Dame USA
Francois Charih ..... Carleton University, Canada
Brian Choi ..... Princeton University, USA
Caroline Correa-Almeida ..... Federal University of Rio de Janeiro, Brazil
Carolynn Davern..... Universisty of North Carolina, USA
Rahul Deb ..... Masaryk University,Czech Republic
Stepan Denisov ..... Maastricht & Cambridge Universities, UK
Andrew M. Encinas..... Purdue University, USA
Juan J. Esteban ..... University of Western Ontario, Canada
Georgina Girt ..... The Rosalind Franklin Institute
Olamilekan Ibukun ..... Indian Institute of Science Education and Research
Corey Johnson ..... Purdue University, USA
Chelsea Jones..... University of California, Irvine, USA
Prasanjeet Kaur ..... Indian Institute of Technology, Delhi, India
Bimal Koirala ..... The Rockefeller University, USA
Jonathan Kwok ..... New York University, USA
Tess D. Lamer ..... University of Alberta, Canada
Muralikrishna Lella..... University of Nevada, Reno, USA
Chloe Mitchell ..... The Hospital for Sick Children, Canada
Danielle Morgan ..... University of Glasgow, United Kingdom
Pankhuri Narula ..... Indian Institute of Technology, Delhi, India
Alexander Lund Nielsen ..... Ecole Polytechnique Federale de Lausanne (EPFL), Switzerland
Mark Nolan ..... Trinity College Dublin, Ireland
Anna Rediger..... Dordt University, USA
Marcey Robins ..... University of Utah, USA
George Saunders ..... University of Toronto
Sudha Shankar ..... University of Glasgow, United Kingdom
Sunit Singh ..... Philadelphia College of Osteopathic Medicine
Christian Stieger ..... FMP Berlin, Germany
Sven Ullrich ..... Australian National University, Australia
Alexis Verwoert ..... Philadelphia College of Osteopathic Medicine, USA
Xiaozheng Wei ..... Universite de Montreal, Canada
Krista Wilson ..... Wingate University, USA
Kejia Yan ..... University of Alberta, Canada
Francine Yanchik-Slade..... University of Rochester, USA

# GENERAL INFORMATION

## On-site Registration/ Information Desk

(Grand Foyer)

**Registration Hours**

| | |
|---|---|
| Saturday | 01:00 pm – 06:00 pm |
| Sunday | 07:30 am – 04:30 pm |
| Monday | 07:30 am – 04:30 pm |
| Tuesday | 07:30 am – 12:30 pm |
| Wednesday | 07:30 am – 04:00 pm |
| Thursday | 07:30 am – 10:30 am |

## POSTER INFORMATION

This year we have two poster sessions in the Grand Foyer.

Both poster groups are encouraged to set up their posters on Sunday morning at 8:00am.

### SESSIONS ONE & TWO

**Set-up:** Prefer **ALL** posters to be set up on Sunday, 08:00 am but *NO LATER than* 8:00 am on the day before your session.

#### Session One

- Posters defend
  Monday, 06:30 pm – 08:30 pm

#### Session Two

- Posters defend
  Wednesday, 06:30 pm – 08:30 pm

**NOTE: All posters must be removed at 8:30 pm on Wednesday**

## Exhibit Information

**Set-up:**

Saturday 01:00 pm – 06:00 pm

**Exhibit Hours:**

| | |
|---|---|
| Saturday | 07:00 pm – 10:00 pm |
| Sunday | 08:00 am – 05:30 pm |
| Monday | 08:00 am – 08:00 pm |
| Tuesday | 08:00 am – 12:50 pm |
| Wednesday | 08:00 am – 08:00 pm |

**Exhibit Teardown:**

Wednesday 08:00 pm

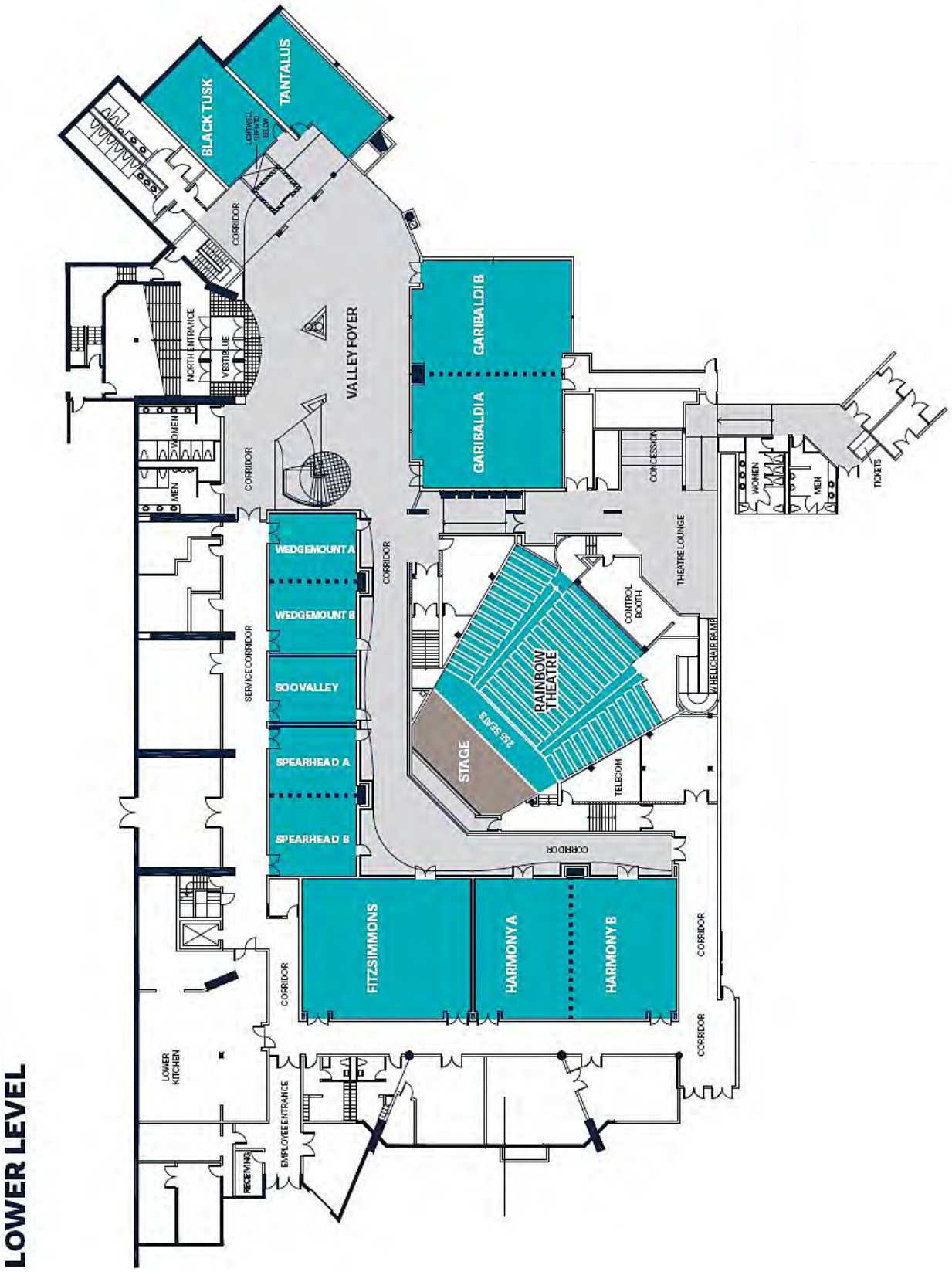
## NAME BADGES

Names badges are your 'ticket' to lectures, poster sessions, exhibits and social events. For security and administrative purposes please wear your name badge in a visible manner to all Symposium functions.

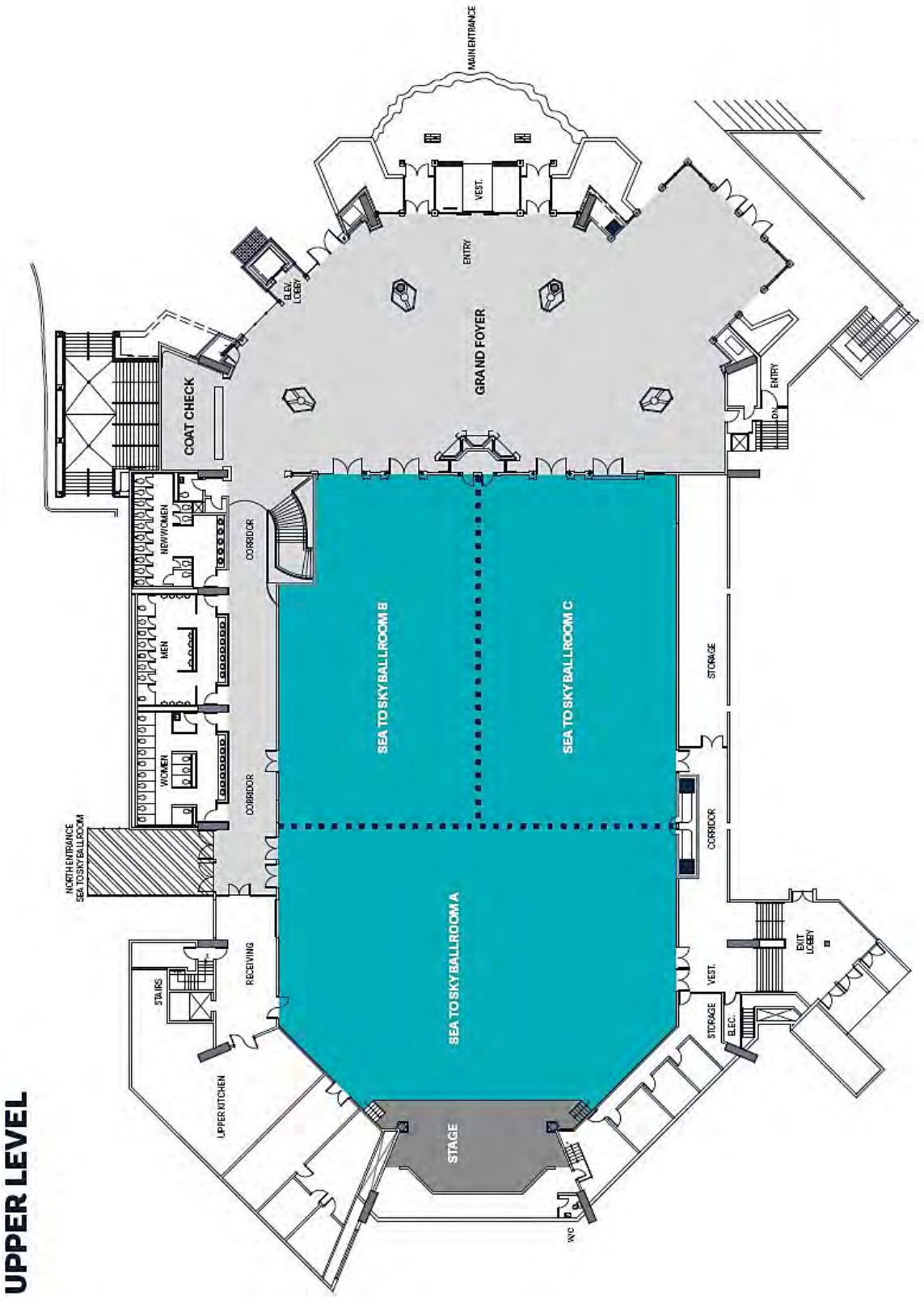
## INTERNET ACCESS

The Whistler Conference Centre is providing APS with complimentary wireless internet access throughout the facility.

# CONFERENCE CENTER LOWER FLOOR



# CONFERENCE CENTER UPPER FLOOR MAP



# LECTURE ABSTRACTS

## L01 mRNA as Medicine

Melissa Moore
*Moderna*

A new class of drugs wherein cells are programmed with synthetic messenger RNAs (mRNAs) to make any desired protein (e.g., cytoplasmic, intraorganelle, membrane-bound, secreted) is an emergent technology with tremendous promise. The ability to simultaneously deliver multiple mRNAs species enables production of multiprotein complexes in their native state. mRNA therapeutics already in or soon to enter the clinic include mRNA-based vaccines (both prophylactic and therapeutic), pro-inflammatory cytokines as anticancer agents, an angiogenic factor for blood vessel regrowth in damaged heart muscle, and protein replacement therapies for treatment of metabolic diseases. Nonetheless, how to combat mRNA's inherent chemical and biological lability, how to direct therapeutic mRNAs to desired cell types, and how to enable repeat dosing without eliciting adverse immune reactions remain challenges for the field. I will discuss recent progress at Moderna in overcoming these challenges, with particular emphasis on our development of new technologies optimized for functional mRNA delivery.

## L02 Illuminating Chromatin: Chemical Biology Tools Light the Way

Tom W. Muir
*Department of Chemistry, Princeton University, Princeton, NJ 08544, USA*

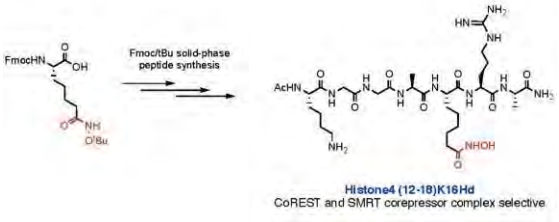
The field of epigenetics has exploded over the last two decades revealing an astonishing level of complexity in the way genetic information is stored and accessed in eukaryotes. This expansion of knowledge, very much ongoing, has been made possible by the availability of ever more sensitive and precise molecular tools, including those grounded in the fields of peptide and protein chemistry. In this presentation, I will discuss the development of new opto-chemical genetic approaches designed to explore spatiotemporal aspects of epigenetic regulation. These methods are helping to expose the remarkable nuances (and vulnerabilities) of epigenetic control mechanisms, providing insights into how these processes become corrupted in disease settings.

## L03 A Hydroxamic Acid-Modified Peptide Library Provides Insight into the Molecular Basis for Substrate Selectivity of HDAC Corepressor Complexes

Lewis J. Archibald,[†] Amit Mahindra,[†] Edward A. Brown,[‡] John W.R. Schwabe[‡] and Andrew G. Jamieson[†]
[‡]*Leicester Institute of Structural and Chemical Biology, Department of Molecular and Cell Biology, University of Leicester, Leicester, LE1 7RH, U.K.*
[†]*School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, UK*

Histone deacetylase (HDAC) enzymes are implicated in several diseases including HIV infection, Alzheimer's and various cancers. Understanding the pathophysiological role of HDACs is of vital importance to drug discovery efforts. Class I HDACs 1, 2 & 3 become catalytically active only when they form multiprotein corepressor complexes, each of which is believed to have a distinct role in the regulation of gene expression. HDACs hydrolyse acetyl groups from a ¤-amine of specific histone-tail lysine residues. However, the mechanism bywhich HDAC corepressor complexes achieve this specificity is currently poorly understood.

In this work we developed novel chemical tool peptides to explore the origin of HDAC corepressor complex substrate selectivity. First an efficient chemical synthesis of Fmoc amino acids having zinc-binding groups was developed.[1] A library of acetyllysine-containing histone tail substrate peptides and hydroxamic acid-containing inhibitor peptides were prepared by solid-phase synthesis. The activity of these peptides was assessed against the full range of class I HDAC corepressor complexes.[2] The data obtained provides strong evidence that site-specific HDAC corepressor complex activity is driven by recognition of the primary amino acid sequence surrounding a particular histone tail lysine site. This information was used to develop HDAC corepressor complex selective inhibitors which are promising lead compounds for further drug development.



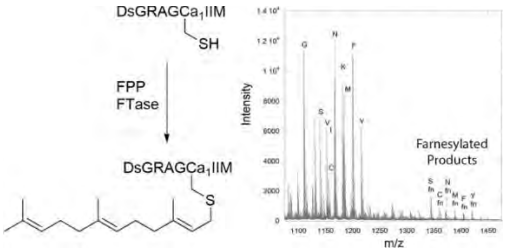

1) Mahindra, A., Millard, C. J., Black, I., Archibald, L. J., Schwabe, J. W.R. and Jamieson, A. G. *Org. Lett.*, **2019**, *21*, 3178-3182.
2) Watson, P. J., Millard, C. J., Riley, A. M., Robertson, N. S., Wright, L. C., Godage, H. Y., Cowley, S. M., Jamieson, A. Potter, B. V.L. and Schwabe, J. W.R. *Nat. Commun.*, **2016**, *7*, 11262.

## L04-YI1 Expanding the Scope of Extended Farnesyltransferase Substrates with a MALDI/MS Screening Approach

G.L. Schey[1], P.H. Buttery[1], E.R. Hildebrandt[2], H.A. Passetti[1], S.X.H. Novak[3], J.L. Hougland[3], W.K. Schmidt[2], and M.D. Distefano[1] [1]*Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota USA.* [2]*Department of Cellular Biology, University of Georgia, Athens, Georgia USA.* [3]*Department of Chemistry, Syracuse University, Syracuse, New York USA.*

Protein prenylation is a post-translational modification where a 15 carbon farnesyl or 20 carbon geranylgeranyl isoprenoid is appended to the C terminal end of a protein by either farnesyltransferase (FTase) or geranylgeranyl transferase type 1, respectively. In the canonical understanding of FTase, the isoprenoids are attached to the Cysteine residue of a four amino acid CaaX box sequence. However, recent work has shown that five amino acid sequences can be recognized, such as the pentapeptide CMIIM. This new discovery greatly increases the number of potential FTase substrates, as the enzyme is already known to tolerate a wide variety of amino acids in the canonical CaaX box. With the goal of developing a more rapid and methodical method to evaluate potential substrates, we envisioned using MALDI to assay libraries of 10 peptides at a time, varying one amino acid in the CaaaX box to all 20 canonical amino acids over two libraries, utilizing both yeast and rat FTase. Through this method we observed over 30 hits in the mass spectrum and chose eleven for further evaluation. Nine of these sequences are novel substrates for FTase, with several meeting or surpassing the *in vitro* efficiency of the benchmark sequence CMIIM. Additionally, *in vivo* experiments in yeast demonstrate that proteins

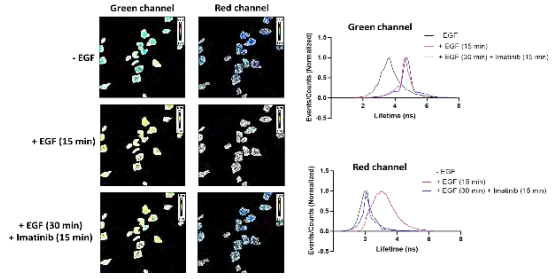
bearing these sequences can be efficiently prenylated in a biological context. Searching the human genome for pentapeptide CaaaX sequences found several hits that prenylated with similar efficiency to a native CaaX sequence, raising the possibility of relevance of these sequences in humans.




L05 Real-time Kinase Assays In Vitro and In Cells using Fluorophore-labeled Peptides

S. Jena, S. Allendorf, J.L. Heier, L.L. Parker
*Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA*

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. Most assays to detect kinase activity are either endpoint- based and/or require lysis of cells (which loses contextual information), or require genetically encoded sensors in engineered cellular systems. We have used synthetic, fluorophore-labeled kinase substrate sequences linked to cell penetrating peptides to deliver peptide-based biosensors to live cells and map more than one subcellular kinase activity at a time via fluorescence lifetime imaging microscopy.[1,2] We have also adapted these approaches for higher throughput using a fluorescence lifetime plate reader for bulk cell suspension analysis. In related systems, we have used the phosphopeptide-SH2 interaction as a sensor for kinase activity detection by fluorescence polarization in a real-time in vitro assay. Overall, we show how synthetic peptides can be used in vitro and in cells to detect dynamic kinase activity

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**L06 Learning the Rules for Selective Protein Glycation**

Rebecca A. Scheck
*Department of Chemistry, Tufts University, Medford MA, 02155*

Research in the Scheck laboratory focuses on understanding protein post-translational modifications (PTMs) that have been difficult to study using traditional tools. Glycation is a PTM that occurs spontaneously and without an enzyme, yet it is known to occur selectively at certain sites on certain proteins. We have uncovered molecular features that govern selective glycation, and we are now using this knowledge to develop new methods that predictably modulate glycation outcomes in living cells. This approach is uniquely suited to explore the biology of glycation by enabling the rigorous study of glycation as a functional PTM.

**L07 Peptide Conformational Engineering using Rigid, Low Molecular Weight Linkers**

Phil Dawson
*The Scripps Research Institute*

The manipulation of peptide structure through macrocyclization has become established as a powerful approach for the development of potent peptide based ligands and inhibitors. We have also developed a robust approach for the introduction of diyne linkers into peptides using ligand optimization to promote the Glaser reaction. The resulting rigid, linear 7 Å linkage is highly effective for linking macromolecules. In addition, when performed in an intramolecular context, diyne linkers can be utilized as tethers or as staples to stabilize alpha helical structures. Importantly, highly strained diyne macrocycles have been synthesized that fully extend the peptide backbone, providing exceptional mimics of beta strand structures. The synthesis, structure and application of diynyl-peptides will be discussed.

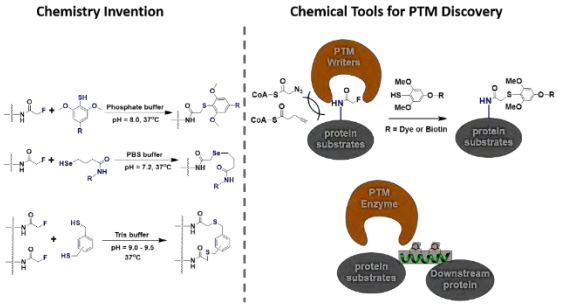
**L08 Fluorine-Displacement Based Probes to Interrogate Novel Protein-Protein Interactions**

Rongsheng (Ross) E. Wang
*Department of Chemistry, Temple University, Philadelphia, PA*

The dysregulation of post-translational modifications (PTMs) has been closely related to the onset and relapse of human diseases.[1] Yet, many PTM-related non-histone proteins remain to be elucidated in terms of their identity, functions, and roles in cellular activities simply due to the lack of tools for characterization.[2] Despite the development of bioorthogonal chemical reactions such as "click chemistry", few research programs have explored protein labeling or tagging with reduced steric. Towards this end, my group has invented a steric-free bioorthogonal reaction (fluorine-thiol displacement (FTDR))[1] and has

developed a novel class of FTDR-based imaging and proteomics probes aimed at a complete dissection of substrate proteins of acetylation that are featured in diseased cells such as cancer cell lines; which for now cannot be systematically profiled due to steric limitations in the current chemical proteomics approach.[1]

Concurrently, to facilitate the studying and targeting of any new protein-protein interactions (PPIs) to be revealed by the aforementioned research investigations in PTM signaling, we also exploited other tool probes[3-4] such as peptide stapling[4] based on the FTDR reaction. The resulting peptides possessed improved folding, stability, and on-target affinity, but also displayed enhanced cell penetration than the peptides stapled by traditionally used ring-closing metathesis.[4]

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## L09 Chemical Approaches to Identify Cell-Cell Signaling Peptide Receptors

Sheryl Sharma, James Checco
*Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588, USA*
*Nebraska Center for Integrated Biomolecular Communication, University of Nebraska-Lincoln, Lincoln, NE 68588, USA*

Small- to medium-sized peptides can act as signaling molecules to carry out complex tasks in living systems, and dysregulation of these signaling events often leads to disease. Characterizing the active forms and molecular-level interactions of endogenous neuropeptides and peptide hormones represents a significant goal to understand how living systems carry out physiological processes (e.g., responding to stress, regulating bodily functions, learning), and may help identify novel therapeutic targets. Understanding cellular communication requires not only knowledge of the transmitter (i.e., the peptide ligand), but also information regarding the cognate receptor that mediates signaling on the partner cell. However, there exist a relatively large number of biologically active peptides whose cell-surface receptor(s) are not known, primarily because of a lack of techniques to reliably identify peptide receptors de novo. Our group is developing new techniques to identify these receptors to better understand the molecular mechanisms of cellular communication. Our research combines approaches from chemical biology, synthetic chemistry, and bioanalytical chemistry to advance our understanding of specific cell-cell signaling pathways, identify new pathways for further exploration, and provide innovative starting points for future therapeutics.

## L10 Chemical Tools for Selective Detection of Methyl Lysine PTMs

Ogonna Nwajiobi, Benjamin Emenike and Monika Raj
*Department of Chemistry, Emory University, Atlanta GA 30322, USA*

Selective modification of biomolecules provides scientists with an effective tool for a multitude of bioanalytical, therapeutic, biological and bioengineering applications. However, chemical strategies that can target a particular functional group at a single site in the presence of reactive amino acid side chains on protein surfaces are limited. We have developed multiple bioconjugation approaches for the selective labeling of proteins containing mono- and di-methyl lysine posttranslational modifications (PTMs).[1-2] This method does not require any genetic engineering of the protein target and protection of the side chains of other amino acids. The resulting bioconjugation reactions lead to the formation of a highly stable bond at the site of the methyl lysine PTMs. The broad utility of these bioconjugation reactions is demonstrated by the conjugation of various affinity probes and fluorophores on methyl lysine PTMs. The dysregulation of mono- and di-methyl lysine PTMs has been linked to a variety of different biological malfunctions, yet the chemical methods for selective detection of these methyl lysine PTMs are still lacking. These selective tagging methodologies can effectively detect mono- and di-methyl lysine PTMs thus have the potential to further our understanding of the role of methylated lysine containing PTMs in regulating various cellular signaling processes and aid in biomarker discovery.

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## L11 Squeezing through the Junctions: From mRNA Display to an Oral Cyclic Peptide PCSK9 Inhibitor

Yuhua Huang
*Merck & Co. Inc.*

Proprotein convertase subtilisin-like/kexin type 9 (PCSK9) is a key regulator of plasma LDL-cholesterol (LDL-C) and a clinically validated target for the treatment of

hypercholesterolemia and coronary artery disease. A series of novel cyclic peptide hits derived from an mRNA display screen were optimized to achieve picomolar level binding peptides through stabilization and rigidificaton of the structure driven by structure-based drug design approach. After metabolic stabilization of both protease degradation and transportater mediated hepatic uptake, optimized molecules demonstrated sufficient oral bioavailability to maintain therapeutic levels in rats and cynomolgus monkeys after dosing with an enabled formulation. We demonstrated target engagement and LDL lowering in cynomolgus monkeys essentially identical to those observed with the clinically approved, parenterally dosed antibodies. These molecules represent the first report of highly potent and orally bioavailable macrocyclic peptide PCSK9 inhibitors with overall profiles favorable for potential development as once-daily oral lipid-lowering agents.

In this talk, we detail the design criteria and multiparameter optimization of this novel series of PCSK9 inhibitors.

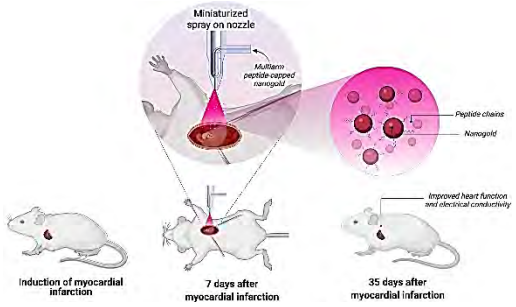
### L12 Next Generation Peptide Therapeutics for Cellular Re-programming

Rami N. Hannoush, Ph.D.
*Chief Scientific Officer (a 'Stealth' Company) and former Senior Group Leader, Genentech*

This talk will describe our efforts in discovering and optimizing peptide-based scaffolds and will highlight some of the challenges and novel technologies for peptide lead identification and development. A few case studies will be presented, including the reprogramming of stem cells in the context of cellular regeneration.

### L13-YI2 Nano-spray Gold-peptide Therapy for Heart Attack Treatment

Marcelo Muñoz[1]†, Cagla Eren Cimenci[1,2†], Keshav Goel[1], Maxime Comtois-Bona[1], Mahir Hossain[1], Christopher McTiernan1, Matias Zuñiga-Bustos[3], Alex Ross[1], Brenda Truong[1], Darryl R. Davis[4,5], Wenbin Liang[4,5], Benjamin Rotstein[6,7], Marc Ruel[1,2], Horacio Poblete[3,8], Erik J. Suuronen[1,2*]& Emilio I. Alarcon[1,7*]
*[1]BEaTS Research, Division of Cardiac Surgery, University of Ottawa Heart Institute, Ottawa, K1Y 4W7, Ontario, Canada. [2]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, K1H 8M5, Ontario, Canada. [3]Departamento de Bioinformática, Centro de Bioinformática, Simulación y Modelado (CBSM), Facultad de Ingeniería, Universidad de Talca, Campus Talca, 2 Norte 685, Talca, Chile. [4]University of Ottawa Heart Institute, Division of Cardiology, Department of Medicine, University of Ottawa, Ottawa, Ontario K1Y 4W7, Canada. [5]University of Ottawa Heart Institute, Cardiac Electrophysiology Lab, University of Ottawa, Ottawa, Ontario K1Y 4W7, Canada. [6]Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, K1H 8M5, Ontario, Canada. [7]Molecular Imaging Probes and Radiochemistry Laboratory, University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, ON, K1Y4W7, Canada. [8]Millennium Nucleus of Ion Channels- Associated Diseases (MiNICAD), Universidad de Talca, 2 Norte 685, Talca, Chile. †These authors contributed equally: Marcelo Muñoz and Cagla E. Cimenci.*We report the development, as well as the in vitro and in vivo testing, of a sprayable nano-therapeutic that uses surface engineered custom designed multi-armed peptide grafted nanogold for on-the-spot coating of infarcted myocardial surface. When applied to mouse hearts, 1- week after infarction, the spray-on treatment resulted in an increase in cardiac function (2.4-fold), muscle contractility, and myocardial electrical conductivity. The applied nanogold remaining at the treatment site 28 days post application with no off-target organ infiltration. Further, infarct size in the mice that received treatment was found to be <10% of the total left ventricle area, while the number of blood vessels, pro-healing macrophages, and cardiomyocytes increased to levels comparable to that of a healthy animal. Our cumulative data suggests that the therapeutic action of our spray-on nanotherapeutic is highly effective and in practice its application is simpler than other regenerative approaches for treating the infarcted heart.[1]

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### L14 Semaglutide; From GLP-1 to Patient

Brian Finan
*Novo Nordisk Research Center Seattle*

The presentation will provide an overview of the journey from discovery to clinic for the semaglutide molecule. The biology of GLP-1, including incretin action and food intake effects will be presented. The semaglutide molecule and mechanism of action will be described as well as the major development activities & milestones, including highlights from the Semaglutide Treatment Effect in People with obesity Programme (STEP) clinical studies.

### L15 Targeting Triple-negative Breast Cancer (Tnbc) and Melanoma with Small Peptide Ligands

Kamaljit Kaur
*Chapman University School of Pharmacy, Harry and Diane Rinker Health Science Campus, Chapman University, Irvine, CA, 92618, USA*

Conventional chemotherapy remains the treatment of choice for many cancers; however, it is constantly challenged by poor selectivity and limited access of drugs to the cancer cells. Targeted drug delivery methods have been explored to improve drug efficacy and selectivity by directing the drug to the cancer site. In recent years, a number of peptides have been identified for delivering drugs and diagnostic elements specifically to the cancer

site. Using peptide array-whole cell binding assay, we have identified several peptides which selectively bind melanoma or triple-negative breast cancer (TNBC) cells. One peptide we identified targets a novel cell-surface receptor, Keratin 1, in TNBC cells. These peptides are either conjugated to chemotherapeutics or co-administered to enhance the cytotoxicity and efficacy of chemotherapy for TNBC or melanoma treatment. Targeting TNBC and melanoma is important because these are more aggressive cancer types, and there are limited treatment options for patients with these cancers. Chemotherapy is the mainstay of treatment for TNBC, while melanoma is notoriously resistant to chemotherapy. Here we will present our results for the design and evaluation of cancer cell targeting peptides, the target receptor in cancer cells, and efficacy of peptide-drug conjugates or co-treatment using cell and mice xenograft models.

### L16 Constraining Peptide Conformations: Finding Aib and Beyond

Padmanabhan Balaram
*Molecular Biophysics Unit, Indian Institute of Science and National Centre for Biological Sciences Bangalore, India*

The report by Mueller and Rudin (1969) that alamethicin, a cyclic peptide, induces voltage-dependent ion channel formation in artificial lipid bilayers, sparked interest in peptides of fungal origin, rich in alpha aminioisobutyric acid (Aib, 2-methylalanine). The structure of alamethicin was corrected in 1976 to reveal an acyclic sequence, rich in Aib, blocked at both N and C-termini. An endeavour to produce alamethicin by chemical synthesis, soon unveiled the unusual and attractive properties of peptides containing Aib.

The alamethicin N-terminal tetrapeptide was quickly reached by solution phase chemistry. Surprisingly, crystals appeared almost instantly after purification. Diffraction photographs, then painstakingly measured, seemed promising enough to move even further forward towards structure determination. Direct methods of phase determination, still in their infancy, revealed a folded structure with two internal 10-atom CO—HN hydrogen bonds, launching the peptide $3_{10}$-helix on its way

The backbone conformational constraints imposed by $C^{\alpha}$ alkylation provided an easy route to helix design and to restrict conformational excursions in biologically active peptides. The growing understanding of polypeptide chain folding in proteins suggested that D-Pro induced prime turns would nucleate ß-hairpins in designed peptides Attempts to probe helix stability by insertion of the backbone expanded ß and γ residues uncovered the wealth of hydrogen bonded folded structures in hybrid sequences, even as the field of ß and γ peptides was established by Seebach and Gellman. This lecture traces the journey taken at Bangalore form the mid-1970s on promoting the use of non-canonical amino acids in peptide design.

### L17 Studies toward Protein Conjugation via Reversible Covalent Sigma Bonds

Qiang Zhang
*State University of New York, Albany*

The site-selective conjugation of a native peptide or protein is arguably the most efficient technologies to functionalize biologics. Although the biological approaches such as enzymatically amend proteins demonstrate high selectivity and capacity. Utilization of sophisticate cell machinery system prevent biological method from rapid, large scale production of modified proteins. Chemical approaches are naturally attractive attribute to their low operational cost and simpler system, yet often suffer from poor chemoselectivity. The development of chemical technology that allows site-specific functionalization of a native protein is critical. We report the synthetic methodologies that achieve chemoselective activation of a peptide or protein C-terminus or N-terminus, which enables subsequent head to tail cyclization to provide cyclic peptide and proteins under physiological conditions. Realize controllable, site-specifically protein bioconjugation at N-termini in a reversible manner.

### L18 Chemoselective Modification of Cysteine and Selenocysteine in Peptides and Proteins

Norman Metanis
*The Institute of Chemistry, The Center for Nanoscience and Nanotechnology, Casali Center for Applied Chemistry The Hebrew University of Jerusalem, Jerusalem 9190401, Israel*

Chemoselective modification of peptides and proteins has wide applications in chemical biology and pharmaceutical development. We report an efficient chemo- and stereoselective cysteine (Cys) functionalization protocol via rationally designed *ß*-addition of ynamides. A substituent of the terminal ynamides offers a handle for functionality diversification. This Cys modification with ynamides proceeds efficiently in a slightly basic aqueous media (pH 8) to provide a series of *Z*- isomer of the corresponding conjugated products with excellent stereoselectivity (> 99%) and superior stability. All the reactive peptide side chain functional groups such as amino, carboxyl, primary amide, and hydroxyl groups, as well as the unprotected imidazole and indole rings are compatible. This method displays a broad substrates scope including linear and cyclic peptides and proteins. The potential application of this method in peptide and protein chemical biology was exemplified by Cys-bioconjugation with ynamides containing different functional molecules, including drug, fluorescent and affinity tags. In addition, this strategy is also compatible with click chemistry (performed in one pot), which remarkably extends the toolbox for further applications. The development of new efficient, chemoselective bioconjugation tools for reactive functional groups is highly desired, especially those that are chemoselective even in the presence of free Cys residues, or disulfides. Herein, we report the chemoselective modification of peptides and proteins via a reaction between selenocysteine residues and aryl/alkyl radicals. *In situ* radical generation from hydrazine substrates and copper ions proceeds rapidly in an aqueous buffer at near

neutral pH (5–8), providing a variety of Se-modified linear and cyclic peptides and proteins conjugated to aryl and alkyl molecules, and to affinity label tag (biotin).

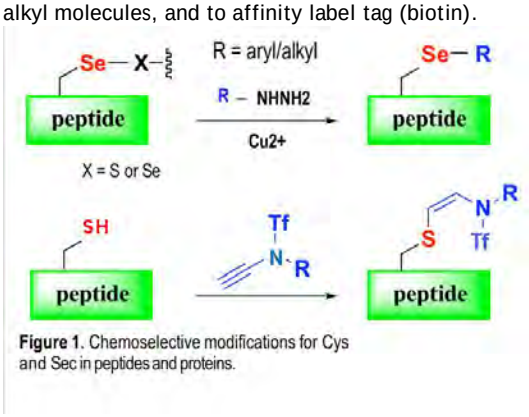


**Figure 1.** Chemoselective modifications for Cys and Sec in peptides and proteins.

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### L19-YI3 A Survey of Stapling Methods to Increase Affinity, Activity, and Stability of Ghrelin Analogues

Juan J. Esteban[1,2], Rithwik Ramachandran[3], Leonard G. Luyt[1,2,4]
*[1]Department of Chemistry, University of Western Ontario, London, ON Canada; [2]London Regional Cancer Program, Lawson Health Research Institute, London, ON Canada; [3]Department of Physiology and Pharmacology, University of Western Ontario, London, ON, Canada; [4]Department of Oncology, University of Western Ontario, London, ON Canada*

The growth hormone secretagogue receptor (GHSR) is a G protein-coupled receptor which regulates various important physiological and pathophysiological processes in the body. The 28 amino acid peptide ghrelin is the primary high affinity endogenous ligand for GHSR and has been used as a structural template for the development of therapeutic and imaging agents. Linear peptides, like ghrelin, have limited secondary structure in solution and are often proteolytically unstable. This inherent instability in ghrelin like peptides can be overcome by incorporating helix- inducing staples that stabilize their structure and improve affinity. We present an analysis of different stapling methods at positions 12 and 16 of ghrelin(1-20) analogues, a previously identified optimal staple location[1], with the goal of increasing proteolytic stability and to retain or improve affinity towards GHSR. Ghrelin(1-20) analogues were modified with a wide range of chemical staples, including a lactam staple, triazole staple, Glaser staple, bis-thioether staple, and hydrocarbon staple. Once synthesized, the analogue affinity and alpha-helicity were measured using competitive binding assays and circular dichroism spectroscopy, respectively. Generally, an increase in alpha-helicity using a flexible staple linker led to an improved affinity towards GHSR. Ghrelin(1-20) analogues with a lactam and a triazole staple resulted in helical analogues (26% and 35% helical character) with stronger affinity towards GHSR (IC50 = 0.94 nM and 1.65 nM) than unstapled ghrelin(1-20), a compound that lacks helical character. Compounds were also investigated for their agonist activity through ß-arrestin 1 & 2 recruitment BRET assays. Incorporating these staples into the structure of ghrelin(1-20) can provide the required stability and affinity to make a robust therapeutic or imaging agent targeting GHSR.

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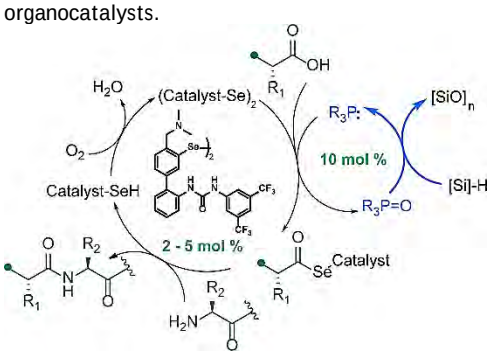
### L20 Rational Design of an Organocatalyst for Peptide Bond Formation

Handoko, Nihar R. Panigrahi, and Paramjit S. Arora
*Department of Chemistry, New York University, 100 Washington Square East, New York, NY 10003, USA*

Amide bonds are ubiquitous in peptides, proteins, pharmaceuticals, and polymers. The formation of amide bonds is a straightforward process: amide bonds can be synthesized with relative ease because of the availability of efficient coupling agents. However, there is a substantive need for methods that do not require excess reagents. A catalyst that condenses amino acids could have an important impact by reducing the significant waste generated during peptide synthesis. This presentation will discuss the rational design of a biomimetic catalyst that can efficiently couple amino acids featuring standard protecting groups. The catalyst design combines lessons learned from enzymes, peptide biosynthesis, and organocatalysts.

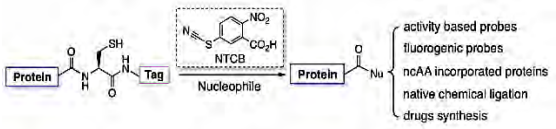



### L21 Expressed Protein Ligation without Intein

Yuchen Qiao and Wenshe Ray Liu
*Department of Chemistry, Texas A&M University, College Station, TX 77843-3255, USA*
*wliu@chem.tamu.edu*

Proteins with a functionalized *C*-terminus such as a *C*-terminal thioester are key to the synthesis of larger proteins via expressed protein ligation. They are usually made by recombinant fusion to intein. Although powerful, the intein fusion approach suffers from premature hydrolysis and low compatibility with denatured conditions. To totally bypass the involvement of an enzyme for expressed protein ligation, here we showed that a cysteine in a recombinant protein was chemically activated by a small molecule cyanylating reagent at its *N*-side amide for undergoing nucleophilic acyl substitution with amines

including a number of L- and D-amino acids and hydrazine. The afforded protein hydrazides could be used further for expressed protein ligation. We demonstrated the versatility of this activated cysteine-directed protein ligation (ACPL) approach with the successful synthesis of ubiquitin conjugates, ubiquitin-like protein conjugates, histone H2A with a *C*-terminal posttranslational modification, RNAse H that actively hydrolyzed RNA, and exenatide that is a commercial therapeutic peptide. The technique, which is exceedingly simple but highly useful, expands to a great extent the synthetic capacity of protein chemistry and will therefore make a large avenue of new research possible.[1-2]

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### L22 Development of Peptide-Based Tools to Study Cell-Cell Signaling in Bacteria

Yftah Tal-Gan
*Department of Chemistry, University of Nevada, Reno, 1664 N. Virginia Street, Reno, NV, 89557, USA*

Quorum sensing (QS) is a cell density-based communication mechanism that bacteria utilize to regulate a variety of symbiotic and pathogenic phenotypes. As such, QS has attracted significant attention as a potential anti-virulence therapeutic target. Gram-positive bacterial species utilize peptides, termed autoinducers, as the signaling molecules for QS. We aim to develop autoinducer-based QS modulators that would be applied to study different Gram-positive bacterial species, with an emphasis on streptococci, investigate the molecular mechanisms that drive signal peptide-receptor binding and lead to QS activation, and interrogate the role of QS in bacterial virulence and inter-species competition.

### L23 Coming Full Circle: Peptide-conjugates for Intracellular Protein and Antibody Delivery

C.P.R. Hackenberger
*Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Robert-Roessle-Str. 10, 13125 Berlin, Germany*
*Humboldt Universität zu Berlin, Institut für Chemie, Brook-Taylor-Str. 2, 12489 Berlin, Germany*

Our lab aims to identify new chemoselective reactions for the synthesis and modification of functional peptides and proteins. We apply these highly selective organic reactions to study functional consequences of naturally occurring posttranslational protein modifications (PTMs) as well as to generate novel peptide- and protein-conjugates for pharmaceutical and medicinal applications.

In this presentation, I will focus on the delivery of semisynthetic protein- and antibody-conjugates into living cells. Here, we employ cylic as well as linear cell-penetrating peptides (CPPs) either to furnish cell-permeable protein conjugates [1] or to enhance cellular uptake in the form of cell-surface bound CPP-additives [2]. For protein modification, we use a combined approach of intein expression as well as recently developed bioorthogonal reactions and enzymatic ligations, for instance the so-called Tub-tag® labeling [2]. We apply this concept to generate cell-permeable nanobodies [3], i.e. small antigen binding proteins that remain active within the reductive milieu inside living cells, as well as the subcellular targeting of protein conjugates and antibodies [4].

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### L24 Synthetic Mucins by NCA Polymerization

Jessica R. Kramer, Ph.D.
*Department of Biomedical Engineering, University of Utah*

Mucin glycoproteins are the major component of mucus. Mucus is essential for life and serves as a physical barrier to hydrate, lubricate, and protect tissues. There are 20+ mucin genes with variable expression patterns, splicing, and post-translational glycosylation that result in structures with discrete biochemical functions. Mucins play roles in infection, immunity, inflammation and cancer. Such diversity has challenged study of structure-function relationships. The Kramer lab is developing scalable methods, based on polymerization of amino acid *N*-carboxyanhydrides, to synthesize glycoproteins that capture the chemical and physical properties of native mucins. We are utilizing these synthetic mucins to engineer the glycocalyx of live cells to shed light on the role of glycans in health and disease. Areas of focus for our lab are progression of epithelial cancers, and infection processes in cystic fibrosis and COVID-19.

### L25 Development of Endosomal Escape Vehicles to Enhance the Intracellular Delivery of Oligonucleotides

Patrick G. Dougherty, Xiang Li, Mahboubeh Kheirabadi, Ajay Kumar, Xiulong Shen, Amy Hicks, Kimberli J. Kamer, Suresh Peddigari, Nelsa L. Estrella, Nanjun Liu, Matthew Streeter, Chance Brandt, Wenlong Lian, Christine Rondeau Waters, Tyler Ironside, Meagan Mycroft, Roshni Mukundan, Vyoma Patel, Phallika Mon, Arianna Bonilla,

Andy Stadheim, Mahasweta Girgenrath, Mohanraj Dhanabal, Natarajan Sethuraman, and Ziqing Qian
*Entrada Therapeutics, Boston, USA*

Biological therapeutics exhibit high target specificity and potency but are limited in their ability to reach intracellular targets of interest. These limitations often necessitate high therapeutic doses and can be associated with less-than-optimal therapeutic activity. One promising solution for the intracellular delivery of biologics is through the use of cell-penetrating peptides (CPPs). However, canonical CPPs are limited by relatively low efficiencies of cellular uptake and endosomal escape, minimal proteolytic stability, and toxicity. To overcome these limitations, we have designed a family of proprietary cyclic CPPs that form the core of our Endosomal Escape Vehicle (EEV™) technology, which is capable of delivering covalently conjugated cargo across all tissue types. To demonstrate the utility of our platform, we employed our EEV technology for the delivery of splice-modulating oligonucleotides and evaluated the EEV-oligonucleotide conjugates in preclinical models of Duchenne muscular dystrophy (DMD). EEV-oligonucleotide conjugates demonstrated durable exon skipping and broad dystrophin protein expression in target tissues including skeletal and cardiac muscles. These results suggest the significant therapeutic potential of our EEV-oligonucleotides for neuromuscular diseases, as well as the broader application of our EEV platform for the delivery of intracellular therapeutics.

## L26 DiaCyt: A Platform Technology for the Discovery of Molecular Transport Systems for Delivery to the Central Nervous System

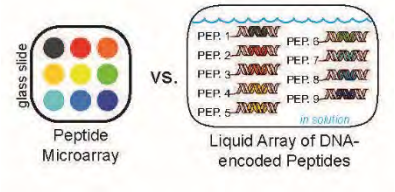
Kathlynn C. Brown, Indu Venugopal, Michael J McGuire, Amanda Powell, Weiliang Xu, Morgan Delong, Yitong Li, John Marafino and Anurag Vyas.
*SRI International, Biosciences Division*

The blood brain barrier (BBB) is a set of specialized and highly selective cellular barriers that protects the central nervous system (CNS). While necessary under normal physiology, the BBB prevents entry of many neurotherapeutics into the brain and biologic therapies such as antibodies and gene therapies are essentially excluded from the CNS. We have developed an unbiased screening platform to identify peptides that mediate delivery throughout the CNS without disruption of the BBB or destruction of biological cargo. This approach, which we have named DiaCyt (Dia: Through and Cyt: Cell), utilizes high diversity phage-displayed peptide libraries to identify peptides that transport cargos. We have identified 3 peptides as lead candidates. The synthetic peptides mediate transport of a protein in an in vitro model with efficiencies of 8-20%. Tight junctions remain intact during peptide transport. Upon intravenous injection into a rat, each peptide is transported into the CNS and is distributed throughout the ventricular system and within the surrounding parenchyma. Transport efficiency is enhanced 3-11-fold into the CSF and 0-22-fold into the brain. We have also identified cell specific peptides to microglia and neuronal cells, capable of intracellular delivery of cargos. Combining these different peptides allows for both transport and cell specific targeting within the CNS.

## L27 In Vitro Selection of DNA-encoded, Synthetic Peptide Libraries for Development of Selective Protein Ligands and Substrates

C. J. Krusemark, S. Wang, B. Cai, Y. Sun
*Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, 47907, US*

We present the use of DNA-encoded libraries of peptides as an approach for discovery and optimization of protein ligands and substrates. We highlight the benefits of a DNA-based approach over traditional peptide library approaches such as cellulose spot and micro arrays. We have synthesized both combinatorial and scanning positional DNA-encoded libraries of trimethyllysine-containing peptides and peptidomimetics for development of selective inhibitors of the CBX family of chromodomains (ChDs). Additionally, we use collections of DNA-encoded peptide ligands to CBX ChDs as a model system to demonstrate the capabilities of selection assays of DNA- encoded peptides. Statistical analysis of selections assays indicates low DNA tag bias and adequate robustness for both ligand discovery and determination of quantitative structure activity relationships. Further, we show how the application of photoaffinity labeling with DNA- linked peptides enables both massively parallel determination of affinity constants of peptides to the CBX ChDs by DNA sequencing and also evaluation of binding within live mammalian cells. In addition to protein binding, we demonstrate selection approaches for selective enrichment of peptide substrates of protein kinases, proteases, and farnesyltransferases. We concentrate specifically on protein tyrosine kinases and investigate the potential of the approach both for new substrate generation as well as the detection of activities in biological samples.




## L28 Passively Permeable Cyclic Peptide Scaffolds: An Abundance of Diversity

R. Scott Lokey, Grant Koch, Panpan Zhang, Justin Faris, Jaru Taechalertpaisarn
*University of California, Santa Cruz*

Cyclic peptide natural products often exhibit the drug-like properties (e.g., cell permeability and even oral absorption) of classic small molecule drugs while showing antibody-like potency and specificity toward "undruggable" targets such as protein-protein interactions. As we continue to outline the factors that govern passive membrane permeability in cyclic peptides, we've been delighted to discover that existing cyclic peptide natural products represent only a tiny fraction of potentially permeable scaffolds. We have developed a powerful mass-encoded library-based approach toward the discovery of novel, passively permeable scaffolds which has enabled illumination of the chemical space that supports passive

membrane permeability in large (> 9-mer) macrocyclic peptides.

I will discuss our latest investigations into the effect of backbone-side chain interactions to enhance solubility without compromising permeability, as well as the use of DNA-encoded technologies to further enhance scaffold discovery.

### L29-YI4 Chemokine-binding Peptides Development using Mirror-Image Clips Phage Display

Stepan S. Denisov[1], Johannes H. Ippel[1], Eline FijIstra[2], Alexandra C.A. Heinzmann[1], Peter Timmerman[2], Tilman M. Hackeng[1], Ingrid Dijkgraaf[1*], Michael Goldflam[2*]
[1]*Department of Biochemistry, University of Maastricht, Cardiovascular Research Institute Maastricht (CARIM), Universiteitssingel 50, 6229 ER, Maastricht, The Netherlands*
[2]*Pepscan Therapeutics, Zuidersluisweg 2, RC Lelystad, 8243, Netherlands*

Chemokines are secreted signaling proteins involved in the development of atherosclerosis, tumor-associated angiogenesis, and neurodegenerative diseases. Therefore, neutralization of chemokines could offer novel beneficial therapeutic strategies. Although peptides are considered potential chemokine-neutralizing agents, they usually suffer from low chemical and proteolytic stability. Peptide cyclization and incorporation of non-natural amino acids inaccessible to enzymes can prevent peptide proteolysis and immunogenicity, leading to more effective drugs. Here, we generated cyclic D-peptides from a combinatorial library for interleukin-8 (CXCL8) neutralization. First, the D-variant of CXCL8 was synthesized using Boc-SPPS and further used as a target in three rounds of CLIPS phage display selection of the combinatorial library with 10 variable residues. Peptide sequences, obtained by NGS of the enriched phage library, were analyzed and clusterized based on sequence similarity. Subsequently, 100 sequences from different clusters were selected and synthesized in L-form for screening against D-CXCL8 by SPR biosensor analysis. The best performing CLIPS peptides had KD values of ~170 nM, and eventually four sequences were selected for synthesis in the D-form. Peptides with inverted stereochemistry bound only L-CXCL8 and not D-CXCL8. Structural features of D-peptides and their complex with L-CXCL8 have been studied using CD and solution NMR spectroscopy, showing that binding caused disruption of CXCL8 dimer, but didn't affect the receptor binding site of the chemokine. Nevertheless, all peptides effectively blocked CXCL8-induced chemotaxis of human monocytes at μM concentrations. In the future, selected peptides will be subjected to sequence optimization to increase the affinity for CXCL8 and improve physicochemical properties.

### L30 Rational Design & Continuous Evolution of Franken-Proteins: Potential Drugs & Synthetic Biology Tools

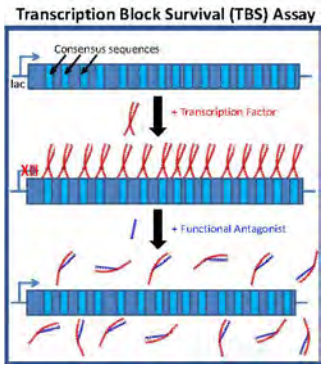
Jumi A Shin
*University of Toronto*

Our transdisciplinary approach borrows from biophysics, molecular biology, and chemistry to explore how nature uses the protein scaffold to target specific DNA sequences. We combine rational design and continuous evolution to develop our DNA-binding minimalist proteins as possiblel drugs against cancer and asthma, and new franken-proteins that can orthogonally target gene circuits in synthetic biology applications. We use our protein design know-how and phage-assisted continuous evolution (PACE) to develop small proteins that target the Myc/Max/E-box network involved in >50% of all cancers. We are now expanding into designing new protein motifs incorporating intrinsically disordered regions that target large DNA sites with utility in synthetic biology.

### L31 Transcription Block Survival: An Intracellular Peptide Library Screening Platform to Derive Functional PPI Antagonists

Jody M Mason
*Department of Biology and Biochemistry, University of Bath, U.K.*

*Transcription Block Survival* (TBS) is a peptide-library screening platform to derive functional transcription factor antagonists within a complex intracellular environment. Our exemplar for the approach is the oncogenic transcriptional regulator cJun, a key component of the Activator Protein-1 (AP-1) system. cJun drives key genes responsible for cell cycle modulation, via TPA-response element (TRE) consensus sequence binding, and is upregulated in numerous cancers. Developing antagonists that bind cJun, but that more importantly ablate function, remains a primary challenge hampering the search for effective molecules. During TBS we exploit this process by introducing fifteen TRE consensus sites directly into the coding region of the essential gene dihydrofolate reductase (DHFR). Introduction of cJun leads to TRE binding, preventing DHFR expression by directly blocking RNAP transcription to abrogate cell growth under selective conditions. TBS proof-of-concept is further demonstrated by an absolute requirement for *i)* the cJun basic domain and *ii)* the presence of TRE sites within DHFR for transcriptional block to occur. Screening a 130,000 member library next identified a sequence that both binds cJun *and* antagonises function as demonstrated by restored cell growth by DHFR transcription. *In vitro* hit validation using CD, ITC and EMSA experiments confirmed high target affinity ($KD$ = 14.4 ± 3.7 nM), importantly demonstrating effective antagonism of the cJun/TRE interaction. TBS is an entirely tag-free genotype-to-phenotype approach, selecting desirable attributes such as high solubility, target specificity and low toxicity within the complex cellular environment. It facilitates rapid library screening to accelerate the identification of therapeutically valuable sequences.



**L32 Blood-Brain Barrier Shuttle Peptides, From Discovery to Applications**

M. Teixidó[1]
[1] *Gate2Brain SL, Baldiri Reixac 4-8, Barcelona, E-08028, Spain*

Gate2Brain shuttle peptides represent salvage for new or previously rejected CNS drug candidates by providing a way to cross the blood-brain barrier (BBB).

Gate2Brain technology consist on a toolbox of peptides able to cross the BBB and carry compounds covalently attached (including small molecules, peptides, proteins, antibodies, plasmids, siRNA or mRNA loaded nanoparticles, etc…) that cannot cross this barrier unaided. They have proofed to carry these cargoes in vitro and in vivo. These peptide shuttles use the existing transport mechanisms at the BBB without affecting the normal functioning of these mechanisms and preserving brain homeostasis.

By improving the delivery of therapeutic candidate to the CNS, we will ensure immediate impact in many CNS diseases patients. In addition, in a broader perspective, Gate2Brain technology may help to repurpose existing therapies previously rejected because of difficulty to reach the brain, accelerating the translation towards clinical development. Gate2Brain will also result in the application of lower concentrations of therapeutic agent, thereby significantly lowering systemic side effects and reducing the cost of the treatment.

Gate2Brain peptides combine protease resistance, capacity to carry a wide range of cargoes thanks to their versatility, low production costs, and low immunogenic risk. They provide a non-invasive, non-antigenic, permeable, stable, soluble and receptor-specific way to transport drugs across the BBB and into the CNS.

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5. B. Oller-Salvia et al. Angew. Chem. Int. Ed. (2016), 55, 572-575

**L33 Design, Mechanism, and Applications of Cell-Penetrating Proteins**

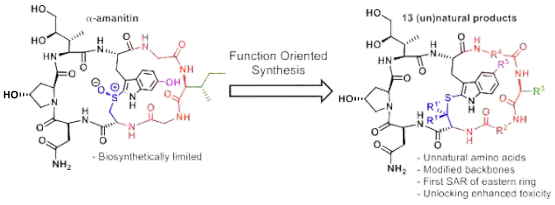
Dehua Pei
*The Ohio State University*

We recently discovered that cell-penetrating peptides and folded proteins (e.g., bacterial toxins) enter the cytosol of mammalian cells by endocytosis followed by endosomal escape via a novel vesicle budding-and-collapse (VBC) mechanism. This mechanistic understanding enabled us to design cell-penetrating proteins by grafting short cell-penetrating peptides into the surface loops of mammalian proteins. Our efforts led to a small, thermodynamically and proteolytically stable, and highly cell-permeable human protein, MTD4. Genetic fusion of MTD4 to the N- or C-terminus of cargo peptides and proteins renders the latter cell-permeable and biologically active. These fusion proteins have demonstrated utility as potential therapeutics by inhibiting intracellular protein-protein interactions or inducing protein degradation in mammalian cells. Effective delivery of biostimulants and biodefense agents
by MTD4 into the cytosol of plant cells provides a novel approach to sustainable agriculture.

**L34 Improving on alpha-Amanitin, One of Nature's Most Toxic Peptides - Synthesis of More Cytotoxic Analogs, Bioconjugation, and Use in Peptide-directed Targeted Therapy**

Mihajlo Todorovic, Alla Pryyma, David M. Perrin
Chemistry Department, University of British Columbia, 2036 Main Mall, Vancouver, BC, V6T1Z1, CANADA

Alpha-amanitin is a classic natural product that was isolated 80 years ago[1] from the notorious death-cap mushroom, *A. phalloides*. Alpha- amanitin is a potent, orally available, highly selective allosteric inhibitor of RNA polymerase II (Pol II) now features as a highly toxic payload for antibody-drug conjugates. Our first total synthesis of amanitin in 2018 has empowered access to new analogs that are more cytotoxic than the natural product. These analogs present discrete modifications to the polypeptide backbone as well as adjustments in "chi" space to rigidify the toxin. Nevertheless, the full basis for their enhanced cytotoxicity is not correlated with in-vitro inhibitory activity of Pol II catalyzed transcription. To begin to address, we have developed the first photo-activatable amanitin whereby light is used to initiate cell death. Finally, we have designed a series of modularly "clickable" linkers by which entirely synthetic toxins are conjugated to octreotate; these show low nanomolar cytotoxicity on Ar42J cells and promising in vivo targeting applications in mice.

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Synthesis and Preliminary Evaluation of Octreotate Conjugates of Bioactive Synthetic Amatoxins for Potential Targeted Therapy of Somatostatin- expressing Cancers, Pryyma et al. *RSC Chemical Biology* 2022 3: 69-78.

### L35 Molecular Design of Peptide Therapeutics for the "Other Pandemics

Krishna Kumar
*Department of Chemistry, Tufts University, Medford MA 02155, USA*

Obesity, Type 2 Diabetes (T2D) and related metabolic disorders afflict hundreds of millions worldwide. State of the art treatments include analogues of the endogenous gut peptide hormones called 'incretins'. The two principal peptides that form this class, Glucagon-like Peptide 1 (GLP-1) and Glucose-dependent Insulinotropic Peptide (GIP) stimulate their cognate receptors (GLP-1R and GIPR) in different tissues with the primary function of maintaining glucose homeostasis in addition to having neuro- and cardioprotective effects. These peptides however suffer from poor metabolic stability and are rapidly degraded by the ubiquitous serine protease, dipeptidyl peptidase IV (DPP-4). We describe here the design and development of potent peptide analogues that are completely refractory to hydrolytic enzyme action while retaining full biological activity, potency, and efficacy. As general modulators of the gut-brain axis, these peptide hormones have also high promise for untreated neurological indications such as Alzheimer's and Parkinson's diseases, and traumatic brain injury (TBI). Furthermore, the platform allows for the design of hundreds of derivatives with the ability to tune the onset and duration of action, potency, efficacy, and providing a method for modulating gut and blood brain barrier (BBB) penetration. This lecture will describe the fundamental design principles, molecular pharmacology and in vivo data. Some of the compounds described here rival or better the compounds used in the clinic today and could serve as a model platform for discovery of clinically relevant molecular entities.

### L36 Stalking Elusive Pathogenic Bacteria: How to Dive into Cells to Treat Infections

Jean Chmielewski
*Department of Chemistry, Purdue University, West Lafayette, IN 47907 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.

### L37 Designing Peptide-based Quorum Sensing Modulators for Bacterial Pathogens

Michael Bertucci
*Department of Chemistry, Lafayette College, 701 Sullivan Rd., Easton, PA 18042;*

### L38-YI5 Genetically Encoded Fragment-Based Discovery (Ge-Fbd) from Covalent and Non-Covalent Pharmacophores

Arunika I. Ekanayake[1], Ratmir Derda[1]
[1]*Department of Chemistry, University of Alberta, Canada.*

Genetically encoded fragment-based discovery (GE-FBD) is a promising approach for the selection of ligands and drug leads from existing GE libraries displayed on phage, DNA, or mRNA. GE-FBD starts with a fragment that interacts with a known site of the target protein but often with low potency and specificity. Covalent incorporation of unnatural fragments or 'pharmacophores' into conventional peptide libraries expands the chemical space and facilitates the discovery of molecules with favorable properties not offered by the fragments alone. This strategy can be applied to both linear and cyclic peptide libraries, using pharmacophores that have covalent as well as non-covalent reactivities towards target proteins. Since cyclic peptides alleviate several caveats presented by linear peptides, macrocyclization strategies that enable the installation of pharmacophores or other chemical moieties are highly desirable. Traditionally, the generation of GE-FBD libraries employs "early-stage" incorporation of unnatural building blocks into the chemically or translationally produced macrocycles. This talk will describe a divergent late-stage modification approach to such libraries starting from readily available starting material: genetically encoded phage-displayed libraries of peptides. Converting these phage-displayed peptides to 1,3-diketone bearing macrocycles provides a shelf-stable precursor for further functionalization with hydrazine through a well-established Knorr-pyrazole synthesis reaction. Ligation of diverse hydrazine derivatives onto diketone macrocyclic peptide libraries displayed on a phage that carries silent DNA barcodes enables genetic encoding of these post-translational chemical modifications. These libraries can be applied against "undruggable" protein targets to discover ligands with improved affinity and specificity.

### L39 Molecular Engineering of Safe and Efficacious Oral Basal Insulin

Martin Münzel[1], Frantisek Hubálek[1], Hanne H.F. Refsgaard[1], Sanne Gram-Nielsen[1], Peter Madsen[1], Erica Nishimura[1], Christian Lehn Brand[1], Carsten Enggaard Stidsen[1], Christian Hove Rasmussen[1], Erik Max Wulf[1], Lone Pridal[1], Ulla Ribel[1], Jonas Kildegaard[1], Trine Porsgaard[1], Eva Johansson[1], Dorte Bjerre Steensgaard[1], Lars Hovgaard[1], Tine Glendorf[1], Bo Falck Hansen[1], Maja Kirkegaard Jensen[1], Peter Kresten Nielsen[1], Svend Ludvigsen[1], Susanne Rugh[1], Mary Courtney Moore[2], Alan D. Cherrington[2] and Thomas Kjeldsen[1]
[1]*Novo Nordisk A/S, Novo Nordisk Park 1, 2760, Maaloev, Denmark*
[2]*Vanderbilt University, Nashville, TN, USA*

Oral administration of insulin represents a formidable scientific and medical challenge. For oral insulin therapy to be a viable option, the insulin molecule must not only survive the aggressive environment of the gastrointestinal tract, but additionally must be effectively absorbed in intact form into the circulation with very little variation. I will present the molecular engineering of the first long-acting insulin analogues for safe and efficacious oral therapy. Molecules were designed to have increased solubility and stability towards proteolytic degradation and additionally exhibit ultra-long pharmacokinetic profiles to minimize variability in plasma exposure following absorption. When formulated in sodium caprate tablets and dosed orally to dogs, these insulin analogues were rapidly absorbed with a bioavailability of 3-4 % and displayed glucose lowering activity.[1] An elimination plasma half-life (~20 h in dogs and ~70 h in man) was achieved by adding a strong albumin binding moiety to reduce clearance, lowering the insulin receptor affinity 500-fold. Furthermore, at peak absorption albumin binding ensured high liver exposure with minimal and slow distribution to peripheral tissues. Thus, the peaked absorption profile mediated an initial liver-centric insulin action that was able to blunt hypoglycaemia even in response to overdosing. These tailor-made long-acting insulin analogues have enabled the first successful once daily chronic administration of basal oral insulin in man, providing effective glucose control with no additional hypoglycaemia incidents compared to injectable insulin glargine.[2]

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### L40 Macromolecular Chemical Biology: Engineering Cell-Cell Interactions and Communication with Chemically Self-Assembled Nanorings

Carston R. Wagner
*Depts Medicinal Chemistry and Chemistry University of Minnesota*

As the basic structural and functional module of the human body, cells play essential roles in almost every aspect of physiological or pathological processes. The role of cell function in maintaining tissue homeostasis is closely associated with organism health. Thus, the manipulation of cell function and cell-cell interactions that are disease related has become a target for therapeutic development. Small-molecule therapeutic agents have achieved significant success in tuning cell function, primarily by interfering with intracellular protein function. Nevertheless, over the last three decades, there has been a rapid rise in the manipulation of cell behavior by targeting extracellular receptors and ligands with macromolecules. Genetic based methods have been developed that alter the natural expression of both ligands and receptors with considerable success, such as chimeric antigen receptors. Nevertheless, these methods can be time consuming to implement, difficult to control and not necessarily applicable to all cells. To address these issues a variety of macromolecular approaches, from modified antibodies to nanoparticles, have been designed and used to direct and redirect cell-cell interactions. Over the past few years, our group has designed, prepared and evaluated the ability of chemically self- assembled nanorings (CSANs) to be deployed for the non-genetic engineering of cell-cell interactions and communication. This seminar will cover our progress in this area, as well as potential therapeutic applications.(R01CA247681, R21CA185627, R01 CA125360, R01CA125360)

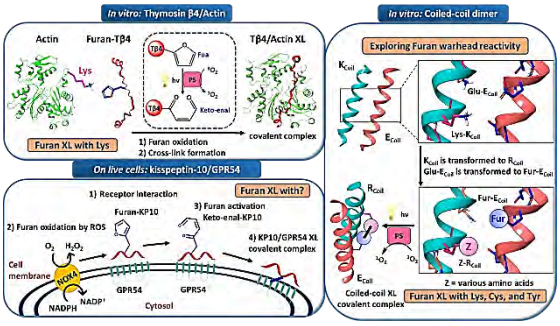
### L41-YI6 Furan-oxidation Mediated Cross-link Technology: From *in vitro* Analysis of Protein-protein Interactions to Covalent GPCR-ligand Interactions on Live Cells

Laia Miret-Casals,[1] Willem Vannecke,[1,2] Marta Vilaseca,[3] Marleen Van Troys,[2] Annemieke Madder[1]
[1]*Organic and Biomimetic Chemistry Research Group, Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281-S4, 9000 Gent, Belgium.* [2] *Department of Biomolecular Medicine, Ghent University, B-9000 Ghent, Belgium.* [3] *Institute for Research in Biomedicine, The Barcelona Institute of Science and Technology, 08028 Barcelona, Spain*

Our research group has developed a novel furan cross-link technology for oligonucleotides, further applicable to the investigation of peptide-protein interactions. As the furan moiety is isosteric with histidine and isoelectronic with tyrosine, the incorporation in peptides is well tolerated. Here, we describe the application of this fast and highly efficient furan-oxidation mediated technology to protein-protein[1], coiled-coil peptide dimer[2], and peptide-protein[3] interactions.

Initially, we studied the weak and dynamic protein-protein interaction between actin**,** the major cytoskeletal protein of the cell that forms filaments**,** and Tß4 that regulates the polymerization of actin and keeps actin in the monomeric form. Furan-armed-Tß4 analogues were shown to

efficiently cross- link to monomeric actin upon singlet oxygen generation[1]. The cross-link in the Tß4-actin covalent complex was to involve Tß4-Fua24 and Actin-Lys61 and a plausible chemical structure of the covalent linkage between Lys and the activated Fua was proposed[1]. A coiled-coil peptide dimer was used as a model system to explore furan reactivity, we described for the first-time reaction of the activated furan warhead with cysteine and tyrosine, besides the previously reported lysine, thus enhancing the versatility of the furan cross-link technology by the possibility to target different amino acids[2]. The furan-oxidation mediated cross-link technology was further optimized to enable cross-linking of furan-modified peptide ligands to GPCR proteins on live cells relying on the spontaneous endogenous oxidation of the furan moiety. We studied the neuropeptide kisspeptin-10 and its G-protein coupled receptor GPR54, which play a role in breast cancer and in the regulation of mammalian reproduction. We described selective cross-linking of a furan-modified kisspeptin-10 analogue to its membrane receptor GPR54 in live cells, with no toxicity and high efficiency[3,4].

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## L42 Chemistry Matched with Mechanical and Computational Machines for Rapid Synthesis, Discovery, and Delivery of Proteins

Bradley Lether Pentelute
*Massachusetts Institute of Technology, Department of Chemistry, 77 Massachusetts Avenue, Cambridge, MA 02139, USA*
*Department of Chemistry and Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States;*
*The Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, United States;*
*Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, United States*

Methods for the automated high-fidelity chemical production of proteins are needed. Proteins manufactured with mechanical machines, as opposed to bio-based systems, can bulletproof production circumventing endotoxin contamination and cell-line variability. Further, chemical synthesis enables the ability to rewire the covalent framework with non-natural amino acids, drugs, and carbohydrates. I will discuss the fully automated single-shot chemical synthesis of protein chains up to 220 amino acids in hours. After purification and folding, the synthetic proteins functioned analogously to ribosomally produced material. In addition, rapid protein synthesis enables accelerated drug discovery when combined with our single-shot affinity-selection mass spectrometry platform. I will discuss our results toward the discovery of high-affinity peptidomimetics that target oncoproteins or SARS-CoV-2.

## L43 New Methods for Construction of Complex Peptide Macrocycles

Gong Chen
*State Key Laboratory of Elemento-organic Chemistry, Nankai University, China*
*gongchen@nankai.edu.cn*

Cyclic peptides have provided an important platform for exploration of biorelevant chemical space between small molecules and biologics. In comparison with the state-of-the-art synthesis of small molecules, chemists' ability to fine-tune the three-dimensional structures and properties of cyclic peptides is much limited. In this talk, I will discuss our recent investigation of various chemical strategies, including metal-catalyzed, radial-mediated and classical polar reactions, for synthesis of peptide macrocycles with different structure features: 1) Construction of cyclophane-braced peptide macrocycles via palladium-catalyzed intramolecular arylation of various C-H bonds. 2) Streamlined construction of S-aryl ether linked peptide macrocycles via palladium-catalyzed intramolecular S-arylation in solution and on DNA template. 3) Cooperative stapling of unprotected peptides at lysine and tyrosine or arginine with simple formaldehyde.

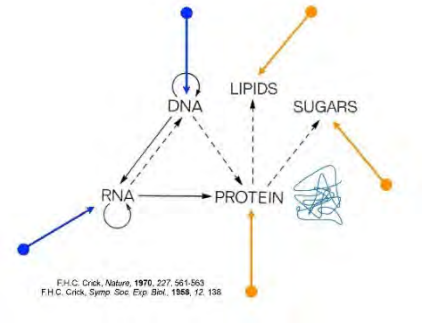
## L44 Sugars & Proteins: Towards a Synthetic Biology

Benjamin G. Davis
*The Rosalind Franklin Institute Harwell, Oxfordshire*
*Department of Pharmacology, University of Oxford*
*Ben.Davis@rfi.ac.uk*

Our work studies the interplay of biomolecules – proteins, sugars and their modifications. Synthetic Biology's development at the start of this century may be compared with Synthetic Organic Chemistry's expansion at the start of the last; after decades of isolation, identification, analysis and functional confirmation the future logical and free-ranging redesign of biomacro- molecules offers tantalizing opportunities. This lecture will cover emerging areas in our group in chemical manipulation of biomoleclules with an emphasis on new bond-forming and -breaking processes compatible with biology as well as new mechanistic methods and their use in unpick- ing associated biological mechanisms.

(i) New methods: The development of efficient, complete, chemo- and regio-selective methods, applied in benign aqueous systems to redesign and reprogramme the structure and function of biomolecules.

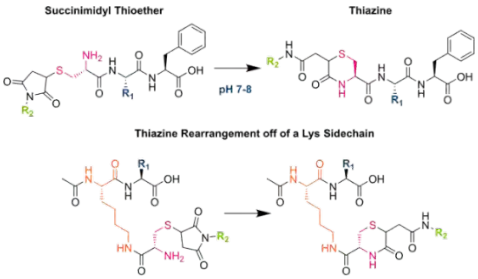
(ii) 'Synthetic biologics', associated biophysical methods and their applications: biomimicry; functional recapitulation; effector [drug/agrochemical/gene/radio-dose] delivery; interrogation of pathogen interactions; non-invasive presymptopmatic disease diagnosis; probes and modula- tors of in vivo function.




### L45 An Underreported Side Reaction in Maleimide Bioconjugation: Thiazine Rearrangement of Maleimido N-Terminal Cys Peptides

I. N. Gober[1], A. J. Riemen[1], and M. Villain[2]
[1]*Research and Development Department, Bachem Americas, Inc., Torrance, California, USA.* [2]*CMC Development Group, Bachem Americas, Inc., Torrance, California, USA*

Thiol-maleimide chemistry is widely used in the preparation of covalently modified peptides and proteins due to the rapid kinetics and selectivity of the reaction. Although thiol-maleimide conjugation has considerable utility, various side reactions are possible which may complicate the preparation of bioconjugates. In this work, we report on the formation of a thiazine impurity during the conjugation of N- terminal Cys peptides to maleimides. NMR spectroscopy and mass spectrometry were used to characterize the structure of the thiazine byproduct. The pH dependence and sequence specificity were investigated using a tripeptide model system wherein the amino acid adjacent to the N-term Cys and the N-substitution of the maleimide were varied to generate a small library of conjugates. Our findings indicate that the rearrangement to the thiazine isomer is general and that the thiazine impurity is the primary byproduct formed when succinimidyl thioether conjugates are incubated at neutral or basic pH. With this knowledge in hand, we demonstrated that the thiazine isomer can also be prepared on the sidechain of a peptide rather than the N- terminus by coupling Cys to a Lys residue prior to the maleimide conjugation and subsequent rearrangement.




Gober, I. N.; Riemen, A. J.; Villain, M. J. Pept. Sci. 2021, 27 (7). https://doi.org/10.1002/psc.3323

### L46 Chemical Labeling/Imaging of Neurotransmitter Receptors in Live Cell and Brain

Itaru Hamachi[1,2]
[1]*Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, Japan, and* [2]*ERATO Hamachi molecular technology for neuroscience project, Japan Science and Techinolgy (JST), Tokyo, Japan*

It is considered live cells and live animals are the multimolecular crowding systems where all of protein molecule are expressed with thier particular functions. Protein labeling is now a powerful tool for studying protein-of-interest (POI) under such natural conditions. However, it is generally defficult to carry out selecitive protein labeling in live cells. While bioorthogonal methods have been reported to date, valuable chemical strategies for selective modification of endogenous protein is yet poorly developed. I describe herein our recent progress in chemistry-based methods for selective labeling of endogenously expressed proteins under live cell/tissues, and even in the brain. Our method, termed ligand-directed chemistry (LDchem), relies on molecular recognition coupled with a (designer) chemical reaction. Molecular recognition can secure the protein selectivity even in the live systems and also accelerate the chemical reaction due to the proximity effect between the designed reactive module and a side chain of canonical amino acid exposed on the surface of POI. The LDchem allows to selectively modify endogenous POI with various synthetic functionalities, which is powerful for analyzing intracellular membrane-proteins such as GPCR, several neurotransmitter receptors including AMPAR, an ionotropic glutamate receptor which controls the excitatory neuronal signal, and GABAaR, a chloride channel located in the inhibitory neuron without genetic manipulation. We are now in progress for studying these receptors localization and functions in the live brains using LDchem.

### L47 Optically Controlled Protein Modification Chemistry

Michael T. Taylor
*Department of Chemistry, University of Wyoming, Laramie, WY 82071*

Biocompatible chemical transformations that are promoted by light have become powerful tools in chemical biology by virtue of enabling spatiotemporal control over activity. Whilst genetically encoded, photoactivatable tools have become mainstays in bio-orthogonal chemistry, photochemistries that covalently label native biomolecular structures (photobioconjugation) that are also biocompatible are comparatively limited. We recently reported an approach to protein photo-bioconjugation process that exploits the inherent photo- and redox-lability of Tryptophan (Trp) residues by pairing a Trp residue with an *N*-carbamoyl pyridinium salt that simultaneously couples photo-induced electron transfer (PET) with Trp to a radical fragmentation/recombination process to carbamylate Trp residues site-selectively with high efficiency. By invoking this unusual reaction mechanism, we are able to access chemical modifications on traditionally non-reactive biological moieties (namely Trp residues) with rapid kinetics and under biologically compatible conditions. Our studies to date have revealed that the pyridinium scaffold provides robust, optically triggered protein labelling in

applications for protein conjugate synthesis as well as for intracellular chemoproteomic profiling of Trp residues. Moreover, we have shown that the pyridinium scaffold is highly tunable; enabling precision control over photophysical and electrochemical properties. We have exploited this tunability to design pyridinium reagents that label Trp residues both by differing reaction mechanisms and at differing optical triggering windows. This, in turn, allows us to couple reaction mechanism to wavelength; enabling optical control over labelling chemistry. We anticipate that this ability will enable the design of reagents and experimental workflows of increasing sophistication and capabilities.

### L48 Expanded Molecular Access with Enhanced Efficiency in Microwave-Assisted Solid-Phase Peptide Synthesis

Wendy J. Hartsock[1], Jonathan Collins[1], Sandeep Singh[1], and Colin Simpson[1]
*[1]CEM Corporation, 3100 Smith Farm Road, Matthews, NC 28104, US*

The ability to drive molecular innovation in medicine and technology is powered by the methods and techniques to move from conception to reality. Here, we present three case studies that demonstrate the power of technology to expand molecular access.

Case Study 1: Rapid production of neoantigens for personalized treatment of cancer
Case Study 2: Chemical synthesis of 100-mer peptides and proteins in under 24 h
Case Study 3: Microwave-Assisted SPPS to expedite the production of peptides at the kilogram scale

These case studies reveal that when engineering and chemistry are optimized together, high-throughput SPPS, long peptide/short protein synthesis, and kilogram production of peptides can be compatible with improved efficiency and the greening of peptide science.

### L49 Design of Proteins and Macrocycles

David Baker
*University of Washington*
*Molecular Engineering and Sciences*

Proteins mediate the critical processes of life and beautifully solve the challenges faced during the evolution of modern organisms. Our goal is to design a new generation of proteins that address current-day problems not faced during evolution. In contrast to traditional protein engineering efforts, which have focused on modifying naturally occurring proteins, we design new proteins from scratch to optimally solve the problem at hand. We now use two approaches. First, guided by Anfinsen's principle that proteins fold to their global free energy minimum, we use the physically based Rosetta method to compute sequences for which the desired target structure has the lowest energy. Second, we use deep learning methods to design sequences predicted to fold to the desired structures. In both cases, following the computation of amino acid sequences predicted to fold into proteins with new structures and functions, we produce synthetic genes encoding these sequences, and characterize them experimentally. In this talk, I will describe recent advances in protein design using both approaches. I will also describe the systematic design of rigid membrane permeable macrocycles 6-12 amino acids in length, and smaller rigid macrocycles built from a wide diversity of backbone chemistries.

### L50 Cleaving Protected Peptides from 2-chlorotrityl Chloride Resin. Moving Away from Dichloromethane

Othman Almusaimi[a], Mahama Alhassan[b], Fernando Albericio[c,d], Beatriz G. de la Torre[e]
*Surfaces and Particle Engineering Laboratory, Department of Chemical Engineering, Imperial College London, London SW7 2AZ, U.K. Email. o.al-musaimi@imperial.ac.uk*
*[a]Department of Chemistry Education, University of Education, Winneba, Ghana*
*[b]Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4001, South Africa.*
*[c]CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, University of Barcelona, 08028 Barcelona, Spain.*
*[d]KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa.*

In recent years, the work of various research groups has allowed the substitution of the hazardous solvents most widely used in solid-phase peptide synthesis, namely DMF, NMP, DCM, DEE, among others, by several much less hazardous solvents. Indeed, greener alternatives have been found for almost all steps of the process, with the exception of the cleavage of protected peptides from 2-chlorotrityl chloride resin.

Given the essential role of protected peptide fragments for key subsequent reactions such as cyclization and fragment condensation, here, after careful screening of several of the so-called green solvents, we propose 2% TFA in either anisole or 1,3-dimethoxybenzene as optimal for the cleavage step. The higher boiling point of these solvents compared with the DCM allows the preparation of protected peptides with less risk of premature removal of the most labile protecting groups, such as the Trt of His. Our findings once again evidence the value/versatility of green solvents in strict chemical terms.

### L51 Peptide Hit Identification and Lead Optimization using Artificial Intelligence Approaches

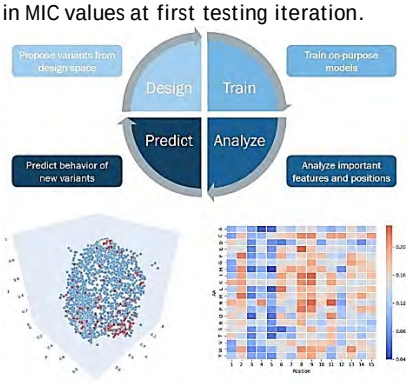
Ewa Lis, Lilly Lindmeier, Leonard Dieguez
*Koliber Biosciences, 12265 World Trade Dr. #G, San Diego, CA, 92128*

Artificial Intelligence / Machine Learning approaches have been widely adopted for small molecule drug discovery however similar approaches for peptides are still in nascent development. The challenges are driven by limited availability of peptide datasets, high dimensionality of the design space as well as poorly developed methods for encoding peptides for deep learning algorithms. Moreover, the performance of the machine learning systems is often evaluated only in a computational fashion and new predictions are rarely validated in the wet lab.

To solve the challenges of encoding peptides to the machine learning algorithms, ten encodings were developed and evaluated on several datasets. The designed encodings outperformed the common method of one-hot-encoding, with largest improvements observed on small datasets. Methods to interpret and visualize the model predictions were also developed to aid in a better understanding of the key performance drivers.

In addition to supervised machine learning methods, approaches to represent and visualize peptide design space in low dimensions were developed and utilized to demonstrate that hits can be identified at 5-fold increased efficiency compared to random screening. Moreover, models built with only 6 positive datapoints were able to correctly prioritize the peptides for the next screening round.

To validate the AI peptide platform, novel anti- microbial peptides were predicted by the machine learning models and shown to have potent activity against gram negative microorganisms. Moreover, multiple substitution mutations were predicted that resulted in up to 32-fold improvement in MIC values at first testing iteration.



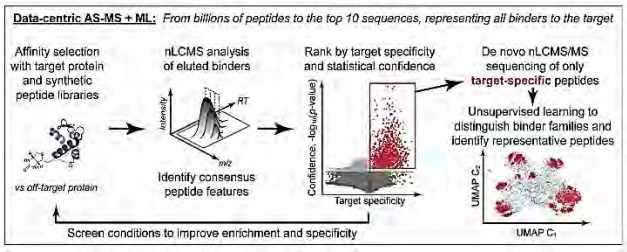

| | ROUND 1 | ROUND 2 | ROUND 3 | ROUND 4 | CUMMULATIVE |
|---|---|---|---|---|---|
| Screened | 100 | + 34 | + 33 | + 33 | 200 |
| RANDOM hits | 6 | 7 | 8 | 10 | 1x |
| ML hits | 6 | 22 | 31 | 41 | 4x |
| DSG-ML hits | 6 | 24 | 43 | 50 | 5x |

### L52-YI7 Targeted Affinity Selection of Peptide Binders Using Machine Learning

Joseph S Brown[1], Somesh Mohapatra[2], Michael A Lee[1], Anthony J Quartararo[1], Nathalie Grob[1], Andrei Loas[1], Rafael Gomez-Bombarelli[2], and Bradley L Pentelute[1,3-5]
*[1]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States; [2]Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States; [3]The Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, United States; [4]Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States; [5]Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, United States*

Affinity selection-mass spectrometry (AS-MS) is a widely used technique for the discovery of high- affinity binding molecules to biomolecular targets. The use of large combinatorial libraries has improved the potential of *de novo* peptide discovery, but remains significantly limited by the capacity of tandem sequencing of affinity selection samples even with high-resolution Orbitrap spectrometers. With 2 x $10^8$- membered libraries in affinity selection, we observe unbiased sequencing results in high-fidelity sequence assignment of only ~0.2% of peptide features in affinity selections samples. Moreover, non-specific binders are simultaneously sampled, requiring individual analysis and validation of each identified sequence.

In this work, we demonstrate a highly-efficient, targeted sequencing workflow that is coupled with machine learning (ML) to significantly advance the discovery capability of AS-MS using peptide libraries. For proof of concept, we use canonical L-peptide libraries (2.4 x $10^9$ members total) and multiple target proteins including an anti-hemagglutinin antibody and Mouse double minute 2 homolog (MDM2). Individual features of affinity-selected peptides are autonomously compared to identify and rank highly-enriched, target-specific features for robust tandem sequencing, greatly expanding the number of true putative binders discovered. With these identified sequences, topological representations of the peptides enable robust encoding for machine learning. Unsupervised learning then distinguishes between the populations of target-specific peptide binders, allowing one to navigate the boundaries and binder families in the peptide sequence space. These efforts will streamline the selection-based identification of target- specific binding molecules and find immediate utility as a powerful tool for drug discovery.




### L53 New Developments for the Solid-Phase Peptide Synthesis (SPPS). Greening the Process

F. Albericio,[1,2] B. G. de la Torre[1]
*[1]Peptide Science Laboratory, University of KwaZulu-Natal, South Africa; [2]Department of Organic Chemistry, University of Barcelona, Spain*

The last few years have witnessed an explosion in the number of peptides approved by regulatory agencies. Peptide chemists are currently facing different challenges. Among them, the synthesis of increasingly complex peptides and the need to make the process greener. In this presentation, we will review our laboratory's efforts in these two directions.

The recently developed SIT for the protection of a Cys allows a chemoselective disulfide formation through a thiol-disulfide interchange approach with a free Cys present in the molecule. Synthesis of several disulfide containing peptides will be discussed.

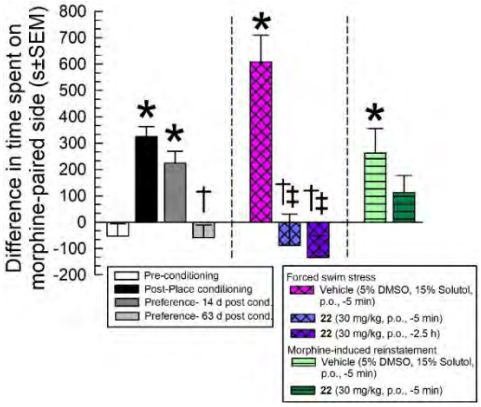
Based on the safety-catch concept, a fourth category of protecting groups, that are stable under the conditions used to remove the first three and that are removed at the end of the synthetic process, has been developed. This step can be performed when the peptide is still anchored to the resin or once the peptide is in solution. This new concept of protecting group facilitate the synthesis and manipulation of difficult peptides.
From a green perspective, SPPS is hampered by high solvent consumption for the washings after each of the two main steps, namely deprotection and coupling. Here we propose to combine the two steps in one. In this regard, once the coupling was completed, piperidine is added up to a concentration of 20% to the coupling cocktail, and after the removal is finished, a few washings with the corresponding solvent in the presence of an acid rectifier - to remove traces of base- completes the synthetic cycle.

Finally, we will illustrate that the Refractive Index is a process analytical tool suitable for the real-time monitoring of different steps of the SPPS including washings.

### L54 Macrocyclic Tetrapeptides Prevent Reinstatement of Opioid- seeking Behavior

J.V. Aldrich,[1] D. Yakovlev,[1] A.C. Brice-Tutt,[2] S.O Eans[2] and J.P. McLaughlin2
*Departments of [1]Medicinal Chemistry and [2]Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA*

Opioid misuse is a serious health problem, with 1.6 million Americans meeting the criteria for an opioid use disorder (OUD) and opioid overdose deaths increasing sharply over the last two years. While medications are available to manage OUD, the relapse rate after detoxification can be up to 80%, with stress being a major factor contributing to relapse of drug-seeking behavior. The endogenous kappa opioid system mediates stress responses, and kappa opioid receptor (KOR) antagonists are known to prevent relapse of cocaine- seeking behavior. We are investigating macrocyclic tetrapeptides related to the KOR antagonist [D-Trp]CJ-15,208 for their potential development as treatments for substance use disorder. A number of analogs of this lead peptide produced KOR antagonism in the CNS after oral administration. Administration of an analog (30 mg/kg, p.o.) prevented stress-induced increases in voluntary consumption of morphine in a two-bottle choice assay, reinstatement of morphine- seeking behavior in a conditioned-place preference assay, and also significantly reduced signs of withdrawal in physically-dependent mice. Moreover, this analog lacked liabilities (respiratory depression, locomotor effects and reinforcing effects) associated with opioid agonists. These results demonstrate the potential for development of these orally active peptides for the treatment of OUD.




This work was supported by the Defense Health Program, through the Peer Reviewed Medical Research Program under Awards W81XWH- 15-1-0452 and W81XWH-15-1-0464. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

### L55 Peptide Mimicry using N-Aminoimidazole-2-ones

Y. Hamdane and W.D. Lubell
*Department of Chemistry, Université de Montréal, Montréal, QC, Canada*

α-Amino lactam residues have been historically used to constrain peptide backbone conformation for studying structure-activity relationships in peptide-based drug discovery [1,2]. N- Aminoimidazol-2-one (Nai) residues are aza-¤-amino lactam counterparts [2-6]. Synthesized by a route featuring proline-catalyzed alkylation of azopeptides [3], Nai residues can adopt the central position of ß- and γ-turn secondary structures as illustrated by X-ray crystallographic and computational methods [4-6]. Moreover, substituents can be readily added onto the Nai heterocycle for mimicry of different side chain orientations [4-6]. With focus on structure- activity relationship studies of biologically active peptides, the synthesis, and applications of Nai residues will be presented [6].

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### L56-YI8 Peptide-Based Disruption ofi Membrane-Embedded Protein-Protein Interactions in Bacterial Efflux Pumps

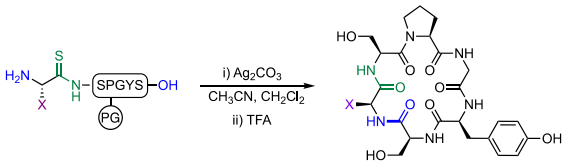
Chloe J Mitchell, Tyler S. Johnson and Charles M. Deber*.
*Division of Molecular Medicine, Research Institute, Hospital for Sick Children, M5G 0A4, Toronto ON, Canada; and Department of Biochemistry, University of Toronto, M5S 1A8, Toronto ON, Canada.*

Targeting membrane-embedded protein-protein interactions continues to be a challenge. We have employed a rational drug design to target the bacterial Small Multidrug Resistant (SMR) efflux protein, that homodimerizes through its 4th transmembrane helix (TM4). The peptide inhibitors contain the TM4 Gly-Gly heptad dimerization motif, to align with - and competitively disrupt - the membrane-embedded TM4-TM4 interface, and thereby disable substrate efflux. The peptide inhibitors also contain two tags: a C-terminal positively-charged Lys tag to direct the peptide to the negatively-charged bacterial membrane, and a non-charged N-terminal peptoid tag to promote membrane insertion. The designed peptides have the prototypical sequence Ac-A-$(Sar)_3$-XXGIXLIXXGVXX-KKK (Sar = N-methyl-Gly). We have earlier shown that these peptides reduce SMR mediated efflux activity, ostensibly through the specifically designed mechanism. However, the architecture of these peptides resembles that of cationic antimicrobial peptides, as both categories of peptides have a hydrophobic and charged domain. Therefore, we explored in structural detail the effects inhibitor peptides have on the bacterial membrane. Through circular dichroism and fluorescence spectroscopy, we
first confirmed that these peptides are inserted and helical in a membrane environment. To determine effects of insertion, we used *in vivo* and *in vitro* dye-based assays and found that the peptides displayed minimal disruption of the membrane *per se*. Interestingly, peptide insertion in absence of protein was seen to cause apparent membrane reorganization in liposomes (3:1 POPE:POPG), as assessed through dual peaks detected by differential scanning calorimetry. Our results show promise for a new approach to target and disrupt membrane protein-protein interactions.

### L57 Exploiting Thioamide Reactivity in Peptide Synthesis

Craig A. Hutton
*School of Chemistry, The University of Melbourne, Parkville, Victoria 3010, Australia*

In recent years a range of novel amide ligation strategies have been developed, many incorporating sulfur-containing reagents as thioesters and other carboxylic acid surrogates. We have recently reported novel methods for peptide cyclization and functionalization based on the reaction of thioamides with carboxylic acids in the presence of silver(I). These methods involve the initial generation of isoimide intermediates, which can undergo a range of acyl transfer processes to generate modified peptides. Recent progress in this area will be highlighted, including novel strategies for macrocyclization, ring expansion and glycosylation of peptide thioamides.[1-5]

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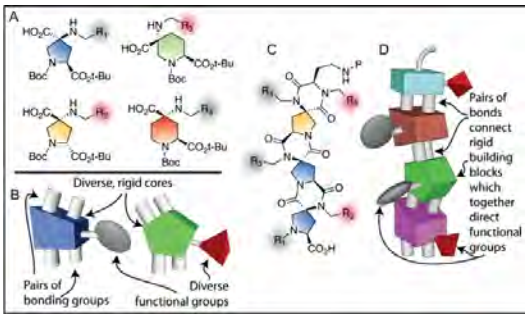
### L58 Automated Synthesis of Spiroligomers: Programmable, Shape- defined, and Cell-permeable Peptidomimetics

Yihui Xie[1], Danni Luo[1], Thomas Paniak[2], Shiying Zhang[3], Justin Northrup[2], Conrad Pfeiffer[2], Joshua A. Kritzer[3], Christian E. Schafmeister[1,2]
[1] *Department of Chemistry, Temple University, Philadelphia, PA 19122, USA*
[2] *ThirdLaw Molecular LLC, Blue Bell, PA 19422, USA*
[3] *Department of Chemistry, Tufts University, Medford, MA 02155, USA*

Spiroligomers are highly functionalized, shape-programmable ladder- oligomers formed by joining functionalized, stereo chemically pure bis-amino acids through pairs of amide bonds. We have previously shown that spiroligomers can modulate protein function by binding grooves on protein surfaces and disrupting protein-protein interactions. Over the last three years we have scaled up the synthesis of bis-amino acids to 30-kilogram scale. We have demonstrated the efficient incorporation of the fluorenylmethyloxycarbonyl (Fmoc) protecting group at hundreds of gram scale and the incorporation of more than 25 diverse functional groups on four stereochemically pure monomers. In the past year, we have prepared more than 600 spiroligomers with molecular weights in the range of 1,300 to 1,500 Daltons using automated peptide synthesizers at a 10-100 milligram scale. These spiroligomers were chosen randomly from the space of 100 million tetramers that we can assemble from our current set of 100 monomers. We have also measured cytosolic penetration of 11 diverse spiroligomers using the chloroalkane penetration assay (CAPA). More than half of the spiroligomers tested appear to penetrate cells by passive diffusion, and most of them penetrate cells better than a poly- arginine peptide. The diversity of 1,500 Dalton spiroligomer structures that we can access synthetically and the observation of cytosolic permeability despite their large size suggests that spiroligomers are an attractive class of “beyond rule-of-five” compounds for therapeutics that inhibit protein-protein interactions.




Spiroligomer Scaffolds – (A) bis-amino acids (B) a cartoon of bis- amino acids; (C) a spiroligomer trimer; (D) a cartoon of a spiroligomer trimer.

**L59** **Manipulating Peptide Self-assembly into Cross-ß-sheet Supramolecular Structures to Design Synthetic Nanovaccines**

X. Zottig, M. Babych, M. Côté-Cyr, S. Bricha, D. Archambault, and S. Bourgault
*Department of Chemistry, Université du Québec à Montréal, QC, Canada*

Amyloid fibrils, historically associated with diseases, have been recognized as a biological structure that performs essential functions in many host organisms, highlighting their potential as life-inspired nanoparticles, soft biomaterials, and matrices. These cross-ß-sheet organized proteinaceous assemblies combine key characteristics, including high mechanical resistance, biocompatibility, biodegradability, and chemical and enzymatic stability. We are currently developing nanoassemblies based on the cross-ß quaternary motif as adjuvanted antigen delivery system. Our strategy relies on the covalent attachment of an immunological epitope to a self-assembling peptide unit. Upon self-recognition of the chimeric peptide, tailored nanostructures displaying multiple copies of the antigen are obtained. These fibrils are efficiently uptaken by antigen-presenting cells, and the cross-ß-sheet architecture readily activates the Toll-like receptor 2 and stimulates dendritic cells, leading to a robust antigen-specific immune response in mice. To address the inherent polymorphism and polydispersity associated with the process of amyloid formation, we conceived a strategy to finely control the architecture from the peptide sequence. Addition of electrostatic capping motifs on the ß-peptide leads to short, rigid, and uniform nanorods. Mice intranasal immunization with these nanorods, decorated with a highly conserved epitope from the influenza A virus, confers complete protection with absence of clinical signs against a lethal infection with the H1N1 strain. We also harnessed this approach to prepare synthetic carbohydrate vaccines to fight bacterial infections. Overall, these studies reveal that by acting as an immunomodulator and an antigen delivery system, peptide cross-ß-sheet assemblies constitute robust and versatile nanoplatforms for subunit and glycoconjugate vaccines.

**L60** **Towards Next Generation Therapeutics: Cell-permeable Macrocyclic Peptides**

Emel Adaligil
*Genentech*

**L61** **Peptide Interactions *in vivo* and *in vitro***

Lynne Regan
*University of Edinburgh*

I will describe how transient peptide-peptide and peptide-protein interactions can be used inside living cells to perform a new type of super-resolution imaging that is accessible to all. I will also describe how by harnessing peptides and proteins with distinctive physical properties we can create new methods of surface display and new classes of biomaterials with user-specified physical and biochemical properties.

**L62** **Determining What Else Trimethyllysine Reader Proteins Can Read**

C. R. Travis‡, K. I. Albanese‡, Kelsey M. Kean, H. C. Henriksen, M. L. Waters
*Department of Chemistry, CB 3290, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599*

Post-translational modification of histone proteins, including lysine methylation, 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 selective inhibitors and probes for this class of proteins. However, with over 200 methyllysine reader proteins in humans, achieving selectivity can be challenging. We have taken a mechanistic approach coupled with high throughput screening to identify unique selectivity patterns in these proteins, establishing new mechanisms of action and providing new opportunities for selective inhibition. These findings will be discussed.

### L63 Catalytic Amyloids Promote Carbon Dioxide Hydration with Efficiencies that Rival those of Native Carbonic Anhydrases

L. R. Marshall, S. Bhattacharya and I.V. Korendovych
*Department of Chemistry, Syracuse University, Syracuse, NY 13244, USA*

Previously we have shown that short *de novo* designed peptides can self-assemble into highly reactive amyloid-like catalysts for hydrolysis of activated esters.[1] Subsequent work by many groups showed broad applicability of this approach to many different model transformations.[2] Here we set out to explore whether catalytic amyloids can replicate native activities of enzymes. We have rationally designed a series 9-residue peptides that self-assemble in the presence of zinc to promote $CO_2$ hydration with $k_{cat}/K_M$ of 6 x $10^5$ $M^{-1}s^{-1}$ at pH 9.5 and 3 x $10^5$ $M^{-1}s^{-1}$ at pH 8. The latter value is at least 100-fold faster than any reported to date artificial catalysts of $CO_2$ hydration. Moreover, given the low molecular weights of the peptides, the designed catalysts have specific activities that are on par with those of natural carbonic anhydrases. To our knowledge, this is the first example of a model system matching enzymes in their own game. This is even more exciting considering that the functional complexity needed to promote efficient catalysis was achieved by supramolecular self-assembly of short peptides containing only natural amino acid residues. Unlike enzymes, catalytic amyloids are extremely robust and can be used under harsh conditions to help promote $CO_2$ capture and fixation.

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### L64 New Tools for Peptide Mimicry and Functionalization

Caroline Proulx
*Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695-8204, United States*

Peptides modulate a variety of biological responses, including as hormones and neurotransmitters. Occupying the space between small molecules and biologics, they have also been pursued to target protein-protein interaction surfaces. However, strategies are needed to convert them into more stable molecules while retaining the bioactivity of the native peptides, ideally employing scaffolds that can adopt precise secondary structures while being amenable to rapid derivatization for library synthesis and screening. Towards that goal, we develop new methods for late-stage peptide functionalizations, and devise new strategies for the synthesis and conformational control of highly substituted peptidomimetics. This includes 1) the synthesis and study of *N*-aryl peptides as tunable precursors for kethydrazone and ketoxime ligations,[1] 2) the development of new methods for chemoselective functionalizations of azapeptides,[2] and 3) the design and application of new conformationally-constrained *N*-substituted glycine (peptoid) monomers.[3] This presentation will provide an overview of the new broadly applicable tools for peptide mimicry developed in the Proulx lab, with emphasis on synthetic method development.

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### L65 Peptides in Materials Science

Joel P. Schneider
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute*

Peptides have, and continue, to impact materials development. They can be used to functionalize the surfaces of existing materials to impart function or serve as building blocks to construct new materials through their polymerization and/or self-assembly. Self-assembling peptides can form a broad array of supramolecule structures including sheets, disks, spheres, barrels, tubes, and fibrils. Fibrils are privileged structures capable of higher-order assembly leading to the formation of networks that constitute the formation of macroscopic gels. The physical, mechanical and biological properties of peptide gels can be finely tuned through molecular design to enable a wide range of biomedical applications. Further, understanding the assembly mechanisms by which these materials are formed and their structures to molecular detail catalyze their development towards targeted applications. We designed a class of peptide hydrogels that allows for the direct encapsulation of therapeutics and their subsequent local delivery to tissue. Peptide assembly leading to fibrillar gels can be triggered in the presence of small molecules, proteins, nucleic acids, cells, and nanoparticles resulting in their direct encapsulation. Resultant gels display shear-thin/recovery mechanical properties, allowing their direct application to targeted tissue by syringe or spray, where they deliver their payload locally. Through the design of over 200 sequences, we have developed a deep mechanistic and structural understanding of this class of material. This has allowed the development of gels that facilitate a broad range of applications including microanastomosis (the suturing of ultrasmall blood vessels), gels that limit tissue rejection after vascularized composite allotransplantation, and as treatments for mesothelioma.

### L66 Designing New Peptide Assemblies for Fun and for In-Cell Applications

Dek Woolfson
*Schools of Chemistry and Biochemistry, & Bristol BioDesign Institute. University of Bristol, UK*

Peptide design has come of age: it is now possible to generate a wide variety stable peptide assemblies from scratch using rational and/or computational approaches. A new challenge for the field is to move past structures offered up by nature and to target the so-called '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 or not used prolifically.

This talk will illustrate what is currently possible in this nascent field using *de novo* designed helical peptides.

Coiled coils are bundles of 2 or more ¤ helices that wrap around each other in rope-like structures. They are one of the dominant structures that direct natural protein-protein interactions. Our understanding of coiled coils provides a strong basis for building new peptide assemblies. The first part of my talk will present this understanding and our current "toolkit" of *de novo* coiled coils.

Next, I will describe how the toolkit can be expanded to generate dark-matter structures. For instance, this has led to the rational and computational design of a completely new $3_{10}$-helical bundle. Then I will turn to in-cell applications. I will describe two new designs for (i) *de novo* cell-penetrating peptides and (ii) high-affinity kinesin-binding peptides. Finally, I'll show how these two designs can be combined to render peptides that can be delivered exogenously to eukaryotic cells and target subcellular processes; in this case, hijacking active protein motors.

### L67 Peptides: Valuable Motifs for Material Design

Ting Xu
*Department of Material Sciences and Engineering, Department of Chemistry, University of California, Berkeley; Materials Sciences Division, Lawrence Berkeley National Laboratory*
*E-mail: tingxu@berkeley.edu*

Peptides are routinely used to understand how biology operates. They are equally valuable as therapeutics and building blocks for biomaterials. We started our journey with investigating biomacromolecule conjugates based on peptides and polymers. We have since benefited from learning how peptides behave when polymers are around. In one case, we investigated the assembly of amphiphilic peptide-polymer conjugates and applied the knowledge to develop a family of sub-20 nm nanocarriers. In the other project, these studies allowed us to "visualize" how synthetic polymers may interact with peptides and the interplay between two classes of materials. This ultimately enlightened us to explore a class of new polymers, called "random heteropolymer" and start the pursue toward recapitulating proteins' behavior using synthetic polymers.

### L68 Development of Cell Adhesive Peptide-Agarose Matrices for Cell Culture

Motoyoshi Nomizu and Yuji Yamada
*School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392, Japan*

Extracellular matrix proteins, such as laminin and collagen, and their active peptides are potential candidates for affording cell adhesiveness to non-cell adhesive materials for three-dimensional (3D) cell culture. Our group has identified various biologically active peptides from laminins by screening more than 3,000 peptides. These peptides recognize various cell surface receptors and have the potential ability to serve as bio-adhesiveness for 3D cell culture matrices. Previously, we demonstrated that "mixing" of syndecan-binding peptides, such as AG73 (RKRLQVQLSIRT), provide
cell adhesiveness to agarose gels while integrin-binding peptides, such as A99 (AGTFALRGDNPQG), do not.

In this study, to conjugate the laminin peptides to agarose, we synthesized agarose-aldehyde by oxidation of primary alcohols of agarose using TEMPO reagent. Two laminin peptides, AG73 and A99, were synthesized with a CGG sequence at N-terminus and conjugated to agarose-aldehyde via thiazolidine formation between the cysteine residue and the aldehyde. Two-dimensional cell adhesion assay using fibroblasts showed that AG73- and A99-conjugated agarose gels have potent cell adhesion activity. Cell encapsulation experiments revealed that only the A99-agarose gels promote elongation and proliferation of fibroblasts in a 3D environment. These results suggest that covalent conjugation of the peptides to agarose is more beneficial to develop a peptide/agarose matrix compared to the previous mixing method, and the peptide-conjugated agarose gels are promising as a matrix for 3D cell culture for anchorage-dependent cells.

### L69 Self-Assembled Protein Vesicles for Drug Delivery and Biocatalysis

Julie A. Champion
*Georgia Institute of Technology, School of Chemical & Biomolecular Engineering*

Protein vesicles incorporating functional, globular proteins have potential in a number of bio-applications such as drug delivery, biocatalysis, and sensing. We have previously created protein vesicles from mCherry-zipper-ELP protein complexes where ELP is a thermo-responsive elastin-like polypeptide, zipper is a coiled-coil, and mCherry is a model folded protein. As we utilize these vesicles, we have replaced mCherry with more useful functional proteins and have engineered the vesicles to provide both stability and stimuli responsiveness. We implemented non-natural amino acid incorporation to enable photocrosslinking strategies to stabilize vesicles and control their swelling and release of cargo as a function of salt concentration. We have modified the ELP amino acid sequence to create vesicles that are pH sensitive and swell or disassemble at acidic pH or form vesicles with different sizes and stabilities. With this information, we have demonstrated assembly of biocatalytic vesicles with significant improvements in activity over soluble enzyme and produced vesicles for drug delivery capable of carrying and releasing therapeutic cargoes. The wide range of vesicle properties and functions exhibited in these examples, highlight the versatility of protein vesicles as functional and responsive protein materials.

### L70 Cancer-Targeting Immunostimulatory Peptides as Synthetic Antibody Mimics for Tumor Immunotherapy Applications

David Sabatino
*Carleton University, Department of Chemistry, 1125 Colonel By Drive, Ottawa ON, K1S 5B6*
david.sabatino@carleton.ca

Tumor immunotherapy involves the activation of lymphocytes by circulating tumor associated antigens, which stimulates inflammatory responses and leads towards the destruction of invasive tumors. We have designed and developed a small set of bifunctional peptides incorporating NKp30-receptor binding and NK cell activating domains as immunostimulatory peptides and the incorporation of GRP78 targeting and tumor cell binding

sequences to generate the so-called cancer-targeting immunostimulatory peptides (CTIPs). A selected CTIP displayed binding to GRP78 presenting HepG2 and A549 tumor cells, which diminished in the presence of an anti-GRP78 peptide blocker, suggesting GRP78-binding specificities. Similarly, the selected CTIP bound to NK cells in an NKp30-dependent manner, which translated into NK cell activation by cytokine secretion. In co-culture, fluorescence microscopy revealed that the GFP-expressing A549 cells were visibly associated with CTIP-activated NK cells. Accordingly, A549 cell death was observed by the loss of GFP signaling and early/late-stage apoptosis. Furthermore, administration of peptide-activated NK cells into A549-tumor-bearing mice resulted in a consistent decrease in tumor growth rate when compared to the untreated control group. Taken together, this presentation will highlight the discovery of CTIPs that serve to mimic antibody targeting and activating functions for tumor immunotherapy applications.

Reference.
Descalzi-Montoya D, Montel RA, Smith K, Dziopa E, Darwich A, Yang Z, Bitsaktsis C, Korngold R, Sabatino D. Synthetic Antibody Mimics Based on Cancer-Targeting Immunostimulatory Peptides. Chembiochem. 2021; 22(9), 1589-1596.

### L71 Microed Conception and Current Practices

Tamir Gonen
*Howard Hughes Medical Institute, Departments of Biological Chemistry and Physiology, University of California, Los Angeles, Los Angeles CA 90095.*

My laboratory studies the structures of membrane proteins that are important in maintaining homeostasis in the brain. Understanding structure (and hence function) requires scientists to build an atomic resolution map of every atom in the protein of interest, that is, an atomic structural model of the protein of interest captured in various functional states. In 2013 we unveiled the method Microcrystal Electron Diffraction (MicroED) and demonstrated that it is feasible to determine high-resolution protein structures by electron crystallography of three-dimensional crystals in an electron cryo-microscope (CryoEM). The CryoEM is used in diffraction mode for structural analysis of proteins of interest using vanishingly small crystals. The crystals are often a billion times smaller in volume than what is normally used for other structural biology methods like x-ray crystallography. In this seminar I will describe the basics of this method, from concept to data collection, analysis and structure determination, and illustrate how samples that were previously unattainable can now be studied by MicroED. I will conclude by highlighting how this new method is helping us discover and design new drugs; shedding new light on chemical synthesis and small molecule chemistry; and showing us unprecedented level of details with important membrane proteins such as ion channels and G-protein coupled receptors (GPCRs) at atomic resolutions.

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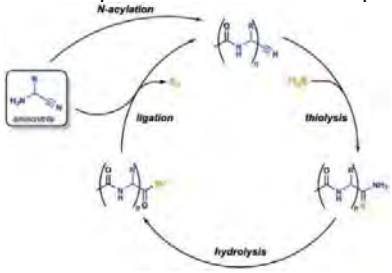
[4] The authors acknowledge funding from the Howard Hughes Medical Institute to T.G. and the National Institutes of Health (P41GM136508)

### L72 Nitriles, Peptides, Cofactors and the Origins of Life

Matthew W. Powner
*Department of Chemistry, University College London, London, WC1H 0AJ, UK*

Living organisms are highly complex chemical systems that exploit a small constellation of universally conserved metabolites. The chemical unity of these metabolites provides compelling evidence that a simple set of predisposed reactions predicated the appearance of life on Earth. Conversely, traditional 'prebiotic chemistry' has produced highly complex mixtures that bear little resemblance to the core metabolites of life. The complexity of prebiotic reactions had, until recently, suggested that elucidating life's origins would be an insurmountable task, but systems chemistry is now providing unprecedented scope to explore the origins of life and an exciting new perspective on a four billion year old problem. At the heart of this new systems approach is the understanding that individual classes of metabolite cannot be considered in isolation from each other or their environment, if the chemical origin of life on Earth is to be successfully elucidated. In this talk recent advances that suggest that proteinogenic peptides are predisposed chemical structures, which can be facilely and selectively synthesized in water, will be presented. Specifically, the role that nitriles may have played in shaping the structure of life's core molecules will be discussed.[1,2,3] These result suggest that *the water paradox* is a false dichotomy, and water is a part of the solution not the problem.

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[3] Foden *et al. Science* **2020**, *370*, 865.

### L73 Beyond N-methylation: Synthesis, Structure, and Function of N-heteroatom-substituted Peptides

J. R. Del Valle*
*Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556*

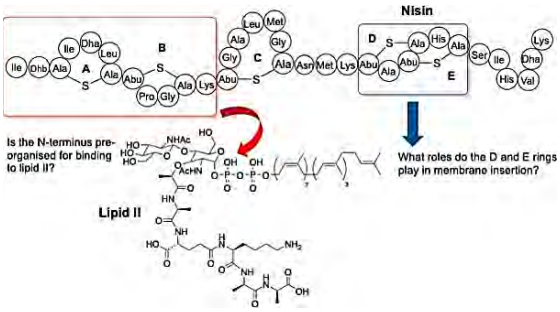
Backbone-oxidized non-ribosomal peptide (NRP) natural products have garnered considerable interest due to their unique conformational preferences and intriguing biological activities. Inspired by these NRPs, our laboratory is exploring the synthesis and properties of designed N-

heteroatom-substituted peptides and proteins. We have examined the impact of hydrazide and hydroxamate backbone replacements on the stability of canonical secondary structures using NMR, X-ray, and circular dichroism. Here, we demonstrate how cooperative non-covalent interactions contribute to help accommodate hydrazide bonds within b-strand, polyproline II, and a-helical folds. In particular, peptide N-amination is shown to stabilize ¤-sheets while enhancing peptide solubility and proteolytic stability, thus addressing a significant challenge in the area of protein mimicry. The conformational and non-aggregating characteristics of N-amino peptides (NAPs) are consistent across distinct models of folding in aqueous solution and in the solid-state. Leveraging these properties, we designed a series of linear and macrocyclic NAPs that target protein fibrilization and block the propagation of amyloid assemblies in a sequence-specific manner. We further show that backbone N-amino substituents within peptides can serve as reactive handles in late-stage macrocyclizations. This enables the incorporation of novel covalent surrogates of sidechain-to-backbone (*sb*) H-bonds that are prevalent motifs in globular proteins. N-Heteroatom-substituted peptides thus represent nature-inspired tools for protein mimicry with broad ranging applications in chemical biology and peptidomimetic drug design.

### L74 A Chemical Biology And Biophysics Approach to Understanding the Molecular Recognition of Lipid Ii by the Lantibiotic Nisin

R. Dickman, A. Murtza, E. Danelius, C. Webley, A. Dorey, S. Mitchell, A. Figuerio, D. F. Hansen, S. Cochrane, M. Erdélyi, S. Howorka and A. B. Tabor
*Department of Chemistry, University College London, UK School of Pharmacy, University College London, UK Structural and Molecular Biology, University College London, UK Department of Chemistry-BMC, Uppsala University, Sweden*
*School of Chemistry and Chemical Engineering, Queen's University Belfast, UK*

The global threat of antimicrobial resistance has generated a renewed interest in natural products, such as the lantibiotics, as a rich source of potent antimicrobial drugs. The best-studied lantibiotic, nisin, is a multiply thioether-bridged polycyclic peptide with high affinity and specificity for binding to lipid II. The currently accepted hypothesis is that nisin and lipid II assemble at an 8:4 ratio to form a pore structure in the bacterial membrane, causing cell death. We are currently studying this interaction at the molecular level using a combination of chemical biology and biophysical approaches. Analogues of the *N*- terminal region of nisin(1-12) have been prepared by solid phase peptide synthesis. We have carried out an NMR ensemble analysis of one analogue and of wild type nisin(1-12), and have demonstrated that rings A and B are pre-organised for binding to the pyrophosphate group of lipid II. Using a semisynthesis approach, we have also prepared partially linearized analogues of nisin with one or more of rings C, D and E replaced by linear sequences. Electrophysiology and *in vitro* antimicrobial testing of these analogues enable the roles of these rings in insertion of the *C*-terminus in the bacterial membrane and binding to lipid II to be probed.




*J. Org. Chem.* 2019, *84*, 11493 – 11512; *Chem. Eur. J.* 2019, *25*, 14572 – 14582.

### L75 Structure and Dynamics of Class B Peptide Hormone G Protein-Coupled Receptors

P.M. Sexton[1], J. Cao[1], X. Zhang[1], S.J. Piper[1], R.M. Johnson[1], M.J. Belousoff[1], R. Danev[2], D. Wootten[1].
*[1]Drug Discovery Biology and ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Vic 3052, Australia. [2]Graduate School of Medicine, University of Tokyo, 113-0033 Tokyo, Japan.*

G protein-coupled receptors (GPCRs) are the largest superfamily of cell surface receptor proteins and a major target class for drug development. GPCRs are inherently flexible proteins that have evolved to allosterically communicate external signals to modulation of cellular function through recruitment and activation of transducer proteins, particularly G proteins. Technological evolution in cryo-EM combined with continuing advances in biochemical approaches for the stabilisation of active-state complexes of GPCRs with different transducer proteins is now enabling structural interrogation of receptor activation and transducer engagement. Moreover, cryo-EM can access conformational ensembles of GPCR complexes that are present during vitrification, which can provide a window into the dynamics of these complexes. Using exemplar class B receptors, I will discuss how we are using cryo-EM to provide insight into GPCR activation by different agonists, and mechanisms of differential transducer coupling. I will also discuss how analysis of conformational dynamics of different agonist-GPCR-transducer complexes can contribute to mechanistic understanding of GPCR pharmacology.

### L76 Protein Delivery to Cells and Animals

Alanna Schepartz
*The University of California, Berkeley*

I will describe the discovery of a miniature protein that guides proteins and enzymes into the cell interior by promoting endosomal escape, a single-molecule tool that quantifies this trafficking event in live cells with accuracy and precision, experiments to probe how endosomal escape occurs, and how this knowledge can be applied for (cytosolic) enzyme replacement therapy.

### L77 Killer Snails: Agents of Change and Innovation

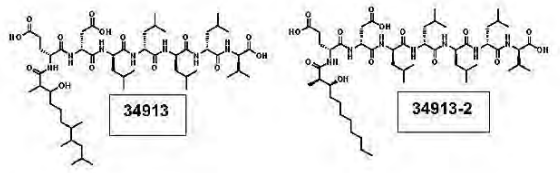
Mandë Holford, PhD
*Department of Chemistry and Biochemistry; Hunter College Belfer Research Building and CUNY Graduate Center*
*413 E. 69th Street, BRB 424, NY, NY 10021 USA*
*Email: mholford@hunter.cuny.edu*
*Sackler Institute for Comparative Genomics, Invertebrate Zoology*
*American Museum of Natural History, Central Park West at 79th Street*
*New York, NY 10024, USA, Email: mholford@amnh.org*

The ubiquitous presence of venomous animals in the seas, on land, and in the skies, demonstrates the evolutionary success of venom in shaping fundamental biological processes related to defense, predation, and competition. *The Holford lab uses venoms and venomous animals as agents of change and innovation.* We are interested in how venoms direct the evolution of organisms and how they can improve human lives. Venom peptides, and the genes from which they are derived, are a resource for investigating biological processes pertaining to organismal evolution (adaptive radiation, diversification), gene development (duplication, neofunctionalization), and cellular physiology involving ion channels (activating/inhibitory ligands). However, with no reliable snail venom sample source outside of the animal itself, there is a bottleneck preventing the investigation of *in vivo* venom production and the evolutionary mechanisms that drive the function of marine snail venom peptides. This talk will demonstrate the scientific path from *mollusks to medicine* examining how venom evolved over time in the terebrid snails (Terebridae), and how we can use this evolutionary knowledge as a roadmap for discovering and characterizing new peptides with therapeutic potential for treating pain and cancer. A venomics strategy, combining genomics, transcriptomics, and proteomics, has been applied to the discovery, characterization and optimization of terebrid venom peptides (teretoxins). In recent efforts, we have focused on developing invertebrate venom gland model systems that can be genetically manipulated to study the molecular innovation of venom. The Holford lab uses inventive tools from chemistry and biology to: (1) investigate the evolution of venom in predatory marine snails, (2) discover disulfide-rich peptides from a venom source, (3) develop high-throughput methods for characterizing structure-function peptide interactions, and (4) deliver novel peptides to their site of action for therapeutic application. Projects highlighting the development of our a *mollusk to medicine* version of *bench to bedside* process will be discussed in this seminar.

### L78 Lipopeptides are a Modular Scaffold for Tunable Targeting of Transcriptional Coactivators

O. Pattelli, E. Martinez Valdivia and A.K. Mapp
*Life Sciences Institute and Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA*

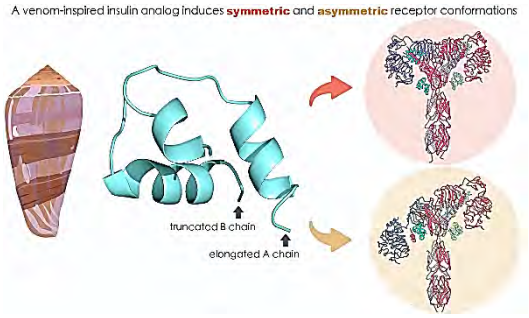
Inhibitors of transcriptional protein-protein interactions (PPIs) have high value both as tools and for therapeutic applications. For example, the PPI network mediated by the complexes formed between the transcriptional coactivator Med25 and its cognate transcription factors regulates stress-response and motility pathways and dysregulation of the PPI networks contributes to oncogenesis and metastasis. The canonical transcription factor binding sites within Med25 are large and have little topology, and thus do not present an array of attractive small-molecule binding sites for inhibitor discovery. We recently identified a novel lipopeptide natural product (NP 34913) that selectively targets Med25 as well as a synthetically more tractable analog with nearly identical selectivity and potency (34913-2). Lipopeptide 34913-2 engages endogenous Med25 in cell lysates and in cell culture, down-regulates key Med25-dependent genes. Through additional mutational analysis, we noted that changes in the peptide sequence lead to changes in the cognate coactivator target, enabling facile development of inhibitors of additional coactivators.




### L79 A Natural Product-inspired Insulin Analog Induces New Insulin Receptor Conformations Upon Activation

Xiaochun Xiong[1], Alan Blakely[2], Helena Safavi-Hemami[3], Christopher P. Hill[2], Danny Hung-Chieh Chou[1]
[1]*Department of Pediatrics, Division of Endocrinology and Diabetes, Stanford University, Stanford, CA 94305, USA*
[2]*Department of Biochemistry, University of Utah, Salt Lake City, UT 84112, USA*
[3]*Department of Biomedical Sciences, University of Copenhagen, 2200 Copenhagen N, Denmark*

Cone snail venoms contain a wide variety of bioactive peptides, including insulin-like molecules with distinct structural features, binding modes, and biochemical properties. Here, we report a fully active humanized cone snail venom insulin with an elongated A chain and a truncated B chain, and use cryo-electron microscopy and protein engineering to elucidate its interactions with the human insulin receptor ectodomain. We reveal how an extended A chain can compensate for deletion of B-chain residues, which are essential for activity of human insulin but also compromise therapeutic utility by delaying dissolution from the site of subcutaneous injection. This finding suggests approaches to developing improved therapeutic insulins. Curiously, the receptor displays a continuum of conformations from the symmetric state to a highly asymmetric low- abundance structure that displays novel coordination of a single humanized venom insulin using elements from both of the previously characterized site-1 and site-2 interactions.




Xiong, X. et al. -Symmetric and Asymmetric Receptor Conformation Continuum induced by an Insulin Analog. Nature Chemical Biology, 2022, in press (DOI : 10.1038/s41589-022-00981-0)

### L80 The Next Generation RaPID System

Hiroaki Suga
*The University of Tokyo, 7-3-1 Hongo, 130033, Japan*

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. 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 (>1 trillion members) of non-standard peptides containing multiple non-proteinogenic amino acids, giving unique properties of peptides distinct from conventional peptides, e.g. greater proteolytic stability, higher affinity (low nM to sub nM dissociation constants similar to antibodies), and superior pharmacokinetics. The field is rapidly growing evidenced by increasing interests from industrial sectors, including small start-ups as well as mega-pharmas, toward drug development efforts on macrocyclic peptides, which has led to several de novo discovered peptides entering clinical trials. This lecture discusses the advanced RaPID system, and several showcases of therapeutic potentials of macrocyclic peptides. This lecture also discusses the next generation of RaPID system involving the display of pseudo-natural products generated by thiopeptide post-translationally modifying enzymes.

### L81 Engineering Enzymes for Green Manufacturing of Noncanonical Amino Acids

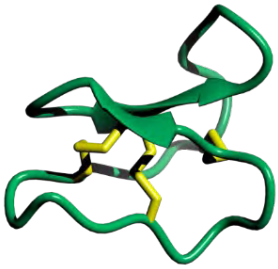
Christina Boville
*Co-founder & CEO, Aralez Bio*

At Aralez Bio, we have developed an enzymatic platform to synthesize noncanonical amino acids quickly, sustainably, and at low cost. We use directed evolution to evolve enzymes that can do this synthesis in a single step with minimal waste while easily mixing and matching chemical moieties for enhanced pharmaceutical diversity. Our proprietary enzymes catalyze a complexity-generating C–C bond-forming reaction with a broad range of readily available substrates, enabling us to manufacture more than 100 enantiopure amino acids at anywhere from mg to kg scale. Through increased efficiency and product scope, Aralez Bio supports the development and manufacture of new peptides more quickly and cleanly than ever before.

### L82 Discovery and Applications of Cyclotides: Nature's Ultra-stable Peptide Scaffolds

David J. Craik
*Institute for Molecular Bioscience, Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, The University of Queensland, Brisbane, Queensland, 4072, Australia*

Our work focuses on the discovery of cyclic peptides in plants and their applications in drug design and agriculture. We have a particular interest in a family of proteins called cyclotides, which comprise ~30 amino acids and incorporate three disulfide bonds arranged in a cystine knot topology, which makes them exceptionally stable. Cyclotides occur in all plants from the Violaceae family and in certain plants from the Rubiaceae, Cucurbitaceae, Solanaceae and Fabaceae, where they are present as host defense agents against insects and nematodes. A single plant may contain dozens to hundreds of cyclotides expressed in a wide range of tissues, including leaf, flower, stem and roots. Their stability and compact structure makes cyclotides an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilise them.[1] Because plants produce cyclotides in large quantities (up to 2g/kg plant weight) we are using crop plants as expression systems for the production of pharmaceutically active cyclotides. This presentation will give an overview on the discovery, biosynthesis and applications of cyclotides, with a focus on recent applications as stable scaffolds in drug design.



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**YI-P001** **If Synthesis and Structural Characterization of Rigid, Diyne-Bridged, Peptide Macrocycles**

Z.C. Adams[a], S.Chiorean[a], B. Balo[a], Y. Shi[a], G. Pierens[b], K.J. Rosengren[b], and P.E. Dawson[a]*
[a] *Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla, CA 92037 (USA)*
[b] *Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD 4072 (Australia)*

Extended backbone conformations are an abundant motif observed in protein-peptide interactions. Along with other secondary structures including turns and helices, extended ß-strand regions define ordered backbone and side chain orientations that contribute to specific recognition of protein targets.1 Designed peptides biased towards a ß-strand conformation have been used to study peptide aggregates,2 and to develop protease inhibitors3 and antibiotics.4 Extending our studies of peptide Glaser couplings5 and their implementation in stapled helical peptides,6 we have investigated the incorporation of diyne linkages into i to i+2 side chain positions. The resulting rigid macrocycles set the peptide backbone and side chains into a variety of extended conformations including ß-strand structures. Optimization of on-resin diyne formation facilitated the synthesis of a variety of strained macrocycles and their incorporation into several peptide contexts. This optimized solid-support synthesis sets the stage for robust high-throughput one-bead one-compound screens for biological activity of this novel class of compounds. The conformational ensemble of a series of dyine macrocycles was determined by NMR and DFT studies. We found that all variations on the size and shape of the stapled ring resulted in an extended backbone conformation, with differences in the conformation dictated primarily by the ring size. Our structural interrogation of this class of compounds provides insights into how these constraints could be used to mimic the backbone structures observed in peptide ligands, inhibitors, and natural products.

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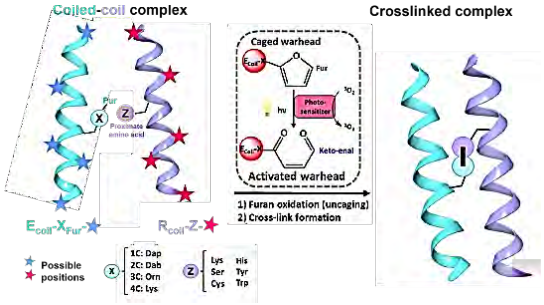
**YI-P002** **Crosslinking Of Furan-Warhead Containing Coiled-Coil Peptides**

Dorien Aerssens,[1] Laia Miret-Casals,[1] Sander Van de Putte, Alex Manicardi,[2] Annemieke Madder[1]

[1] *Organic and Biomimetic Chemistry Research Group, Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281-S4, 9000 Gent, Belgium*
[2] *Department of Chemistry, life sciences and environmental sustainablility, University of Parma Viale delle Scienze, 17/A, I-43124 Parma, Italy*
¶
The heterodimeric (EIAALEK)3/(KIAALKE)3 coiled-coil system offers stability in the nanomolar range ($K_D$=70 nM), making it ideally suited for labelling applications in which a protein is extended with the K-coil sequence to make site-selective reaction with the complementary E-coil peptide possible.[1],[2],[3] Additionally, one could also fixate the coiled- coil structure by crosslinking the two coiled-coil forming peptides which can be useful for subsequent biochemical analysis.

The furan-oxidation strategy has been successfully used for crosslinking and labelling purposes in previous research within our group. [4,5],[6],[7],[8] The strength of this crosslink strategy lies in the fact that it can be triggered, in a selective and biocompatible way, through singlet oxygen mediated furan oxidation.
In this work, we looked at crosslinking of the furan modified E-coil towards the K-coil in more detail. Not only do we also report on the co-existence of the parallel and anti-parallel conformation of this coiled-coil system in solution. but we also demonstrate that replacement of weak interhelical ionic contacts with a furan moiety and its potential cross-link partner affords covalently connected coiled-coil motifs upon furan activation. Furthermore, we also describe novel cross-link partners of furan warheads for site-specific cross-linking.

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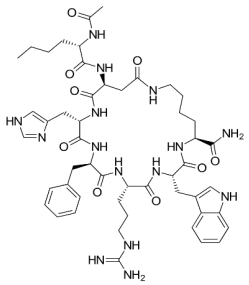
**P003** **From Small to Large Scale: Optimizing and Upscaling the Synthesis of Melanotan II (MT-II) - Melanocortin Receptor Agonist, on PurePep® Chorus and PurePep® Sonata+**

*Luísa Aguiar, Colton Quick, Juan Bello, Victor Nikolaev, Scott Harlow and Łukasz Frankiewicz*
*Gyros Protein Technologies, 4675 South Coach Drive, Tucson, Arizona, 85714, U.S.A.*

α-Melanocyte Stimulating Hormone (α-MSH) is responsible for triggering the production of melatonin – body's natural protection against ultraviolet (UV) radiation. α-MSH production is prompted, in itself, by the exposure of the skin to UV rays; however, stimulating the biosynthesis of melatonin prior to UV exposure could possibly prevent UV-induced skin cancer.

Considering that the native form of α-MSH was too unstable in vivo to be administered as a therapeutic agent, a wide range of analogues were synthesized; one of them is MT-II (Figure 1), a cyclic pseudopeptide with high resistance to enzymatic degradation and an extraordinary potency. Given the relevance of MT-II and the need for its production at a larger scale, our work focused on the optimization of a fully automated synthetic pathway, i.e., synthesis of the linear sequence and on-resin lactamization of the final peptide. In this work, we report the fully automated synthesis of MT-II on PurePep® Chorus using different coupling conditions and orthogonal protection schemes, for different cyclization strategies. Furthermore, we describe the upscale of MT-II synthesis, using the new PurePep® Sonata+ large scale peptide synthesizer.

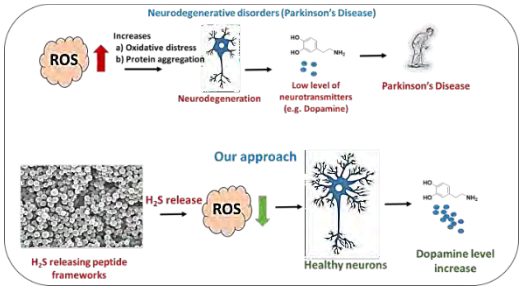


Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-NH2
Figure 1. Structure of pseudopeptide MT-II.

**YI-P004** **Design and Synthesis of $H_2S$ Releasing Peptide Frameworks and their Therapeutic Application in Neurodegenerative Disorders**

Rafat Ali[a], Hilal Ahmad Pal[a], Rohil Hameed[b], Aamir Nazir[b], and Sandeep Verma[a]
[a]*Department of Chemistry, Indian Institute of Technology Kanpur, Kanpur-208016, INDIA*
[b]*Division of Neuroscience and Ageing Biology, CSIR-Central Drug Research Institute, Lucknow 226031, India*

Neurodegenerative disease such as Parkinson's Disease (PD), Alzheimer's Disease (AD), etc., impact millions of people globally and in these disorders impairment in the functions of nerve cells of the brain or peripheral nervous system have been observed. Currently available therapies only treat the symptoms but not the root cause and progression of the disease. Therefore, there is an urgent need to develop new therapeutic strategies which can act upon the disease- causing pathways instead of providing mere symptomatic relief. $H_2S$ an endogenous signaling molecule regarded as toxic gas initially, but now its antioxidant, neuroprotectant and neuromodulator property is very well established. Therapeutic potentials of this gaseous molecule largely rely on its duration of release and its local concentration. We have successfully design and synthesize the peptide frameworks which on conjugating with hydrogen sulfide releases the $H_2S$ gas in slow and controlled manner. These peptides will form distinct nanostructures in aqueous solution and have the ability to inhibit the aggregation of protein (for e.g $A\beta_{1-42}$ protein aggregates in AD) responsible for the progression of these neurodegenerative disorders. These peptide nanostructures successfully deliver the $H_2S$ gas inside the Caenorhabditis elegans (*in-vivo* system used in our studies) and significantly decrease the oxidative distress and increases the level of critical neurotransmitters which is otherwise depleted in the neurodegenerative diseases. In my presentation I will mainly discuss the design and synthesis of peptide-based frameworks which delivers $H_2S$ in slow and sustained manner in *in-vivo* system and their application in Alzheimer's and Parkinson's Disease.

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**YI-P005** **Cleaving Protected Peptides from 2-Chlorotrityl Chloride Resin. Moving Away from Dichloromethane**

Othman Almusaimi[a], Mahama Alhassan[b], Fernando Albericio[c,d], Beatriz G. de la Torre[e]
[a]*Surfaces and Particle Engineering Laboratory, Department of Chemical Engineering, Imperial College London, London SW7 2AZ, U.K.*
*Email. o.al-musaimi@imperial.ac.uk*
[b]*Department of Chemistry Education, University of Education, Winneba, Ghana*
[c]*Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4001, South Africa.*
[d]*CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, University of Barcelona, 08028 Barcelona, Spain.*
[e]*KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa.*

In recent years, the work of various research groups has allowed the substitution of the hazardous solvents most widely used in solid-phase peptide synthesis, namely DMF, NMP, DCM, DEE, among others, by several much less

hazardous solvents. Indeed, greener alternatives have been found for almost all steps of the process, with the exception of the cleavage of protected peptides from 2-chlorotrityl chloride resin.
Given the essential role of protected peptide fragments for key subsequent reactions such as cyclization and fragment condensation, here, after careful screening of several of the so-called green solvents, we propose 2% TFA in either anisole or 1,3-dimethoxybenzene as optimal for the cleavage step. The higher boiling point of these solvents compared with the DCM allows the preparation of protected peptides with less risk of premature removal of the most labile protecting groups, such as the Trt of His. Our findings once again evidence the value/versatility of green solvents in strict chemical terms.

### YI-P006 High Scalability and Kinetic Control in Polyelectrolyte Complex Assembly of microRNA/peptide Nanoparticles

Caleb F. Anderson, Poulami Majumder, Joel P. Schneider
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD, 21702, USA*

Current treatment options for mesothelioma are not effective, as complete surgical tumor resection is nearly impossible due to the complicated sheet-like anatomy of these tumors. Therefore, we previously engineered a surface-fill hydrogel (SFH) that can be syringe- or spray-delivered to surface cancers during surgery or as a primary therapy to deliver loaded tumor-specific miRNA-peptide polyelectrolyte complex nanoparticles (NPs) that attenuate the oncogenic signature of cancer cells.[1] Once applied, SFH can shape-change in response to alterations in tissue morphology and locally release NPs. However, the NPs exhibit broad size ranges and are metastable, where NPs will begin aggregating within ~2 h if not encapsulated within the gel phase, complicating their translation to large-scale, clinical settings. Thus in this report, we utilize a scalable process called "flash nanocomplexation" (FNC) that facilitates shortening of the diffusion paths of the assembly components, yielding highly uniform and stable NPs. Using a confined impinging jet mixer, we achieve controlled turbulent micromixing
of cationic peptide and miRNA, producing NPs with extremely narrow size distributions and enhanced stability. We systematically vary FNC processing parameters (such as inlet flow rates, charge ratios, pH, and assembly component concentrations) and peptide composition to assess their influence on resulting NP size, polydispersity, surface charge, colloidal stability, and their ability to internalize into cells, escape endosomes, and silence target genes important in mesothelioma.

1. Majumder, P. et al. Surface-fill hydrogel attenuates the oncogenic signature of complex anatomical surface cancer in a single application. *Nature Nanotechnology* 16, 1251-1259 (2021).

### P007 Potent Inhibition of SARS-CoV-2 and Its Variants by a Lipopeptide Broadly Inhibiting Coronavirus Fusion

Y.M. Angell[1], Y. Wang[1], M. Adler[1], Y. Matsui[2], M. Montano[2], R. Schwarzer[2], E.W.A. Helmy[2], R. Suryawanshi[3], M. Ott[3], and W.C. Greene[2]
*1 ChemPartner, Co. Ltd., South San Francisco, CA 94080*
*2 Greene Lab, Gladstone Institute of Virology, San Francisco, CA, 94158*
*3 Ott Lab, Gladstone Institute of Virology, San Francisco CA, 94158, USA*

In order to contain the COVID-19 pandemic, it is necessary to control the transmission of the virus. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection starts with membrane fusion between the viral and host cell membranes, which is mediated by Spike protein. Here, we developed a new lipopeptide pan-coronavirus fusion inhibitor, CGM23, which targets the HR1 domain in the Spike protein and can potently inhibit infection by divergent human coronaviruses tested, including seasonal coronaviruses (229E and NL63), SARS-CoV, MERS-CoV, and SARS-CoV-2. CGM23 is also effective against infection of SARS-CoV-2 variants, including Omicron. The broad-spectrum fusion inhibitor is based on the amino acid sequence of SARS-CoV-2, but three specific substitution mutations at the N-terminus and the addition of PBA (4-phenylbutanoic acid) increased the helicity of CGM23 and enhanced its stability. Furthermore, CGM23 has 16 carboxylic acids at the C-terminus with a PEG linker. This lipidation enhanced the viral suppression effect. In pharmacokinetic studies, after intranasal administration, CGM23 diffused rapidly to the lower respiratory tract and was absorbed into the bloodstream with a half-life of 7 hours. In fact, during In vivo challenge experiments, simultaneous intranasal administration of CGM23 (0.5 mg/kg), with live SARS CoV-2 virus significantly suppressed infection in mice. These studies suggest that CGM23 could be applied prophylactically to prevent human coronavirus infections, or as a treatment to mitigate viral proliferation during the active infections.

### YI-P008 Relationship Between Structure and Antibacterial Function of Short Peptides Derived from Rabbit Cathelicidin (CAP18)

Aróstica M.[1], Guzmán F.[2], Acosta G.[3], Albericio F.[3,4], Viñas M.[5], Sierra J.[5], Mercado L.[7].
*[1]Doctorado en Biotecnología. Pontificia Universidad Católica de Valparaiso (PUCV) y Universidad Técnica Federico Santa María, Valparaiso, Chile.[2] Núcleo de Biotecnología Curauma, PUCV, Valparaíso, Chile. [3]CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine & Department of Organic Chemistry. University of Barcelona (UB), Barcelona, Spain. [4]School of Chemistry & Physics, University of KwaZulu-Natal, Durban 4001, South Africa. [5]Department of Pathology and Experimental Therapeutics, Faculty of Medicine and Health Sciences, Campus Bellvitge, University of Barcelona, Barcelona, Spain. [7] Institute of Biology, PUCV.*

Currently, the search for novel agents to combat infectious diseases and prevent resistance to pathogens is of vital importance. Since the last decade, interest has increased in the use of antimicrobial peptides (AMPs) as an alternative to conventional antibiotics, mainly due to their versatility and therapeutic properties.
The cathelicidin family is interesting for its wide range of action, effectiveness and for being part of the immune

system of many organisms. The problem with its use is that the antimicrobial domain of this family usually has medium toxicity, Also, its large molecular size compared to conventional antibiotic molecules make it difficult and expensive to produce in large quantities which reduces its applicability as an antibacterial agent, mainly due to its molecular size.

In this work, short peptides derived from rabbit cathelicidin were obtained and the relationship between secondary structure and antibacterial function was determined for their potential use as antibacterial agents. The peptides were synthesized by Fmoc solid phase synthesis and subsequently their antibacterial activity was obtained in a wide range of bacteria (4 bacterial strains). Its secondary structure was determined by circular dichroism in different media (TFE 0-40%, H2O, PBS and DMPG), concentration and temperature. The peptides showed activity in the strains studied with MIC close to 7.8 uM and the predominant secondary structure was α-helix. In this work, the bases for bioactive peptides, derived from long cathelicicin, are obtained, which will allow their future use in treatments of bacterial diseases.

Acknowledgement: ANID-PFCHA/Doctorado Nacional/2019-21191654

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### P009 Optimization of Antimicrobial Peptides as Alternative Antimicrobials in Poultry Production

Alana Babcock, Kirsty Sinclair, Darcy Sutherland, Anat Yanai, Inanc Birol and Fraser Hof*
*acDepartment of Chemistry, University of Victoria, Victoria, B.C. Canada.*

Antibiotic resistance is an emerging global health crisis. Strategies to mitigate this threat are currently concentrated on curbing the misuse of antibiotics in healthcare and in agriculture, however this has generated unintended consequences for the farming industry. Antibiotics are critical for maintaining animal health and without them, animal mortality has increased and farm productivity has declined. This is especially true in the poultry industry, where chicks hatch with under-developed immune systems and are extremely vulnerable to infection. Antimicrobial peptides (AMPs) are promising alternatives for antibiotics in poultry production. AMPs simultaneously attack multiple bacterial structures and modulate the host immune system to aid bacterial killing. These variable mechanisms of action make AMPs less likely to exert specific selective pressures for bacteria to exploit and develop resistance. Despite their acknowledged potential, AMPs require pharmacological optimization to improve their therapeutic applications. We report on the high-throughput production of modified AMPs identified from machine-learning-driven genomic screens[1]. We will also report on using synthetic optimizations to improve AMP stability and activity against drug-resistant and/or highly pathogenic bacterial strains. This set of results expands our knowledge of AMP and aids in the development of effective alternatives to conventional antibiotics.

[1] Li, C., Sutherland, D., Hammond, S.A. *et al.* AMPlify: attentive deep learning model for discovery of novel antimicrobial peptides effective against WHO priority pathogens. *BMC Genomics* 23, 77 (2022). https://doi.org/10.1186/s12864-022-08310-4

### YI-P010 Optimizing Robotic Solid-Phase Synthesis of Polypeptoids for Materials Applications

J. Babi[†], A.M. Clapperton[†], Ł. Frankiewicz[*], and H. Tran[†‡]
[†]*Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H4, Canada*
[‡]*Department of Chemical Engineering & Applied Chemistry, University of Toronto, Toronto, ON, M5S 3E5, Canada*
[*]*Gyros Protein Technologies, 4675 South Coach Drive, Tucson, AZ 85714, USA*

Peptoids have broad applicability as a peptidomimetic material for biosensing applications.[1] The submonomer synthesis approach on robotic synthesizers remains the most commonly used method for the preparation of peptoids, enabling the automated synthesis of polymers in the range of 40 repeat units. However, while a range of syntheses with varying reaction times and temperatures, reagent concentration and delivery ratios, and post-reaction resin cleavage are reported in literature, these programs do not apply uniformly across disparate instruments or desired synthetic targets.

Here we share our experiences in the optimization of solid-phase peptoid synthesis on a Gyros Protein Technologies PurePep Chorus synthesizer. For example, our results suggest that using equivalent volumes and concentrations of bromoacetic acid (BrAc) and N,N- diisopropylcarbodiimide (DIC) during bromoacetylation increases overall coupling yield and can sufficiently correct for inherent deficiencies in automated reagent delivery. The purity and yields observed with several combinations of reagent concentrations and delivery volumes are compared, and considerations for effective displacement with bulky amines and resin cleavage/protecting group deprotection strategies are explored. This work represents an approach which is broadly applicable to optimize solid-phase peptoid synthesis for materials chemistry applications.


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*Journal of Polymer Science* **2021**, 59(21), 2378-2404.

### YI-P011 New Developments in Solid Phase Peptide Chemistry Facilitate the Study of Protein Prenylation

T. K. Bader[1], A. Cardillo[2], J. Morstein[3], C.A. Hrycyna[2], and M.D. Distefano[1]
[1]*Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA*
[2]*Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA*
[3]*Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA 94158, USA*

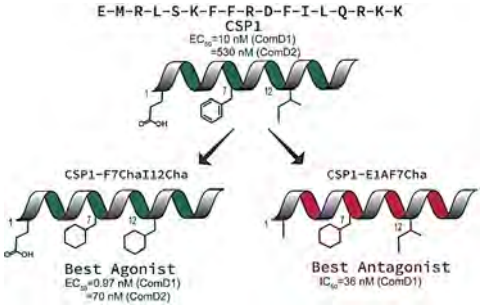
Prenylation is an essential biological process involved in many signal transduction pathways. Prenylation generally involves three enzymatic steps; transfer of an isoprenoid moiety to the cysteine of a C-terminal CaaX sequence (C is cysteine, a is an aliphatic amino acid, and X is a variable amino acid dictating whether a farnesyl or longer geranylgeranyl chain is added), proteolytic removal of the aaX sequence by ZMPSTE24 or RCE1, and finally carboxymethylation of the newly exposed C-terminal cysteine by ICMT. The system historically used for studying prenylation is the yeast mating pheromone a-Factor, a dodecameric peptide. Producing this and other prenylated peptides presents three key challenges: The C-terminal-methyl-ester is not readily available through traditional SPPS, the terminal cysteine must be chemoselectively modified with prenyl chain, and often there are several cysteines in the sequence which necessitates additional orthogonal protecting groups. In this work, the syntheses of two classes of prenylated peptide probes overcoming these challenges are described. The first class is based on the structure of a-Factor and contains a photoswitching diazobenzene within the prenyl chain, which is introduced using an optimized Zn mediated thiol alkylation reaction. These peptides are used to control prenylation through light-switching. Another class of peptides include the hexadecameric C-terminal fragment of the prenylated and palmitoylated N-Ras protein. This peptide contains a prenylated C-terminal methyl ester cysteine produced through side chain anchoring methodology, and a second upstream free thiol cysteine obtained though photoremovable nitrodibenzofuran protecting group. These peptide probes highlight efficient strategies for overcoming synthetic challenges associated with the production of lipidated peptides.

### P012 Employing Bulky, Hydrophobic Nonproteogenic Amino Acids to Create Potent Quorum Sensing Modulators in *Streptococcus Pneumoniae*

T. A. Milly[c], A. R. Buttner[b], E. R. Engler[b], N. Rieth[b], E. Hutnick[b], K. Chichura[b], A. R. Campanella[a], B. Koirala[c], M. Lella[c], Y. Tal-Gan[c], M. A. Bertucci[a]

[a]*Department of Chemistry, Lafayette College, 701 Sullivan Rd., Easton, PA 18042;* [b]*Department of Chemistry, Moravian University, 1200 Main Street, Bethlehem, PA 18018;* [c]*Department of Chemistry, University of Nevada, Reno, 1664 North Virginia Street, Reno, NV 89557*

The notorious human pathogen *Streptococcus pneumoniae* uses quorum sensing, a density-dependent means of chemical communication, to regulate the unified expression of genes that enhance its pathogenicity. The chemical signals that mediate quorum sensing in this genus are known as Competence-Stimulating Peptides (CSPs). CSP-1 is a 17-amino acid ¤-helical peptide that contains a patch of hydrophobic residues critical in binding its cognate receptor (ComD1) and initiating QS. Using bulky, hydrophobic nonproteogenic amino acids, we saw an opportunity to design CSP-1 derivatives to strengthen the receptor binding interaction and increase the proteolytic stability of the peptide. In doing so, a series of QS modulators with low nanomolar and even picomolar potency were produced with reduced rates of proteolysis in the presence of trypsin and chymotrypsin. In addition to yielding novel QS inhibitors for *S. pneumoniae*, our work also highlights the benefits of employing specific nonproteogenic substitutions, like cyclohexylalanine for phenylalanine, as part of routing structure-activity screens.




### YI-P013 Systematic Development and Biological Evaluation of Novel Peptide Inhibitor Targeting KDM5C Demethylase

V. Lukinović, H. Adhikary, M. Hoekstra, A. Chopra, K.K.Biggar

*Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada*

Development and progression of many types of cancer are under the epigenetic and cell signaling control of gene expression through post-translational modifications (PTMs) of proteins, dysregulation of which often occurs in human pathologies. Lysine methylation signaling is a dynamic and reversible PTM, that regulates biological functions through specific activities of lysine methyltransferases (KMT) and demethylases (KDM) enzymes. With continuously increasing evidence of activity of these enzymes related to disease progression, there is a need for implementing novel approaches to modulate their activity. So far, investigations are mainly oriented towards development of small molecule drugs. However, with the high off-target effect as their main disadvantage, there is a need for novel therapeutics approaches.

Here we bring novel methodology to develop peptide-based inhibitors. Our strategy involves use of lysine-oriented peptide libraries (K-OPL), whereby systematically reducing degeneracy enables us to identify amino acids critical for binding to our target protein – demethylase enzyme KDM5C. Final peptide candidates were biochemically characterised, where the most potent peptide exhibited an $IC_{50}$ of 0.97 nM, a $K_d$ of 0.11 ± 0.03 nM for KDM5C, and specificity within the KDM5 family. We further show that this peptide inhibitor is able to penetrate the cell and affect the activity of KDM5C in colorectal carcinoma cell line. Finally, we show that peptide is also able to significantly inhibit tumor growth (TGI) by nearly 50% in HCT116 xenografts. Our approach to inhibitor development offers novel strategy to identify target specific peptide-based therapeutics for various enzymes that exhibit abnormal activity in diseases.

### P014 Synthesis on Solid Phase of Antiviral HPIC-Containing Cyclohexapeptides

Ryan Baker and Antoine Blanc*
*Department of Chemistry and Biochemistry, SUNY Oneonta, Oneonta, NY 13820, USA.*
**antoine.blanc@oneonta.edu*

In Asia, tea or traditional Chinese medicine granules of *Melicope pteleifolia* are employed for the treatment of various diseases or symptoms, including cerebritis, eczema, dermatitis, rheumatoid arthritis, cold, and flu. In late 2020, scientists may have identified the Melicope pteleifolia's medicinal active ingredients, named Melicoptelines.[1] Among Melicoptelines, Melicoptelines C and D are 3*a*-hydroxyhexahydropyrrolo[2,3-*b*]indole-2-carboxyl (HPIC)-containing cyclohexapeptides displaying an antiviral activity against Influenza A Virus. HPIC-containing cyclopeptides, have shown promising, antiviral, bactericidal and tumoricidal properties.[2] So far, chemists have accomplished synthesis of only a few HPIC-containing cyclopeptides, due to the tedious extraction process from natural resources and challenging synthesis. Consequently, their potential medicinal properties are difficult to unravel, and scientists seek to develop a robust method to achieve their synthesis. Here we report the solid phase synthesis of Melicoptelines C and D, as well as Melicopteline analogs. The synthesis of Melicoptelines represent a good starting point in our chemical and biological exploration of HPIC-containing cyclopeptides. Moreover, this work lays down the foundation for a one-bead-one-compound (OBOC) combinatorial HPIC peptide library to improve the Melicoptelines antiviral activity.

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[2]Blanc, A. & Perrin, D. M. *Pept. Sci.* 2019, *111*, e24082.

### P015 Late-stage N-alkylation of Azapeptides

Maxwell Bowles, Caroline Proulx
*Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204, USA.*

Azapeptides are unnatural peptide mimics in which one or more α-carbon is substituted for a nitrogen. This substitution has been shown to improve protease stability, restrict the conformation of the peptide backbone, and perturb the pKa's of the surrounding nitrogen atoms (NH).1 In this work, we take advantage of the variations in acidity to chemoselectively introduce diverse side chains onto internal aza-amino acid residues, using Leu-enkephalin as a model peptide. We demonstrate that dialkylations and sequential mono-alkylations are also possible, providing access to underexplored N1,N2-disubstituted azapeptides. Through a combination of MS/MS sequencing, Edman degradation and NMR, we demonstrate that the installation of these side chains is highly selective for the aza-amino acid over any other backbone nitrogen. This late-stage functionalization of azapeptides allows for the expeditious creation of a library of >15 highly substituted peptidomimetics from a single compound.1Proulx, C.; Sabatino, D.; Hopewell, R.; Spiegel, J.; Ramos, Y. G.; Lubell, W. D. Azapeptides and Their Therapeutic Potential. Future Med. Chem. 2011, 3, 1139.

### YI-P016 Elucidating the Role of the Competence Regulon Quorum Sensing Circuitry in *Streptococcus Cristatus*

Alec Brennan*, Yftah Tal-Gan*
**University of Nevada, Reno*

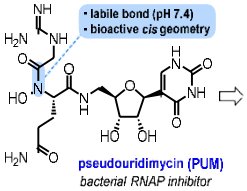
Understanding bacterial communication mechanisms is imperative to improve our current understanding of bacterial infectivity, as well as find alternatives to antibiotic treatment, which is rapidly becoming less effective. Of particular interest are Gram-positive bacteria known as streptococci. These bacteria accomplish competence via the competence stimulating peptide (CSP)-mediated quorum-sensing (QS) pathway known as the *comABCDE* regulon. Many streptococci are considered opportunistic pathogens and possess natural competence for genetic transformation that allows for the acquisition of antimicrobial resistance through the use of the competence regulon. *Streptococcus cristatus* is a recently reclassified species that is incredibly understudied, thus elucidation of the specific QS mechanisms used by this species is of great importance. Natural competence in this species indicates that it can serve as a reservoir for antibiotic resistance genes, aiding in the development of antibiotic resistance in other, more pathogenic species. Additionally, as a member of the mitis group of streptococci, *S. cristatus* shares many biological mechanisms and QS features with closely related species, including *S. oligofermentans*. As such, *S. cristatus* has the potential to serve as a biotherapeutic by inhibiting the pathogen *S. mutans* in a similar fashion as *S. oligofermentans*. Modulation of the QS pathway through modification of the CSP is imperative in gaining a comprehensive understanding of QS in *S. cristatus*. This study seeks to develop peptide-based analogs derived from the native CSP signal to directly modulate QS, ultimately attenuating bacterial infectivity and virulence, exploring beneficial proliferative phenotypes, and ultimately circumventing the development of antibiotic resistant bacterial species.

### YI-P017 Transcription Block Survival: A Novel Peptide Library Screening Assay

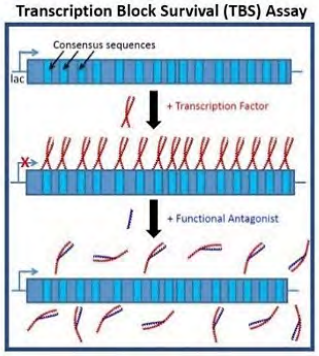
Andrew Brennan and J.M. Mason
*Department of Biology & Biochemistry, University of Bath, Bath BA2 7AY, U.K.*

Transcription Block Survival (TBS) is a high-throughput, intracellular peptide screening platform to derive functional transcription factor antagonists. This assay has been utilised to target the transcriptional regulator exemplar cJun which interacts with TRE sites on DNA and is upregulated or overexpressed in a range of diseases. Protein-DNA interactions are compelling yet underexplored targets and harbour significant promise in drug development.

During TBS, TRE sites are introduced into the coding region of the essential gene dihydrofolate reductase (TRE-mDHFR). Introduction of cJun into cells containing TRE-mDHFR causes RNA polymerase to be sterically hindered producing a transcriptional block, leading to cell death. *In vivo* library screening of ~130,000 peptides



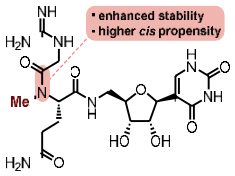

identified a sequence that both binds cJun *and* antagonises function as demonstrated by restored cell growth due to TRE-mDHFR transcription, and subsequent *in vitro* hit validation. TBS is an entirely tag-free genotype-to-phenotype approach, selecting desirable attributes such as high solubility, target specificity and low toxicity within a complex cellular environment. It facilitates rapid library screening to accelerate the identification of therapeutically valuable sequences.




### YI-P018 Synthesis of the RNAP Inhibitor Pseudouridimycin and Dipeptide-modified Analogues

Christopher F. Cain, Aaron M. Scott, and Juan R. Del Valle*
*Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA*

RNA polymerase (RNAP) catalyzes the transcription of DNA into RNA in both eukaryotic and bacterial cells. Due to protein evolutionary divergence, bacterial RNAP is a viable target for antibiotic treatment in humans. Pseudouridimycin (PUM) is a naturally occurring C-nucleoside dipeptide antibiotic isolated from *Streptomyces albus* in 2014. PUM inhibits bacterial RNAP *in vitro* in the mid-nanomolar range, with greater than 10-fold selectivity over human and viral RNAP. It exhibits antibacterial activity against Gram-positive, Gram-negative, and drug-resistant strains without significant toxicity to human cells and elicits a 10-fold lower rate of spontaneous resistance in *S. pyogenes* RNAP versus rifampicin (Rif). Structural features of this natural product include a ß-pseudouridyl moiety and a dipeptide tail consisting of N-guanidinoglycine and N-hydroxyglutamine residues. Here, we describe the total synthesis of PUM and the structure-based design of dipeptide analogues with improved physiochemical and biological properties. These analogues specifically address two significant decomposition pathways that limit PUM's therapeutic potential. We further explore key conformation-activity relationships through modifications to the hydroxamate bond within the dipeptide subunit.




### YI-P019 Aspartimides in Ribosomally Synthesized and Posttranslationally Modified Peptides (RiPPs)

Li Cao[†] and A. James Link[†,‡,§]
[†]*Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544, United States*
[‡]*Department of Chemistry, Princeton University, Princeton, NJ 08544, United States*
[§]*Department of Molecular Biology, Princeton University, Princeton, NJ 08544, United States*

Aspartimides are known to be notorious in the solid-phase peptide synthesis and in pharmaceutical formulations as undesired side products. In our work, we discovered natural products, specifically ribosomally synthesized and posttranslationally modified peptides (RiPPs), in which aspartimide is installed intentionally. This abstract will focus on the discovery, characterization, and biosynthesis of two aspartimidylated lasso peptides, cellulonodin-2 and lihuanodin. The aspartimide in these peptides is installed by a dedicated *O*-methyltransferase, a protein L-isoaspartyl methyltransferase (PIMT) homologue. We confirmed the existence of the aspartimide moiety by solving the solution nuclear magnetic resonance (NMR) structure of lihuanodin and by analyzing the hydrolysis and hydrazinolysis products of both peptides. The aspartimides in cellulonodin-2 and lihuanodin exhibit some stability, even at elevated temperatures, in low ionic strength environments. In the presence of high ionic strength buffers, surprisingly, the hydrolysis of aspartimides is regioselective to give aspartate and essentially no isoaspartate. In addition, we reconstituted PIMT-mediated aspartimide formation in vitro, showing that lasso peptide-associated PIMTs transfer methyl groups very rapidly as compared to canonical PIMTs. Furthermore, the cognate methyltransferases could re-dehydrate the hydrolyzed peptides, suggesting that the aspartimide-containing cellulonodin-2 and lihuanodin are the intended natural products in the native hosts. Given that these RiPP-associated PIMTs have a unique C-terminal domain of ~110-180 aa compared to canonical PIMTs, these enzymes provide a handle for discovering novel aspartimide-containing peptides in other RiPP families, including examples shown in lanthipeptides and graspetides.

**P020** **Quantitative Estimation of Serum Half-Life of Linear Unconjugated Peptides using Amino Acid Residues Sequence**

Marco Cavaco, Javier Valle, Isabel Flores, David Andreu, Miguel A. R. B. Castanho[1]
*1. Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal*

Proteolytic instability is a critical limitation for peptide-based products. Although significant efforts are devoted to stabilize sequences against proteases/peptidases in plasma/serum, such approaches tend to be rather empirical, unspecific, time-consuming, and frequently not cost-effective.

First, we conducted a meta-analysis of curated literature reporting experimental data on the lifetimes of unmodified peptides exposed to proteolytic conditions. Then, we developed a multivariable regression model to unravel those peptide properties with most impact on proteolytic stability and thus potential $t_{1/2}$ predicting ability. Model validation was done by two different approaches: (i) a library of peptides spanning a large interval of properties that modulate stability was synthesized and their $t_{1/2}$ in human serum were experimentally determined; and (ii) the $t_{1/2}$ of 21 selected peptides approved for clinical use or in clinical trials were recorded and matched with the model-estimated values. With both approaches, good correlation between experimental and predicted $t_{1/2}$ data was observed.

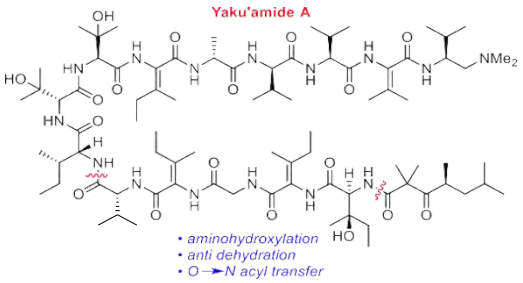
From a set of sequence-dependent variables, four were found to impact significantly on $t_{1/2}$, namely, the presence of nonpolar residues, the presence/absence of Trp and/or Tyr, and electric charge as gauged by the isoelectric point, pI. Computing this characteristics on a single equation enables estimation of $t_{1/2}$ of any peptide sequence in an easy-to-use straightforward way. This equation is therefore not only a valid explanatory tool, but also has proven predictive power for use in peptide drug development strategies.

**P021** **Synthesis and Anticancer Activity of Yaku'amide A and Analogs**

Concordia C.-L. Lo, David W. Kastner, Daniel Joaquin, Diego A. Moyá, Alexander Ramos, Stephen M. White, Blake L. Christensen, and Steven L. Castle*
*Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT*

Total synthesis of the anticancer peptide yaku'amide A and two full-length analogs will be described. The ß-*tert* hydroxy amino acids were constructed via regioselective aminohydroxylation involving a chiral mesyloxycarbamate reagent. Stereospecific construction of the *E*- and *Z*-ΔIle residues was accomplished via a one-pot reaction featuring *anti* dehydration, azide reduction, and O→N acyl transfer. Alkene isomerization was negligible during this process. These methods enabled a highly convergent and efficient synthetic route to the natural product.

Then, two promising analogs were identified by computational studies in which the three *E*- and *Z*-ΔIle residues of the natural product were replaced by the more accessible dehydroamino acids ΔVal and ΔEnv. Anticancer screening revealed that the two analogs mimic the potent anticancer activity of yaku'amide A, thereby validating the computational studies.




**YI-P022** **Towards Biochemical Characterization of Protein Pyrophosphorylation**

Arif Celik [1,2], Jeremy A. Morgan [1], and Dorothea Fiedler [1,2]
[1] *Leibniz-Forschungsinstitut für Molekulare Pharmakologie, 13125 Berlin, Germany*
[2] *Humboldt University Berlin, Institute for Chemistry, 12489 Berlin, Germany*

Protein pyrophosphorylation is a poorly characterized posttranslational modification (PTM) with unknown functions in living organisms. Compared to kinase mediated protein phosphorylation, pyrophosphorylation is thought to be installed non-enzymatically, when a highly phosphorylated inositol pyrophosphate (PP- InsPs) transfers its high energy ß-phosphoryl group onto the pre-existing phosphoryl group of a protein substrate.[1] The Fiedler research group recently investigated mammalian cells using a novel MS/MS approach[2] and identified numerous endogenous pyrophosphorylation sites. These included the nucleoside diphosphate kinase A (NME1), the only known mammalian histidine kinase which is linked with metastasis suppression activity. In this work, the synthesis of site-selectively pyrophosphorylated NME1 (ppNME1), stoichiometrically modified at the endogenous site was attempted in order to assess the effect of this modification on protein activity. Biochemical characterization showed high Nucleoside diphosphate kinase A activity for the wildtype, whereas the phospho- and pyrophospho mutants had clearly reduced activity. *In vitro* autophosphorylation demonstrated the formation of the 1-phosphohistidine (1-pHis) for all mutants, however, only ppNME1 displayed an unreported hyperphosphorylation event. Besides enabling the generation of the first pyrophosphorylated histidine kinase NME1, this work highlights the impact of the pyrophosphorylation on the protein activity and indicates a novel and unreported auto hyperphosphorylation activity for ppNME1.

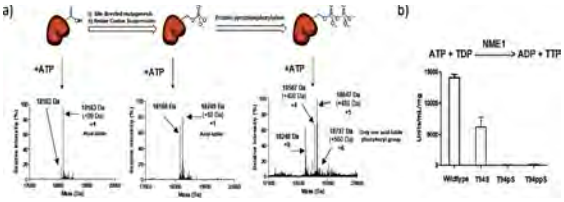


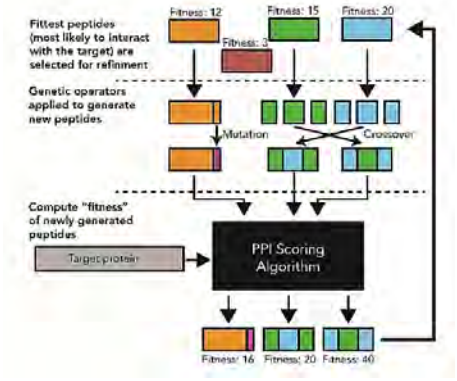

*Figure 1: a) Expression and synthesis of NME1 (wildtype, T94pS, and T94ppS) with subsequent in vitro autophosphorylation assay, b) Nucleoside diphosphate kinase activity of all generated NME1 mutants.*

References:
[1] Saiardi, A., Science, 2004, 306, 2101-2105.
[2] Penkert, L., Anal. Chem., 2017, 89, 3672-3680.
[3] Marmelstein, A. M., Chem. Sci, 2018, 9, 5929-5936.

### YI-P023 Darwin: An Evolution-Inspired Algorithm for Target-specific Peptide Inhibitor Engineering

François Charih[1,2], James R. Green[2] and Kyle K. Biggar[1]
[1]*Institute of Biochemistry, Carleton University, Ottawa, ON, Canada* [2]*Department of Systems and Computer Engineering, Carleton University, Ottawa, ON, Canada*

Owing to recent advances in deep learning, a variety of computational models capable of producing bioactive peptides have been developed. These methods generally model the distributions of peptides with a desired bioactivity (eg. anti-microbial, anti-cancer, *etc.*) to then sample from these distributions to produce slightly different peptides. These methods are limited, however, as they do not allow for the development of peptides that interact with a specific target. Building on top of the *In Silico* Protein Synthesizer (InSiPS)[1] we developed Darwin, a computer program that leverages knowledge encoded in validated protein-protein interactions to develop target-specific peptide inhibitors. Darwin combines a state-of-the-art protein-protein interaction predictor and a genetic algorithm to produce peptide candidates ready for validation within a matter of days on a reasonably powered personal computer or hours, on a computer cluster. Our hope is that Darwin will enable biologists to design peptide inhibitors for therapeutic applications or biological investigations faster than its predecessor.

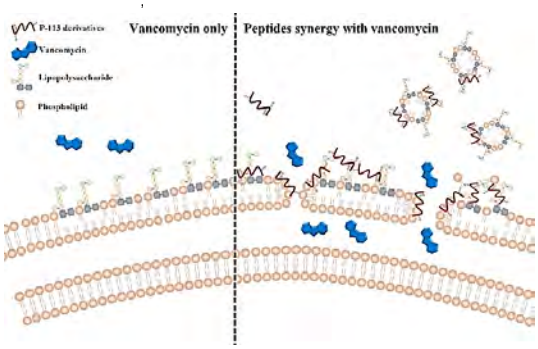



[1]Schoenrock, A. *et al.* Engineering inhibitory proteins with InSiPS: the in-silico protein synthesizer. *Sc15 Int Conf High Perform Comput Netw Storage Analysis* 25 (2015) doi:10.1145/2807591.2807630.

### P024 Antimicrobial Peptides Display Strong Synergy with Vancomycin Against Vancomycin-Resistant *E. faecium*, *S. aureus*, and Wild- Type *E. coli*

Jya-Wei Cheng
*Institute of Biotechnology, National Tsing Hua University, Hsinchu 30043, Taiwan*

There is an urgent and imminent need to develop new antimicrobials to fight against antibiotic-resistant bacterial and fungal strains. In this study, a checkerboard method was used to evaluate the synergistic effects of the antimicrobial peptide P-113 and its bulky non-nature amino acid substituted derivatives with vancomycin against vancomycin-resistant *Enterococcus faecium, Staphylococcus aureus*, and wild-type *Escherichia coli*. Boron-dipyrro-methene (BODIPY) labeled vancomycin was used to characterize the interactions between the peptides, vancomycin, and bacterial strains. Moreover, neutralization of antibiotic-induced releasing of lipopolysaccharide (LPS) from *E. coli* by the peptides was obtained. Among these peptides, Bip-P-113 demonstrated the best minimal inhibitory concentrations (MICs), antibiotics synergism, bacterial membrane permeabilization, and supernatant LPS neutralizing activities against the bacteria studied. These results could help in developing antimicrobial peptides that have synergistic activity with large size glycopeptides such as vancomycin in therapeutic applications.




### YI-P025 Design and Synthesis of R-spondin-1 (RSPO1) Mimetics

Ramesh M. Chingle, and Terrence R. Burke, Jr.*
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute-Frederick, Frederick, MD 21702, USA*

The leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is highly over-expressed in various stages (I and II) of colorectal cancers. R-spondin-1 (RSPO1) is a protein that binds to the extracellular domain of LGR5 proximal to the binding site of the transmembrane E3 ubiquitin ligase RING finger 43 (RNF43) (PBD: 4KNG;

Figure 1A). Major binding interactions between LGR5 and RSPO1 have been shown to occur via residues F106 and F110 within the bis-disulfide-containing K96 - K113 sequence situated in the C- terminal Fu2 domain (we term this the "Right Side"; Figure 1B). Secondary interactions also play a role in the binding of RSPO1. These are provided by the R87 residue contained within the P77 - I95 sequence in the mono-disulfide cyclized N-terminal Fu1 domain (we term this the "Left Side"; see Figure 1B). We are designing and synthesizing RSPO1 mimetics that entail use of SPPS to construct first the Right Side 18-mer and the Left Side 19-mer. These two segments are then ligated together using azide-alkyne click chemistry to form a full "37-mer" construct. Tagging these RSPO1 mimetics with suitable dyes may render useful in intraoperative fluorescence molecular imaging of colorectal cancer during surgical resection procedures.

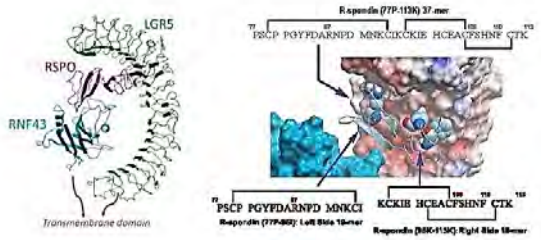



Figure 1. (A) Crystal structure of the LGR5–RSPO1–RNF43 ternary Complex.
(B) Surface and Ribbon depictions of residue P77-K113, important key residues R87, F106, F110 are shown in space filling.

1 Steege, E. J. T.; and Bakker, E. R. M. Oncogene, 2021, 40, 6469–6478.
2 Kumar, K. K.; Burgess, A. W.; Gulbis, J. M. Protein Sci. 2014, 23, 551 565. 3 Peng, W. C.; Lau, W. de; Forneris, F.; Granneman J. C. M.; Huch, M.; Clevers, H.; Gros, P. 2013, Cell reports, 3, 1885–1892.

### YI-P026 Synthesizing Oxy-Labelled Micelles for NMR Structure Probing of Membrane-Interacting Antimicrobial Peptides

S. Chiorean[a,b], I. Antwi[a,c], and J. C. Vederas[a]
[a] *Department of Chemistry, University of Alberta, Edmonton, AB, CAN*
[b] *Department of Chemistry, Scripps Research, La Jolla, CA, USA*
[c] *Department of Chemistry, Yale University, New Haven, CT, USA*

As the threat of antimicrobial resistance grows, antimicrobial peptides have shown great potential as alternatives to currently available antibiotics. A significant number of these peptides interact with a receptor on the bacterial membrane surface prior to exhibiting their lethal effects on the cell.[1] These membrane-interacting peptides often undergo a structural change when they encounter their target receptor or a lipid-based solvent system. We endeavored
to probe the peptides' mechanism of action at the cell surface membrane by observing changes in their nuclear magnetic resonance (NMR) profile when exposed to membrane-mimicking micelles.

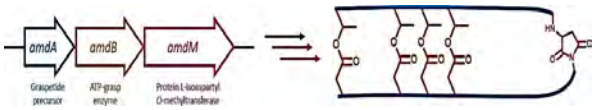
A series of deuterated dodecylphosphocholine (DPC) oxy-analogues were synthesized, with oxygen replacing a methylene at several positions along the DPC lipid chain. This allowed us to observe the chemical shift changes of peptides as they interact with the surface, middle, and inner part of the DPC micelle. Tridecaptin $A_1$, a lipopeptide that targets cell membrane precursor lipid II,[2] was chosen as the first peptide to be studied by NMR in our various oxy-DPC micelle systems. We found that chemical shift perturbations across the different micelle systems vary, indicating that the microenvironments created by the oxy-modified lipids can be detected by the residues within the peptide closest to the oxygen. This brought insights into how tridecaptin $A_1$ is oriented within the micelle and which residues could be involved in membrane interaction and receptor binding. Future work will look at expanding this methodology to other membrane-interacting antimicrobial peptides.

[1] M. Mahlapuu *et al. Front Cell Infect Microbiol*. 2019, *6*, 194.
[2] S. A. Cochrane *et al. Proc Natl Acad Sci*. 2016, *113*, 11561.

### YI-P027 Enzyme-mediated Sidechain and Backbone Cyclization of a Tetracyclic Graspetide

Brian Choi[*], Li Cao[*], and A. James Link[*†‡] *Department of Chemical and Biological Engineering, Princeton University, Princeton, New Jersey 08544, United States*
[†] *Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States*
[‡] *Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, United States*

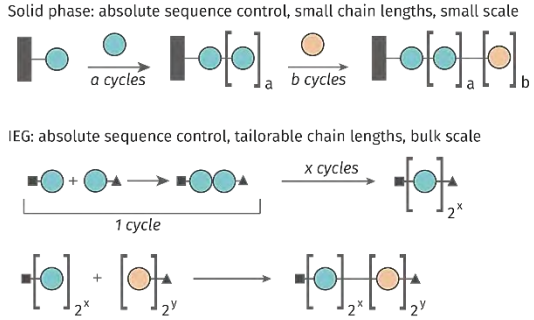
Both synthetic and natural macrocyclic peptides have shown intriguing activities against pharmaceutical targets, steadily gaining attention as drug candidates. Graspetides are bacterial macrocyclic peptides with protease inhibition activities and diverse patterns of sidechain-sidechain ester and amide linkages. Here, we characterize a 29-aa long graspetide with a novel backbone aspartimide. This additional post-translational modification is performed by an enzyme homologous to protein L-isoaspartyl *O*-methyltransferase (PIMT). The 2D NMR experiments, comprised of TOCSY, NOESY, HSQC and HMBC, show that the aspartimidylated graspetide has a tetracyclic hairpin structure, which is novel amongst the graspetide structures characterized so far. Each macrocycle contains 38, 22, 22 and 34 atoms, from the innermost to outermost macrocycles. Additionally, we demonstrate that the ATP-grasp enzyme installs the four macrolactone linkages vectorially, starting from the inner loop, then outwards. On the other hand, the methyltransferase shows an exceptional specificity to the pre-aspartimidylated amycolimiditide. This is in stark contrast to canonical PIMTs, which exhibit broad substrate tolerance.



**YI-P028** **Iterative Exponential Growth for Sequence-Controlled Peptoid Polymers**

A.M. Clapperton[a] and H. Tran[a,b]
[a] *Department of Chemistry, University of Toronto, 80 St. George Street LM 514, Toronto, ON, M5S 3H6, Canada*
[b] *Department of Chemical Engineering, University of Toronto, 200 College St, Toronto, Toronto, ON, M5S 3E5, Canada*

The precise structural organization and presentation of functional domains within sequence- defined biopolymers such as proteins allows for highly selective interactions with various ligands involved in signalling and recognition pathways. Peptoids, a class of peptidomimetic polymers, draw inspiration from these ordered protein scaffolds and can self-assemble into two- dimensional nanosheets with highly tunable surfaces for the display of molecular recognition elements relevant for biosensing. The stepwise solid-phase synthesis of peptoids offers unmatched sequence-control but is limited by the reaction scale (typically 100 milligrams) and attainable chain lengths (typically a maximum of 30 repeat units). Solution phase alternatives can be run on larger scales but sacrifice the sequence-control necessary for the precise ordering of recognition motifs for biosensing. To address the need for bulk samples in materials applications while maintaining sequence-control, we present the use of iterative exponential growth as a scalable method for the sequence-specific synthesis of peptoid polymers with chain lengths greater than the 30 repeat units typically obtained with solid-phase techniques.




**YI-P029** **A Secreted Bacterial Protein Mediates the Neutralization of Cationic Antimicrobial Peptides by Sequestration into Phase Separated Droplets**

Gregory B. Cole[1] Nicholas K. H. Ostan[1], Gaelen Moore[1], Hyun Kate O. Lee[1], and Trevor F. Moraes[1]
[1] *University of Toronto, Department of Biochemistry*

Mammalian hosts combat bacterial infections by the production of defensive cationic antimicrobial peptides (CAMPs). These immune factors are capable of directly killing bacterial invaders, however many pathogens have evolved resistance evasion mechanisms such as cell surface modification, as well as CAMP sequestration, degradation, or efflux. We have discovered that several pathogenic and commensal proteobacteria, including the urgent human threat *N. gonorrhea*, secrete a protein (LbpB) that presents a low complexity, disordered, anionic domain capable of inhibiting the antimicrobial activity of host CAMPs. This study focuses on a cattle pathogen, *Moraxella bovis* that expresses the largest anionic domain of the LbpB homologues. We used an exhaustive structural biology approach employing CD, cross-linking mass spectrometry, and SEC-MALS-SAXS to understand the structure and dynamics of LbpB. We found that the anionic domain of LbpB displays alpha helical secondary structure but is highly dynamic and lacks a rigid tertiary fold. The addition of antimicrobial peptides derived from lactoferrin (i.e. lactoferricin) to the anionic domain of LbpB or full length LbpB results in the phase separation of LbpB and the antimicrobial peptides into coacervate droplets that can be visualized under bright field microscopy and characterized using FRAP and turbidimetry. The presence of full length LbpB or its intrinsically disordered anionic domain was able to protect bacteria from CAMP mediated killing. Our data suggests that pathogens like *Moraxella Bovis* leverage anionic intrinsically disordered domains for the broad recognition and neutralization of antimicrobials via formation of biomolecular condensates.

**P030** **Fiber Optic Array Scanning Technology (FAST) Mega-Throughput Screening of Large OBOC Non-Natural Peptide Libraries for the Discovery of SARS_CoV2 S1 Protein Affinity Agents and Therapeutics**

M. Avital-Shmilovici, C. Apostol, P.B. Madrid, X. Liu, D. Wang, T. Shaler, N. Collins
*SRI Biosciences, SRI International, Menlo Park, CA 94025 USA*

We have recently reported a new methodology that enables the production and screening of libraries of natural and non-natural peptides up to 50+ residues in length comprising $10^7$ to $10^9$ compounds[1]. Using a self-readable polymer design that facilitates sequencing sensitivity at fmol scale of peptide material, libraries can be synthesized on 10 – 20 micron diameter beads to maximize library size while minimizing cost of synthesis. The screening method utilizes a Fiber-optic Array Scanning Technology (FAST) for screening of OBOC libraries against fluorescently labeled targets, with an unprecedented throughput of 5 million beads per minute. This method has been demonstrated to identify non-natural peptide affinity agents and PPI inhibitors with sub-nanomolar to pico-molar affinities for challenging targets such as KRAS, IL6 and TNFa. Here we describe the synthesis of libraries with increasing incorporation of synthetic drug like building blocks combined with peptide backbones to modulate biostability, immunogenicity and pharmacokinetic properties that are more similar to small molecules and antibodies than peptides alone. We screened these hybrid peptide libraries for the rapid discovery of high affinity agents for the SARS_CoV2 S1 receptor binding domain (SRBD) in the presence and absence of ACE2 to identify general and competitive binders. Synthetic optimization of these non-natural peptide hits for drug like properties as well as aproaches to developing therapeutics from these types of affinity agents will be presented.

[1] M. Avital-Shmilovici, X. Liu, T. Shaler, A. Lowenthal, P. Bourbon, J. Snider, A. Tambo-Ong, C. Repellin, K. Yniguez, L. Sambucetti, P. B. Madrid, N. Collins. *ACS Cent. Sci.* 2022, 8, 1, 86–101

### P031 Identification of Lactate-Induced Lactylation in Human Skeletal Muscle

L. A. Consitt, D. Maschari, G. Saxena, T. Law, and E. Walsh.
*Department of Biomedical Sciences, Ohio University, Athens, Ohio 45701, USA*

Background: Skeletal muscle insulin resistance is a characteristic feature of type 2 diabetes and plays a prominent role in the development of this disease. Lactate, a metabolite by-product of anerobic metabolism is associated with insulin resistance and is responsible for the novel post-translational modification, lactylation which involves the addition of lactyl groups to lysine (K) residues. To date, no known research has determined if lactylation occurs in skeletal muscle, the main source of lactate production. Purpose: 1) determine if lactate-induced lactylation occurs in primary human skeletal muscle cells (HSkMC) and 2) determine if lactylation in skeletal muscle is associated with insulin resistance in humans. Methods: Human myotubes were incubated with sodium lactate for 24 hours to examine the effects of exogenous lactate on lactylation levels using a pan anti-Kla antibody and western blot technology. Lactylation levels were measured in response to the manipulation of endogenous lactate levels via pharmacological and siRNA technology. Lactylation levels were measured in the skeletal muscle from 15 lean, insulin sensitive individuals and 15 obese insulin resistant individuals. Results: Treatment of primary HSkMC with exogenous lactate increased protein lactylation in a dose-dependent manner (P<0.01). Myotube lactylation decreased (P<0.05) in response to pharmacological inhibition of glycolysis (2-DG) and inhibition of LDH-A activity (oxamate) and expression (LDH-A siRNA). Skeletal muscle lactylation was negatively associated with insulin sensitivity in lean and obese individuals (P<0.05). Conclusion: These findings demonstrate lactate-induced lactylation occurs in human skeletal muscle and is elevated in the skeletal muscle of obese, insulin-resistant individuals.

### YI-P032 Inhibition of *Aspergillus Nidulans* Biofilm by *Ps*d2 Pea Defensin

C. Corrêa-Almeida[1], L. P. Borba-Santos[1], R. Rollin-Pinheiro[2], E. Barreto-Bergter[2], S. Rozental[1], E. Kurtenbach[1]
[1] *Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, RJ, Brazil.*
[2] *Institute of Microbiology Paulo de Goés, Federal University of Rio de Janeiro, RJ, Brazil.*

Approximately three million people have *Aspergillus* infections worldwide and due to the global pandemic scenario, these co-infections have an important impact on the morbidity and mortality of COVID-19 patients. Moreover, the ability of these fungi to form biofilms in catheters and the increase of fungi resistance to the therapeutic arsenal is required the development of new drugs that are effective in inhibiting the biofilm. *Ps*d2 is a pea defensin that possesses selective activity against planktonic cells of representative pathogenic fungi. Its activity is characterized by the interaction with lipid rafts enriched with glucosylceramide and ergosterol. Thus, this study aimed to evaluate the effect of *Ps*d2 on *Aspergillus nidulans* biofilm formation. *Ps*d2 was expressed in the recombinant yeast *Pichia pastoris* GS115. By phase contrast and spinning disk confocal microscopy it was observed that *A. nidulans* adhered to the polystyrene surface at only 4 h and from 24 h formed a robust extracellular matrix (ECM) producing biofilm, increasing in thickness, which was observed until 48 h. The optical density at 540 nm, cell viability by XTT, biomass by crystal violet, and ECM production by safranin O were also evaluated. 10 µM of pure and active *Ps*d2 inhibited 50% biofilm viability and biomass and 40% ECM production. By scanning electron microscopy was observed that *Ps*d2 decreased the colonized area in central venous catheters, reducing the length and diameter of hyphae and inhibiting the conidiophore formation. Preliminary results showed that 20 µM *Ps*d2 reduced significantly the adhesion of conidia in polystyrene surfaces.

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### P033 Discovering a D-peptide Inhibitor of SARS-CoV-2 Entry

Z.S. Cruz, S.E. Apple, J.L. Evangelista, D. M. Eckert, and M.S. Kay
*Department of Biochemistry, University of Utah, Salt Lake City, Utah 84112, United States*

SARS-CoV-2 has been responsible for >300 million infections and >5 million deaths worldwide. Despite the rapid development of vaccines and therapeutics, there is still a need for better therapeutics for the current and future coronavirus pandemics. We are developing D-peptides that bind the spike protein's highly conserved heptad repeat 1 (HR1) region to inhibit viral entry. The HR1 region forms a parallel trimeric-coiled coil that interacts with the HR2 region to form a trimer-of-hairpins (6-helix bundle) that mediates membrane fusion. Targeting HR1 with a D-peptide will prevent hairpin formation and thus viral entry. To discover such D-peptides, we used mirror-image phage display to screen random libraries of phage displaying L-peptides against a mirror-image HR1 mimic (synthesized using D-amino acids). Deep sequencing of enriched phage outputs provided sequences of tight-binding "winning" peptides. We synthesized these sequences with D-amino acids and measured their binding to L-HR1 using surface plasmon resonance. Our initial screenings of naïve peptide libraries against D-HR1 discovered an alpha-helical D-peptide, AN1, with low micromolar binding affinity to L-HR1. A crystal structure of AN1 binding to L-HR1 reveals its mode of binding and will inform affinity maturation of this initial hit. To improve AN1's affinity, we are screening secondary phage libraries based on AN1's sequence, including a statistical light mutagenesis (SLiM) library and a groove-crawl library that extends its sequence further down the HR1 groove.

### YI-P034 Submonomer Synthesis of Peptoids Containing Trans-inducing N-imino- and N-alkylamino-glycines

Carolynn M. Davern, Brandon D. Lowe, Adam Rosfi, Elon A. Ison and Caroline Proulx
*Department of Chemistry, North Carolina State University, Raleigh, NC 27695, USA*

Peptoids are a class of peptidomimetics in which the side chain is bonded to the backbone nitrogen rather than the α-carbon, giving *N*-substituted glycine oligomers.[1] Generally, these peptide mimics possess greater flexibility than their peptide counterparts because of the lack of chiral center and backbone hydrogen bond donor. Some side chains have been previously reported to restrict conformational freedom of N-substituted glycines in the *trans*- conformation, such as *N*-aryl-, *N*-hydroxy-, *N*-alkoxy-, and *N*-acylamino-glycine monomers. While these monomers are structure-inducing, they are not all easy to incorporate following the standard submonomer method and do not always have a lot of side chain diversity. In our recent work,[2] we demonstrate the use of various hydrazones as submonomers in solid phase peptoid synthesis to give *N*-imino glycine containing oligomers. Conditions to promote a one-pot cleavage of the peptoid from the resin and reduction to the corresponding *N*-alkylamino glycine were also identified. Both the *N*-imino- and *N*-alkylamino glycine residues were found to favor the *trans*-amide bond geometry by NMR, X-ray crystallography, and computational analyses. Additionally, *N*-imino- and *N*-alkylamino glycines have been patterned with positively charged *N*-substituted glycines to access peptoid oligomers that promote ribbon like structures or possess antimicrobial activity.

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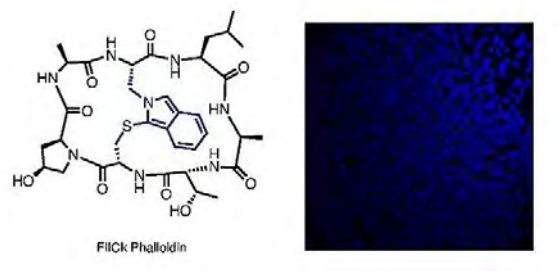
### YI-P035 FIICked Phalloidins: Staining Actin with Inherently Fluorescent Peptide Bicycles

Mihajlo Todorovic, Naysilla L. Dayanara, David M. Perrin*
*Department of Chemistry, University of British Columbia, Vancouver, BC V6K4W5, Canada*

The study and synthesis of novel peptidic constructs is highly attractive in the development of therapeutics. The way peptides interact with targets can be modulated for the regulation and probing of biological functions[1]. Herein we report the study of an emerging peptide scaffold in the form of a fluorescent isoindole intra-annular bridge and its potential application as an imaging tool. This scaffold was achieved through a novel stapling methodology called FIICk[2], where an ortho-phthalaldehyde (OPA) reacts chemoselectively with a free amine and thiol. We apply this fluorescent staple on Phalloidin, a strained bicyclic peptide ubiquitously used for staining actin. Natural Phalloidin is often conjugated to a dye, however our technology allows us to bypass conjugation entirely owing to the inherent fluorescence of the isoindole staple. This has the potential to provide facile access to molecular probes with high utility.

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### P036 Optimization of ProTx-II based Peptides as Selective Blockers of $Na_v1.7$ Channel for the Treatment of Pain

Daniele De Simone*[1], Gregory L. Adams[2], Parul S. Pall[2], Steven M. Grauer[2], Xiaoping Zhou[2], Jeanine E. Ballard[2], Marissa Vavrek[2], Richard L. Kraus[2], Pierre Morissette[2], Nianyu Li[2], Stefania Colarusso[1], Elisabetta Bianchi[1], Anandan Palani[3], Rebecca Klein[2], Christopher T. John[2], Deping Wang[2], Matthew Tudor[2], Andrew F. Nolting[2], Mirlinda Biba[4], Timothy Nowak[3], Alexey A. Makarov[4], Mikhail Reibarkh[4], Alexei V. Buevich[3], Wendy Zhong[4], Erik L. Regalado[4], Xiao Wang[4], Qi Gao[4], Aurash Shahripour[2], Yuping Zhu[3], Tommaso Frattarelli[1], Nicolo' Maria Pasquini[1], Paola Magotti[1], Roberto Iaccarino[1], Yuxing Li[2], Kelli Solly[2], Keun-Joong Lee[4], Weixun Wang, Feifei Chen[2], Haoyu Zeng[2], Jixin Wang[2], Hilary Regan[2], Rupesh P. Amin[2], Christopher P. Regan[2], Christopher S. Burgey[2], Darrell A. Henze[2], Chengzao Sun[2], and David M. Tellers[2]
*[1]Peptides and Small Molecules R&D Department, IRBM Spa, Via Pontina km 30.600, 00071 Pomezia (RM), Italy.*
*[2]Merck & Co., Inc., West Point, Pennsylvania 19486, United States.*
*[3]Merck & Co., Inc., Kenilworth, New Jersey 07033, United States.*
*[4]Merck & Co., Inc., Rahway, New Jersey 07065, United States.*

ProTx-II is a toxin derived from tarantula venom able to inhibit the human voltage-gated sodium channel 1.7 (hNaV1.7) which has been reported to be involved in nociception[1,2]. ProTxII is a 30 amino acid peptide, containing 3 disulfide bonds in a ICK (inhibitor cystine knot) motif which confers plasma stability and subcutaneous bioavailability. Despite this, ProTxII still displays poor in vivo efficacy and shows adverse effects when dosed in rats intravenously or intrathecally.

We here report the synthesis and SAR of a series of ProTxII analogues designed to optimize in vitro ion channel blocking selectivity. We investigated the effect of natural or unnnatural mutations on the large hydrophobic surface of ProTxII , which is involved in the interaction with NaV1.7 (Figure 1 in yellow) with the goal to design peptides selective for blocking of Nav1.7 over closely related NaV channels[3]. The impact of some of these mutations on the folding capacity of the linear peptide was also investigated. Furthermore, given the highly cationic character of ProTxII, SAR efforts were also focused on limiting the potential toxicity due to mast cell degranulation and histamine release in vivo[3,4].

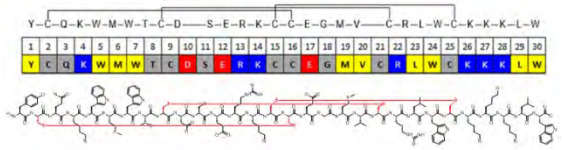


Figure 1

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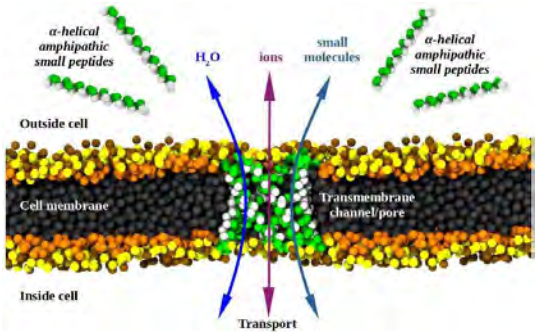
## P037 De Novo Design of Peptides that Form Transmembrane Barrel Pores Killing Antibiotic Resistant Bacteria

Rahul Deb and Robert Vácha
*CEITEC - Central European Institute of Technology, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic.*

*De novo* design of peptides that self-assemble into stable transmembrane barrel pores is challenging due to the complexity of several competing interactions involving peptides, lipids, water, and ions. Optimization of transmembrane barrel pores for specific functions is even more challenging because the generalized design principles are still missing. Here, we develop a computational approach using molecular dynamics simulations for the *de novo* design of α-helical peptides that self-assemble into stable transmembrane barrel pores with a central functional channel, i.e., capable of conducting water, ions, and small molecules across the lipid membranes. We formulate the previously missing design guidelines and report 52 sequence patterns of the peptides that can be tuned for specific applications using the identified role of each residue. Atomic force microscopy and fluorescent dye leakage experiments confirm that the designed peptides form leaky membrane pores *in vitro*. We customize the peptides to potent pore-forming antimicrobial agents able to kill even antibiotic-resistant ESKAPE bacteria at micromolar concentrations by rapid permeabilization of bacterial membranes[1], while exhibiting low toxicity to human cells. The designed peptides can be similarly fine-tuned for other medical and biotechnological applications[2], including anticancer agents[3], single molecule sensing and sequencing[4].

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## P038 How Sorbent Selection Impacts Peptide Library Clean up with SPE

Elizabeth Denton, PhD
*Biotage, LLC*

Peptides have returned to the forefront of drug discovery efforts as unbiased screening technologies have improved and the rules defining cell permeability have been clarified. As a result, the demand for synthetic peptide libraries has increased significantly, particularly during secondary screening as lead compound programs progress. At these early discovery phases though, highly pure peptide samples may not be necessary when evaluating these compound libraries in assays. With this in mind and given the improvements in automated peptide library synthesis strategies, a solid phase extraction methodology may be appropriate to parallelize peptide library cleanup and significantly reduce library purification time.

Herein we present data demonstrating that sorbent selection, specifically the sorbent parameters themselves, impacts the predictability of elution profile when using solid phase extraction to improve the purity of synthetic peptide libraries across a wide range of therapeutically relevant peptides.

## YI-P039 Biotin Orthogonal Streptavidin System (BOSS): An Application of Mirror-Image Streptavidin and Biotin

S. R. E. Draper, P. Spaltenstein, R. J. Giesler, and M. S. Kay
*Department of Biochemistry, University of Utah, Salt Lake City, UT 84112, USA*

Streptavidin (SA) and biotin have one of the strongest known binding interactions, with a $K_D$ in the femtomolar range. This extraordinary binding affinity has led to their ubiquitous use in biomedical research and diagnostics. Though SA/biotin enjoys success in many applications, several challenges limit its utility. Abundant endogenous biotin can limit assay sensitivity, especially in the context of increasingly common biotin supplementation. Most importantly for therapeutic applications, streptavidin is a highly immunogenic foreign protein. Mirror-image proteins (D-proteins, composed of D-amino acids) offer an elegant solution to these problems. D-proteins are generally inert to proteases and therefore cannot be digested for MHC presentation to the immune system. These properties mean that D-SA will have greatly decreased immunogenicity and increased half-life compared to L-SA. Additionally, by the law of mirror-image symmetry, D-SA and mirror-image biotin (L-biotin) will have identical exceptional affinity as the natural pair (L-SA and D-biotin). We have discovered through isothermal titration calorimetry that this interaction is highly stereospecific, with nearly a billion-fold preference for the natural ligand over the mirror-image ligand. This result suggests that D-SA and L-biotin can be used in living systems without endogenous biotin

interference, thus acting as a biotin orthogonal streptavidin system (BOSS). As a first step towards developing BOSS, we have synthesized L-SA using a three-segment native chemical ligation strategy. The insoluble first segment required an N-terminal solubilizing "helping hand" tag to increase solubility. After synthesizing the full-length product, we removed the solubilizing tag in a scarless manner to yield full-length native SA. We are now repeating this optimized synthesis to make D-SA.

### P040 Prodrug-Based Intracellular Delivery of Zerumbone for Breast Cancer Therapy

Eltayeb E.M. Eid
*Department of Pharmaceutical Chemistry and Pharmacognosy, Unaizah College of Pharmacy, P.O.BOX 5888 Unaizah 51911, Qassim University, Saudi Arabia Email: eem.eid@qu.edu.sa*

The idea relates to breast cancer therapy in animal model. More specifically is for breast cancer targeting therapy by releasing zerumbone (ZER) as a toxic agent intracellularly through a prodrug strategy using cell penetrating peptide as a carrier.
Brief description of the idea

A prodrug for treating breast cancer or inhibiting metastasis using an animal model is proposed. The prodrug is prepared by conjugating iRGD with ZER. iRGD is a cell penetrating peptide that has amino acids sequence (CRGDK/RGPD/EC), whereas ZER

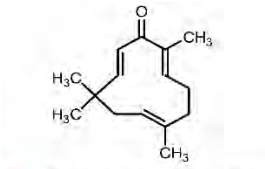



Fig. 1: The structure of zerumbone (ZER)

**(Fig 1.),** is a sesquiterpene compound isolated from tropical ginger. The conjugation will be through an acid labile or pH – responsive linker. Examples of acid labile linkers include imine group, hydrazone, cis-aconityl, ketal bond, acetal bond, carboxylic hydrazone bond and trityl bond. The conjugated delivery system, as a prodrug, will be internalized into the cancer cell via the attachment of iRGD into the αß integrin receptor in the cancer cell surface. The internalization of the designed prodrug is investigated by entry mechanism and intracellular process of iRGD-ZER into breast cancer tumor cells to determine whether iRGD carrier peptide could disturb the cancer cell biochemistry by which ZER is endocytosed. Endocytosis is well reported entry mechanism for different nanoscale drug carriers. Both iRGD and ZER will be label with two different fluorescent dyes for tracking the cleavage of iRGD- zerumbone bond.

The intracellular release of ZER as a toxic agent will be in late endosome process. In this process the pHi, being 4.0-6.5, enables the cleavage of the linker between iRGD & ZER. When the zerumbone is inside the cancer cell it will disturb the cell cycle through the elevation of caspase 3 or / and also inhibiting the activation NF-κB, resulting in cancer cell apoptosis and stop the proliferation, respectively. The iRGD will be degraded and digested in the lysosome process. The illustration in **Fig. 2**. explained the clear delivery system pathway.

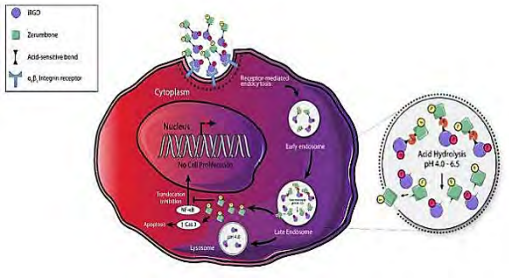



**Fig. 2:** Zerumbone (ZER) (cargo, indicated as a green square), will be conjugated with iRGD, a cell-penetrating peptide (carrier, indicated as a purple ball). The conjugation will be through an acid-labile linker or pH-responsive cleavable bond (indicated as a black line). Both iRGD and ZER should be label precisely by two different fluorescent dyes (indicated as a circle F) to track the cleavages and their sites in the intracellular regions and to validate the traceless of ZER in the extracellular compartments. Also, to avoid an altered cytotoxic activity as the result of ZER traces, the acid-labile linkers that will be used for conjugation should be stable at pHe range 6.5-7.4. The internalization of the conjugated delivery system onto the cancerous cell will be through receptor- mediated endocytosis, in which the iRGD peptide (carrying, ZER) binds to αvβ3 Integrin receptors in cancer cell surface that is usually overexpressed in the cancer cells more than the normal cells. The release of ZER as a toxic agent will take place in the late endosome process by exploiting the pHi value range 4.0 - 6.5, that considered being as a unique physical property for acid liable linker's cleavage. Then, ultimately, ZER will stop cell proliferation, or in a more explicit scientific term, it will disturb the cancer cell biochemistry.

### YI-P041 Stereochemical Modifications to Cationic Amphiphilic Polyproline Helices to Enhance Targeted Intracellular Bacteria Treatment

Andrew M. Encinas, and Jean Chmielewski
*Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA*

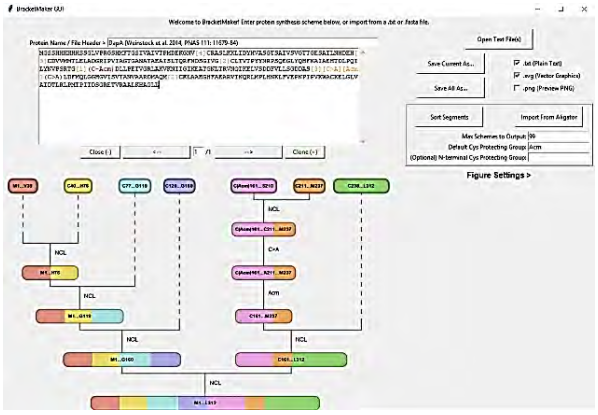
Antibiotic-resistant bacteria cause almost three million infections and 36,000 deaths each year.[1] Additionally, numerous bacteria have evolved to reside inside mammalian cells where countless antibacterial drugs cannot enter. Various efforts to circumvent these issues have been made through designing both cell penetrating peptides (CPPs) and antimicrobial peptides (AMPs). Our lab has previously reported on cationic amphiphilic polyproline helices (CAPHs), incorporating both CPP and AMP properties. These peptides are composed of modified proline residues with alternating hydrophobic and cationic hydrophilic side chains. One CAPH peptide, P14LRR, showed promising cell penetration and great antibacterial activity.[2] Recently, a peptide composed of more rigid cationic side chains, P14GAP, showed an increase in antibacterial activity compared to P14LRR. By modifying the stereochemistry of the 4-position on the proline ring, the location of charges

around the peptide is altered. The effects of this modification on cell-penetrating efficacy and antimicrobial activity of these new peptides are discussed. [1] CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019. [2] Kuriakose, J.; Hernandez-Gordillo, V.; Nepal, M.; Brezden, A.; Pozzi, V.; Saleem, M. N.; Chmielewski, J. *Angew. Chem. Int. Ed.* 2013, *52*, 9664-9667.

### YI-P042 BracketMaker: Simple and Fast Visualization of Chemical Protein Synthesis

J.L. Evangelista & M.S. Kay
*Department of Biochemistry, University of Utah, Salt Lake City, UT, USA*

When planning any chemical protein synthesis (CPS), one must carefully consider the location of ligation junctions (typically at Cys or Ala), the ligation order, and the timing of side chain reactions (e.g., desulfurization or deprotection). Synthetic strategies are typically visualized with a bracket-style scheme showing the exact route from starting peptides to final protein. Although these figures are a common shorthand language in the CPS field, producing them by hand or in standard graphics software is a tedious and error-prone process. Here we introduce BracketMaker, an easy-to-use program with built-in GUI for rapid drawing of protein synthesis schemes. From a simple text input specifying the location and order of ligation junctions and side chain reactions, BracketMaker generates an informative PNG or publication-quality vector image. Furthermore, BracketMaker's sorting algorithm suggests the most efficient synthetic route(s) for a set of segments (such as segments with optimal ligation sites found with our program Aligator [Jacobsen, M.T. et al. *Bioorg Med Chem* 2017]) by analyzing all possible orders of ligation, adding appropriate desulfurization and deprotection steps, and ranking by predicted final protein yield. BracketMaker provides a simple framework to draft CPS strategies and compare multiple approaches, especially important as the field aims for larger and more ambitious protein targets. The program is open-source and available for download (Windows/Mac executable) at github.com/kay-lab.




### YI-P043 Discovery of Peptide-Based Candidates for a Placental Malaria Vaccine

Daniel Ferrer Vinals[1], Bernard Tornyigah[1], Bart Hazes[2], Stephanie K. Yanow[1,2]
[1] *School of Public Health, University of Alberta, Edmonton, AB, T6G 2H5, Canada.*
[2] *Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, T6G 2H5, Canada*

Placental malaria (PM) is caused by the sequestration of *Plasmodium falciparum* infected erythrocytes (iEs) in the placenta. During PM, iEs display the parasite-encoded ligand VAR2CSA that recognizes chondroitin sulphate A (CSA) in the placenta. The presence of antibodies against VAR2CSA is associated with protection from PM. We demonstrated previously that the *P. vivax* Duffy binding protein (PvDBP) can elicits antibodies that cross-react with VAR2CSA. Additionally, the monoclonal antibody 3D10, raised against PvDBP, recognizes VAR2CSA and inhibits the binding of iEs to plate-immobilized CSA. Here, we screened peptide arrays of PvDBP and VAR2CSA with 3D10 to map epitopes recognized by this cross-reactive antibody in both the source and target proteins. 3D10 recognized two epitopes, RKRR and CIPDR, within subdomain 1 (SD1) of PvDBP, and several epitopes in VAR2CSA. However, our prior data suggested that the CIPDR region is immunodominant and antibodies to this region are not functional. Based on these results, we designed a chimeric SD1 peptide ($SD1_{ch}$) where we exchanged the CIPDR sequence for linear epitopes identified in VAR2CSA that were also recognized by 3D10 in the peptide array. We conjugated $SD1_{ch}$ to KLH using click chemistry, raised antibodies in rats and characterized their ability to inhibit binding of iEs to CSA. Our goal is to focus the immune response on the conserved epitopes shared between PvDBP and VAR2CSA as a novel approach to a vaccine against placental malaria.

### YI-P044 Cytosolically Cleavable Linker for the CPP-mediated Delivery of Therapeutically Relevant Nanobodies in Living Cells

L. Franz*#, A.F.L. Schneider, C.P.R. Hackenberger*
**Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany*
#*Institut für Chemie und Biochemie Freie Universität Berlin, Berlin, Germany*

Addressing intracellular targets with functional protein therapeutics is rapidly gaining recognition for therapeutic applications. However, the pharmacological potential is still limited by their lack of cell-permeability. Modification with cell penetrating peptides (CPPs) is considered a promising strategy to increase the cell-permeability of proteins[1]. To achieve CPP mediated cytosolic delivery of a functional protein this CPP modification needs to be cleavable inside the cell[2].

Here, we demonstrate two different cytosolically cleavable linker systems for the modification of functional proteins to achieve cytosolic delivery without impacting their function.

The first system relies on the existence or incorporation of an accessible cysteine in the nanobody that can be modified with a CPP via a well-established glutathione cleavable disulfide linker[3]. In the second system, we introduce a recombinantly expressed linker system, which can be modified with a CPP. The protein-CPP conjugates are stable

in serum but the linker is enzymatically cleavable inside mammalian cells. We demonstrate that a co-expression of a nanobody with the CPP-modifiable small protein linker can be used to induce cytosolic-CPP mediated uptake into cells without impacting the function of the nanobody by allowing the intracellular cleavage of the linker.

These intracellular cleavable linker strategies present a widely applicable modification approach for CPP-mediated delivery of functional therapeutically relevant proteins[4] that increases the applicability of therapeutic proteins for intracellular targets.

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### YI-P045 Synthesis of Cyanobactin-Derived Photoaffinity Probes for Target Identification

Georgina C. Girt†§, Brunello Nardone‡ and James H. Naismith†§
*† Structural Biology, The Rosalind Franklin Institute, Harwell Science and Innovation Campus, Oxon, OX11 0QS, UK.*
*§ Research Complex at Harwell, Rutherford Laboratory, Didcot, Oxfordshire OX11 0FA, UK.*
*‡ Biomedical Sciences Research Complex, University of St Andrews, St Andrews, Fife KY16 9ST, U.K.*

Patellamides are cyclic ribosomally synthesized and post-translationally modified peptides (RiPPs) of the cyanobactin superfamily, produced by Prochloron sp., an uncultured obligate symbiont of L. patella.1 These natural products have been found to display a number of valuable biological activities, such as cytotoxicity and reversal of multidrug resistance in human leukaemia cells.2 Other members of the cyanobactin family possess immunomodulating, antimalarial, antibacterial activity, but for many the exact cellular mechanisms are unconfirmed.

Biosynthetic machinery and structural features are highly conserved amongst cyanobactins. Of the seven genes of the patellamide pathway, patE encodes a 71-residue precursor peptide comprising of two core sequences, flanked by leader and follower sequences that act as recognition motifs. Previous work in the Naismith group has included the engineering of a modified heterocyclase that can process synthetic core peptides without any leader, as well as discerning minimal tripeptide recognition sequences (which are cleaved upon macrocyclization). 3,4

These findings, combined with the natural promiscuity of the enzymes of the patellamide biosynthetic pathway, allow for the chemoenzymatic synthesis of a diverse range of macrocyclic peptides, including ones containing unnatural residues and non-amino acid moieties such as polyethers, polyketides and sugars.5
Photoaffinity labelling (PAL) is a powerful tool for the elucidation of protein-ligand and protein-protein interactions. Here, we present work on the synthesis of patellamide derived photoaffinity probes via the combination of solid-phase peptide synthesis (SPPS), enzymatic modification and chemical conjugation which can be used to determine cellular targets, verified through secondary assays.

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### P046 Methodologies for Designing, Synthesizing, and Formulating Peptide Nucleic Acids to Achieve Allele Selectivity for Genetic Targets

Jeremy Gleaton, Barry Badeau, Nathan Tavenor, Ramesh Batwal, Dani M. Stoltzfus, Anthony Rossomando, Dietrich A. Stephan
*NeuBase Therapeutics Inc. 350 Technology Drive, Pittsburgh, PA, 15219, U.S.A.*

Peptide nucleic acids (PNAs) have been studied extensively in academia but due to their poor 'drug-like' properties have failed to be developed into therapeutics. NeuBase's PATrOL™ platform is based upon the ability to design, synthesize, and formulate peptide nucleic acids (PNAs) to Drug the Genome™. We have developed ultra-high binding affinity PNAs that can target the genome with allele-selectivity.

The PATrOL™ platform utilizes a cationic delivery shuttle to achieve broad and enduring biodistribution following systematic administration, including into the brain and skeletal muscle. The delivery shuttle also imparts excellent water solubility onto drug candidates, which allows for PNAs to be formulated in a highly concentrated, stable, and monodisperse state. Utilization of the delivery shuttle has enabled us to manufacture drug candidates with high purity at the multi-gram scale and demonstrate allele-selectivity both *in vitro* and *in vivo*.

This presentation will focus on the advancements we have made in the manufacturing and formulation of PNA drug candidates.

### YI-P047 Rapid, High-Yielding Solid-phase Synthesis of Cathepsin-B Cleavable Linkers for Targeted Cancer Therapeutics

Alla Pryyma, Shanal Gunasekera, Joshua Lewin, David M. Perrin*
*Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, B.C., V6T 1Z1, Canada*

Antibody Drug Conjugates (ADCs) are amongst the fastest growing drug classes in cancer therapy. These therapeutics deliver cytotoxic payloads selectively to tumors by exploiting the overexpression of target antigens on

cancerous cells thus minimizing systemic toxicity and harnessing a wider therapeutic window. An ADC is composed of a tumor-targeting monoclonal antibody bridged to a cytotoxic payload via a serum stable linker which efficiently releases the payload in tumor cells upon internalization. A protease cleavable valine-citrulline (Val-Cit) linker technology, which demonstrates specific cleavage via Cathepsin-B protease upregulated at tumor sites, is widely employed in ADC therapy and featured in four FDA approved ADCs. Herein we report a scalable, high yielding and facile synthetic strategy to access this versatile peptidic linker via standard Fmoc solid-phase synthesis, thus avoiding multiple protecting group manipulations, challenging chromatographic purifications and epimerization associated with previous solution-phase synthesis strategies. Our methodology supersedes previous solid-phase synthesis strategies with up to10-fold amplification of yield and significant improvement of Cit loading on 2-chlorotrityl chloride resin. This novel approach employs Oxyma as a pH modulatory agent during Fmoc-removal and a mild DIC/Oxyma coupling strategy, thus obviating basic conditions during synthesis which is critical to avoid premature linker cleavage from the resin. This strategy provides facile access to a wide repertoire of cleavable linkers for ADC generation by introduction of diverse conjugation handles during late stages of the synthesis and has potential to accommodate library generation of linker analogues featuring unnatural amino acids as a selectivity tuning tool.

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### P048 Anti-Proliferative Macrocyclic Peptides Regulate Proteins in the c-Myc Degradation Pathway

L.E.Hanold, A.L. Cheek, and J.V. Aldrich
*Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA*

Overexpression of the oncoprotein c-Myc contributes to increased growth and cell survival in a variety of cancers, and knockdown of c-Myc has been shown to decrease proliferation and promote apoptosis. Thus, downregulating c-Myc is a promising approach for the development of anti-cancer therapeutics. We have previously demonstrated that the macrocyclic tetrapeptide [D-Trp]CJ-15,208 downregulates c-Myc and decreases proliferation in multiple c-Myc-overexpressing cancer cell lines. However, the mechanism by which this peptide regulates c-Myc and inhibits cell proliferation is unknown. Initial data indicates that the peptide alters post-translational modification of protein phosphatase 2A (PP2A), a protein responsible for the dephosphorylation of c-Myc to promote its degradation. This data suggests a role for the c-Myc degradation pathway in the activity of [D-Trp]CJ-15,208, and therefore we are exploring the effects of [D-Trp]CJ-15,208 on expression and post-translational modification of proteins that regulate this pathway. The results of these studies led to the identification of multiple proteins affected by treatment with [D-Trp]CJ-15,208, including Akt and proteins that regulate PP2A. Furthermore, preliminary results for a more potent analog we previously identified in an anti-proliferative screen indicate it has similar effects on a protein that regulates PP2A. Since the proteins affected by these macrocyclic peptides are often dysregulated in cancer, the peptides may have therapeutic potential against these cancers. These results improve our understanding of pathways affected by these macrocyclic peptides, which will help to guide future mechanistic evaluation and the development of promising compounds as potential anti-cancer therapeutics.

This work was supported by the Defense Health Program, through the Prostate Cancer Research Program under Award No. W81XWH-14-0330. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

### P049 Structural Insights into the Activation Mechanism of Vasopressin V2 Receptor

Nidhin Thomas and Sarah Harrison
*Peptilogics, Inc.*

Arginine-vasopressin (AVP), a neurohypophyseal cyclic nonapeptide, plays an essential role in maintaining water homeostasis in the body. In the kidney, AVP binds to the vasopressin type 2 receptor (V2R), a G protein-coupled receptor (GPCR), at the renal collecting ducts, which promotes water reabsorption back into circulation. Numerous mutations in V2R have been identified that result in severe water balance disorders linked to shifts in the V2R activation state, making V2R attractive for targeted drug design. A detailed understanding of the structural basis of V2R is paramount to guide discovery of novel drug candidates. However, the unavailability of an inactivate structure for V2R hinders the analyses of its underlying ligand binding mechanism and activation dynamics. In this work, we constructed the inactivate structure of V2R through homology modeling and validated the structure using equilibrium simulations of the GPCR-peptide complex. Comparison of this inactivated structure with recently published cryo-EM structures in the activated state provides deeper insight into the activation mechanism of V2R. Extensive molecular dynamics (MD) simulations reveal the existence of water networks within the transmembrane segments of V2R that are significant in facilitating activation. We demonstrate that the absence of G protein from the intracellular binding domain destabilizes the activated state and allows multiple metastable intermediate conformations. In addition, the intracellular regions of the transmembrane segments are potential phospholipid binding sites, suggesting the role of anionic lipids as allosteric modulators.

### YI-P050 Bioinspired Organic Corrosion Inhibitor in the Marine Environment

Bui My Hanh, S. H. Hiew and A. Miserez
*School of Material Science and Engineering, Nanyang Technological University (NTU), Singapore 639798*.

Abstract
Corrosion damage is a ubiquitous issue for metal structures submerged in the sea, as the high-salt environment causes surface material erosion and crevice corrosion by dissolution of the metal ions during the complex electrochemical and anaerobic corrosion processes.

The useful lifespan of metals become significantly reduced when exposed to saltwater, requiring frequent and costly maintenance. Although effective, synthetic coatings are not ideal options as toxic coating chemicals eventually get released to the environment by wave action, causing water pollution and interfering with the geochemistry balance of the marine environment.

Often, barnacles have demonstrated the ability to adhere strongly to metal substrates and minimize corrosion events where they attach. Hence, drawing inspiration from a common marine biofouler, the barnacle *Megabalanus rosa*, we present the intriguing anti-corrosion attributes of its adhesive protein, MrCp20, which holds promising potential for further developing into a green anti-corrosion coating alternative. Previously, our team has solved the structure of the protein [1] and identified that the impressive underwater adhesive properties stem from its high content of cysteines (Cys) and hydrophobic residues. At lower concentrations, MrCp20 was reported to accelerate corrosion of marine-grade steel [2], and herein, we report the concentration-dependent anti-corrosion property of MrCp20 protein at higher concentrations, showing that the adhesive characteristic of the protein work in conjunction with the anti-corrosion action [3]. With systematic electrochemical studies and careful investigation of protein and substrate interactions, we present a plausible action of anti-corrosion mechanism. This study will help pave the path to develop MrCp20-inspired anti-corrosion coating materials.

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### YI-P051 Bioinspired Peptide Materials and Peptide Materials for Therapeutics Delivery

S. H. Hiew and A. Miserez
*School of Material Science and Engineering, Nanyang Technological University (NTU), Singapore 639798.*

Sucker ring teeth (SRT) of the Humboldt squid (*Dosidicus gigas*) are unusual hard tissues that have inspired the design of several protein-based and peptide-based materials for potential biomedical applications [1]. The teeth are natural "biotools" that are hard and robust, non-mineralized and fully proteinaceous, comprising of proteins named "suckerins" with high sequence modularity.

With previous work unraveling the molecular-scale interactions, self-assembly mechanisms, the smart selection of protein sequences that drive the hierarchical assembly of natural SRT material in a unique fashion [1], we now adapt the modular peptide sequences to fabricate useful materials for real-world solutions.

This presentation highlights the study of peptide materials inspired by SRT peptides, including the molecular-scale interactions, self-assembly mechanism, material properties and exploration of their potential as therapeutics delivery vehicles. The unique self-assembly property of a short SRT-bioinspired peptide, GV8 [2], enables a simple one-pot encapsulation of therapeutics under mild aqueous conditions and the delivery of complex growth factors such as stem cell secretome. Delivery of these complex regenerative therapeutic candidates have been faced with multiple challenges that impede their application and GV8 hydrogels demonstrate the capability to address these issues, hence a promising candidate for wound-healing applications [3,4].
The boundless lessons we learn from a single structural protein can equip us with a plethora of possibilities toward engineering new peptide-based biomimetic materials, from a detailed study of SRT-bioinspired peptides, GX8. GX8 peptides are found in many structural proteins in nature too, and here we present first-hand the intriguing self-assembled materials from GX8 [5].

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### P052 Integration of a Chymotrypsin Filter into *In Vitro* Selections for Macrocyclic Peptide Ligands

Christopher J. Hipolito, Sabrina E. Iskandar, Rumit Maini, Sanjay A. Saldanha, Adam Weinglass
*Screening & Compound Profiling, Quantitative Biosciences, Merck & Co., Inc., 2000 Galloping Hill Rd, Kenilworth, NJ, USA, 07033.*

Macrocyclic peptide ligands identified using *in vitro* selection may serve as hit compounds that could be further optimized into lead compounds for drug discovery. Providing chemists with hit compounds bearing the desired pharmacological properties would reduce time spent on optimization and increase the number of hits that pass through the hit-to-lead triage funnel. A common strategy for identifying ligands of interest is via direct target

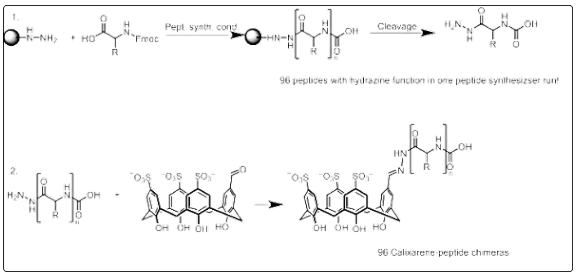
engagement using panning in high-throughput screens or *in vitro* selections. Identification of confirmed ligands could lead to hits that would then be assessed for additional related properties, such as inhibition of protein-protein interaction or inhibition of enzymatic activity. Strategies for enriching properties orthogonal to target binding, such as stability, could also be integrated into a panning-based campaign by carefully optimizing the combined selective pressures. We focus on resistance against chymotrypsin activity for the development of orally available macrocyclic peptide therapeutics and endeavor to find sequence motifs that bestow enhanced stability. In addition, the constrained scaffold of the macrocyclic peptide is thought to provide higher stability over its linear counterpart. Here, we discuss optimization of a chymotrypsin filter for integration into a round or rounds of selection and the evolutionary trajectory of clones enduring the combination of binding and stability selection pressures.

### YI-P053 Molecular Chimeras: Use of Peptide-Calixarene Conjugates for Drug Detection

A.Y. Kliuchynskyi and Fraser Hof
*University of Victoria*

*Department of Chemistry, University of Victoria, 3800 Finnerty Road, Victoria, BC V8P 5C2, Canada*

Due to the ongoing overdose crisis, designing selective hosts for drug detection in aqueous media is an emerging goal of supramolecular chemistry. Water-soluble calixarenes could serve as good drug-binding hosts on their own and are easy to use in various assays, but generally lack selectivity towards specific targets. Peptides often suffer from poor solubility and lack of quantitative assays that would allow for determination of drug content, but can provide structural diversity that can enhance specificity. This work concentrates on combining the favorable properties of these types of compounds in an attempt to create selective chemosensors for cocaine by attaching short peptide chain to an upper rim of a calixarene. First we created a library of peptides made up of most common amino acid residues that are responsible for binding in various natural receptors and antibodies. Then we used solid-phase peptide synthesis (SPPS) technique to make 96 different peptides terminated with a hydrazone moiety[1], which were then attached to a formyl handle on sulfonated calix[4]arene. Following the synthesis, these peptide conjugates were studied in an indicator displacement assay (IDA) to screen for the best-binding candidates. This approach could be easily applied to any desirable biologically active molecule, so could lead to an entirely new family of prospective chemoreceptors.

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### P054 Identity Confirmation of GLP-1, Liraglutide, Semaglutide with High Confidence using High-resolution Mass Spectrometry

Seoyeon Hong
*PolyPeptide Group, Torrance, 365 Maple Ave, Torrance, CA 90503*

The glucagon-like peptide I (GLP-1) (7-36) is a 30-amino acid peptide hormone attributed to glycemic control and body weight regulation. Liraglutide and Semaglutide are GLP-1 analogs that contain side chains to improve the properties such as half-life and binding to albumin (e.g., binding affinity). Liraglutide exhibits a major modification compared to GLP-1 at Lys in position 26, which is attached to a palmitic acid (C-16 fatty acid) through γ-Glu linker. Semaglutide displays the substitution of Gly with Aib at position 2 and the attachment of the octadecanoic diacid (C-18 fatty acid) to Lys in position 26 through a short polyethylene glycol (PEG) spacer and γ-Glu acid linker. The accurate identification of these drugs is mandatory by the U.S. Food and Drug Administration to ensure the safety and efficacy. Here, electrospray ionization (ESI) coupled to high-resolution mass spectrometry (HRMS) is used to confirm the identity of GLP-1 (7-36), Liraglutide and Semaglutide products by monoisotopic mass. Lastly, the amino sequence of the drugs is probed using ESI coupled to tandem mass spectrometry (MS/MS) and matrix-assisted laser desorption/ionization (MALDI)-MS/MS analysis.

### P055 Synthesis of Peptide Dsiplaying Bacteria for the Decontemination of Fenitrothion

S.H. Hong, J. Jeong and A. Kumaravel
*Department of Chemical Engineering, University of Ulsan, Ulsan 44610, Korea*

Organophosphorus pesticides have been used extensively all over the world, and currently about 100 organophosphorus pesticides, such as chlorpyrifos, fenitrothion, and parathion are in use, since they are considered to have specific toxicity towards insects. Fenitrothion, or O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate, is a nitrophenolic pesticide that has been widely used in agriculture.

A codon-optimized, pesticide-binding peptide was attached to the C-terminus of OmpC at loop 7 (993 bp) to remove fenitrothion. The efficiency of fenitrothion binding by the monomer peptide was evaluated under different temperatures, pH levels, and fenitrothion concentrations.

### P056 An Ensemble-Centric Approach for Studying Peptide Behavior in Solution

Parisa Hosseinzadeh
*University of Oregon*

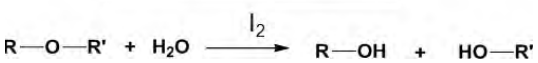
Peptides play essential roles in our body as hormones and signaling molecules. They are attractive therapeutic modalities complementary to small molecules and antibodies. However, predicting peptide features that are of interest, such as permeability, binding affinity, and binding orientation has been a challenging task. To better understand the behavior of peptides in solution, and to reduce the cost of down-stream optimization of peptides

which is currently necessary for peptide binders, robust and general predictors of peptide behaviors are required. Our lab combines computational peptide sampling using Rosetta, molecular dynamics simulations, and learning methods to give a holistic picture of peptide conformations in solution and use these models of peptide ensembles as a starting point for predicting their properties such as binding affinities. In this conference, I will present our latest work on studying peptide conformations in solution. I will then show our efforts in applying these conformations to predicting peptide binding affinities and permeabilities. Finally, I will talk about future work aimed at combining these ensembles with design to generate an accurate pipeline for designing peptides that can bind to protein targets of interest.

### YI-P057 Structure and Glycosidase Activity of an Enzyme Mimetic

Olamilekan Joseph Ibukun and Debasish Haldar*
*Department of Chemical Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur-741246, West Bengal, India.*
*deba_h76@yahoo.com ; deba_h76@iiserkol.ac.in*

Glycoside hydrolases are ubiquitous in nature as intracellular or extracellular enzymes that are largely involved in nutrient acquisition.[1] In E. coli bacteria, glycoside hydrolases is the enzyme beta-galactosidase (LacZ), which is involved in regulation of expression of the lac operon. In higher organisms glycoside hydrolases are found within the endoplasmic reticulum and Golgi apparatus where they are involved in processing of N-linked glycoproteins, and in the lysosome as enzymes involved in the degradation of complex carbohydrates. Deficiency of glycoside hydrolases can lead to a range of lysosomal storage disorders.[2] Herein we have designed and synthesized a enzyme mimetic containing L-phenylalanine, ¤- aminoisobutyric acid (AiB), L-leucine and *m*-Nifedipine. From X-ray crystallography, the peptide adopts a ¤-hairpin conformation. The ¤-hairpin structure is stabilized by two 10- members and one 18-member NH….O=C hydrogen bonds. Moreover, the peptide was found to be highly efficient to hydrolases ethers and glycosides in presence of iodine. Further, from X- ray crystallography, the backbone conformation of the enzyme mimetic is almost unchanged after the glycosidases reaction. This is the first example of an iodine supported glycosidase activity with an enzyme mimic at room temperature.

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### YI-P058 A Refined Photo-switchable Cyclic Peptide Scaffold for Use in ß-turn Activation

Corey Johnson,[a] John S. Harwood[a], Mark Lipton*[a] and Jean Chmielewski*[a]
*Department of Chemistry, Purdue University, 560 Oval Drive West Lafayette IN, 47906*

The ability to reversibly modulate peptide secondary structures, such as the ß-turn, allows for precise control of biological function, including protein interactions. Herein we describe the design of two scaffolds containing an azobenzene moiety with flanking alanine or ß-alanine residues to probe essential features for photo-control of a ß-turn within a cyclic peptide. To efficiently cyclize the designed linear peptides, prior isomerization of the azobenzene-containing amino acid from the trans to the cis form was necessary. The two cyclic peptides (TAp and TApß) were found to undergo rapid photochemical conversion to the cis isomer of the azobenzene, with a more gradual thermal reversion to the trans isomer over the course of a week at 37 °C. Spectroscopic analysis and restrained molecular dynamics simulation of the cis form of TAp and TApß revealed type II and type II' ß-turns within the cyclic peptides, respectively. The trans isomer of the TAp cyclic peptide was found to have a kink within the peptide structure, whereas the longer trans-TApß contained a more extended conformation. TApß, therefore, demonstrates a clearer difference in the cyclic peptide conformations when in the cis versus trans form, a feature that may prove beneficial for use with biologically active ß-turn sequences.

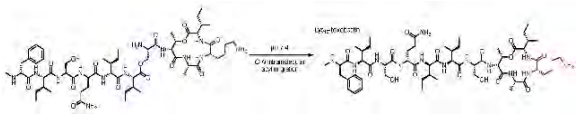
### YI-P059 *O*-Acyl Isopeptide Prodrugs of Teixobactin Analogues

Chelsea R. Jones[1], James S. Nowick[1,2]
[1]*Department of Chemistry, University of California, Irvine, Irvine, CA 92697, USA*
[2]*Department of Pharmaceutical Sciences, University of California, Irvine, Irvine, CA 92697, USA*

In 2015, Lewis and coworkers discovered teixobactin, a non-ribosomal undecapeptide consisting of a linear tail and a macrolactone ring.[1,2] Teixobactin inhibits gram-positive bacteria—including MRSA, VRE, and MDR-TB—by binding the prenyl-pyrophosphate groups of lipid II, a precursor to peptidoglycan cell wall synthesis.[1] Teixobactin and active analogues of teixobactin form gels in aqueous conditions.[3,4] This tendency to gelate in aqueous conditions is a limitation to teixobactin and administration of the compound. Particularly, at high concentrations required for IND-enabling toxicity studies, teixobactin aggregates and forms gels.

In the present work, we have synthesized and investigated novel antibiotics comprising of *O*-acyl isopeptide prodrugs of teixobactin analogues. These teixobactin prodrugs vary in the position at which the *O*-acyl isopeptide linkage is present, either between $Ile_6$ and $Ser_7$, $Ile_2$ and $Ser_3$, or between both $Ile_6$ and $Ser_7$ and $Ile_2$ and $Ser_3$. The teixobactin *O*-acyl isopeptide derivatives undergo conversion to the corresponding teixobactin analogue when exposed to neutral or basic conditions. The *O*-acyl isopeptide

prodrug analogues have exhibited comparable or improved antibiotic activity to their parent compounds. Gelation assays of the analogues demonstrate that these derivatives to not gelate immediately upon exposure to buffer, suggesting improved solubility in aqueous conditions. The enhanced solubility of the prodrugs imparts better pharmacological properties than the parent antibiotics, which are difficult to administer intravenously, making them superior drug candidates.



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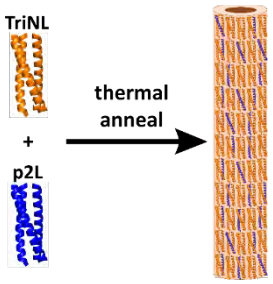
### YI-P060 Self-assembled Coiled-coil Peptide Nanotubes with Enhanced Stability and Metal-dependent Cargo Loading

M.D. Jorgensen and J. Chmielewski
*Department of Chemistry, Purdue University, West Lafayette, IN, 47906, USA*

Nanotube biomaterials have drawn great attention for their applications ranging from piezoelectric devices to cargo delivery. Previously, we reported a nanotube composed of the coiled-coil peptide **TriNL** based on the GCN4 leucine zipper.[1] While capable of encapsulating cargo, these nanotubes quickly dissolved in phosphate buffer saline (PBS). Herein we report a stabilized **TriNL** nanotube through the introduction of a coiled-coil peptide with metal-binding ligands, **p2L,** to control the rate of degradation in a metal-dependent fashion. The intermixing of **TriNL** and **p2L** was achieved either through thermal annealing for heterotrimeric coiled-coils or through a stepwise addition of homotrimeric coiled-coils. While maintaining nanotube morphology with small amounts of **p2L**, the material began to adopt a more crystal-like structure at higher **p2L** concentrations. The location of the metal- binding ligands was confirmed using His-tagged cargo with a more uniform distribution of metal-binding ligands from the heterocoiled-coil assembly. This stabilized nanotube not only allows for dual cargo incorporation but also shows promise for controlled cargo release.




[1] Nambiar, M.; Nepal, M.; Chmielewski, J. Self-Assembling Coiled-Coil Peptide Nanotubes with Biomolecular Cargo Encapsulation. *ACS Biomater. Sci. Eng.* 2019, *5* (10), 5082–5087. https://doi.org/10.1021/acsbiomaterials.9b01304.

### YI-P061 Development of Allosteric Constrained Peptide Inhibitors Targeting the C-tail of LRRK2

Krista K. Barbour and Eileen J. Kennedy
*Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA, 30602, USA*

Parkinson's Disease (PD) is the second most commonly occurring neurodegenerative disorder following Alzheimer's Disease. Mutations in the Leucine Rich-Repeat Kinase 2 (LRRK2) protein account for the majority of familial Parkinson's and frequently result in upregulated LRRK2 dimerization and kinase activity. While multiple small molecule kinase inhibitors have been developed to target LRRK2, their translational potential has been limited due to LRRK2 mis-localization and lung and kidney toxicities. As an alternative approach, we sought to design constrained peptides targeting the LRRK2 dimer interface as a strategy to allosterically downregulate LRRK2 activity. We developed a class of peptides mimicking the C-terminal tail of LRRK2 which forms intermolecular interactions with the kinase domain of LRRK2 as a strategy to inhibit dimerization, LRRK2 activity and disease pathologies seen in PD. Using this targeting strategy, we have identified two constrained peptides that are cell permeable and downregulate LRRK2 dimerization and its activity. This work demonstrates, for the first time, the significant role of the C-terminal tail on LRRK2 dimerization and regulation and highlights a novel strategy that may be applied for the treatment of Parkinson's.

### P062 BRK1-derived Constrained Peptides Targeting the WASF Regulatory Complex in Metastasis

M.K. Whittaker*, A.J. Limaye*, and E.J. Kennedy
*Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA 30602, USA*

Wiskott-Aldrich syndrome protein family (WASF) members are key regulators of actin cytoskeleton dynamics at the leading edge of the cell membrane. Of the three WASF family members, only WASF3 was found to directly promote cancer invasion and metastasis. WASF3 is incorporated into a hetero-pentameric protein complex with BRK1, CYFIP1/2, NCKAP1/1L, and ABI1/2/3 termed the WASF Regulatory Complex (WRC) that links upstream signaling pathways to Arp2/3-mediated actin nucleation. Disruption of the complex inhibits actin remodeling and presents a novel approach to targeting cancer invasion and metastasis. Large portions of the complex are mediated by α-helical

interactions that are amenable to targeting with constrained, α-helical peptides. Here we report the development of a first-generation all-hydrocarbon stapled BRK1 mimetic peptide, BASH-2, designed to disrupt binding of BRK1 to the WRC to disrupt WRC assembly and function. BASH-2 was developed using a combination of structure-guided rational design and homology modeling. BASH-2 was found to permeate cells and inhibit cancer cell migration with greater potency than a related WRC peptide mimetic, WAHMIS-2. Current studies aim to characterize BASH-2 binding and degradation of WRC components as well as its effects on invasive potential of cancer cells. Targeted disruption of BRK1 from the WRC may serve as a novel strategy for suppression of cancer metastasis.

### YI-P063 A Versatile Peptidic Probe for Conventional and Super-Resolution Microscopy Reveals Inhibitory Synapse Ultrastructure and Brain Connectivity

Vladimir Khayenko, Clemens Schulte, Sara L Reis, Orly Avraham, Cataldo Schietroma, Rafael Worschech, Noah F Nordblom, Sonja Kachler, Carmen Villmann, Katrin G Heinze, Andreas Schlosser, Ora Schueler-Furman, Philip Tovote, Christian G Specht, Hans M Maric
*Center for Integrative and Translational Bioimaging; University of Wuerzburg*

Visualization of inhibitory synapses requires protocol tailoring for different sample types and imaging techniques and relies either on genetic manipulation or on antibodies that underperform in tissue immunofluorescence. Starting from an endogenous activity-related ligand of gephyrin, a universal marker of the inhibitory post-synapse, we developed a short peptidic binder and dimerized it, significantly increasing affinity and selectivity. Parallelly, we tailored fluorophores to the binder, yielding "Sylite" – a probe with outstanding signal-to-background ratio that outperforms antibodies in tissue staining, since it penetrates rapidly and efficiently, does not produce staining artefacts and benefits from simplified handling. In super-resolution microscopy Sylite precisely localizes the post-synapse and enables accurate pre- to post- synapse measurements. Combined with complimentary tracing techniques Sylite reveals inhibitory connectivity and profiles inhibitory inputs and synapse sizes of excitatory and inhibitory neurons in the periaqueductal gray brain region. Taken together, Sylite is a versatile probe for conventional and super-resolution microscopy, enabling multiplex studies of neuronal inhibition on the synaptic, cellular and circuit level.

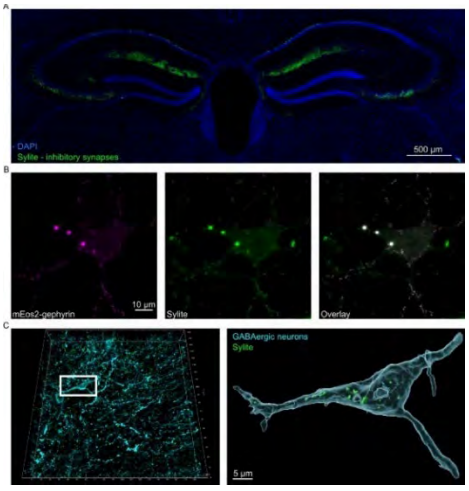



**Figure 1. A.** A wide- field image of mouse hippocampal brain section stained with Sylite to visualize inhibitory synapses (green) and nuclear DAPI staining (blue). **B.** Wide-field images of primary cortical neurons. Left: mEos2-gephyrin expression in cortical neurons (magenta) after lentiviral infection. Middle: Sylite staining (green) of the neuronal culture. Right: Overlay of the mEos2- gephyrin and Sylite signals. A high degree of overlap is observed. Note that Sylite labels also native non-fluorescent gephyrin. **C.** Confocal microscopy of a fixed brain section of recombinant mice expressing eYFP in GABAergic neurons (turquoise) with Sylite staining (green). Left: Volumetric representation of the section. Frame 185x185x24 µm. Right: 3D reconstruction of the neuron body in the white box. Multiple inhibitory synapses are observed in the cell soma.

### YI-P064 Property-Based Selection of Membrane Permeable Macrocyclic Scaffolds in a DNA-Encoded Library

Grant Koch[1], Panpan Zhang[1], R.S. Lokey[1]
[1]*Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95060, USA*
[2]*Ra Pharmaceuticals, Massachusetts 02140, USA*

Over the past decade, DNA-encoded libraries (DELs) have emerged as a promising new strategy to greatly increase the size and diversity of chemical libraries for drug screening. DELs of macrocyclic peptides and peptidomimetic scaffolds have already produced hit compounds against a wide range of protein targets, but these hits tend to be the more polar members of the library and have no guarantee to access their targets *in vivo*, especially as macrocycle size increases. Inspired by recently published findings from our lab, we have been investigating the potential to identify cell-permeable compounds within a DNA-encoded libraries of macrocyclic peptides. We have obtained exciting data demonstrating that small molecule and peptide macrocycle model compounds bound to DNA can be selected for based on their lipophilic efficiencies by utilizing simple RP-HPLC. This phenomenon is now being investigated in a scaffold-diverse library of cyclic peptides to determine if permeable members can be identified within a DNA-encoded library. This study will provide a tool to guide the resynthesis of hits, greatly increase the ability to search for permeable macrocyclic scaffolds, and expedite the hit-to-lead process for encoded libraries.

### YI-P065 Discovery of Naturally Inspired Peptide Antibiotics

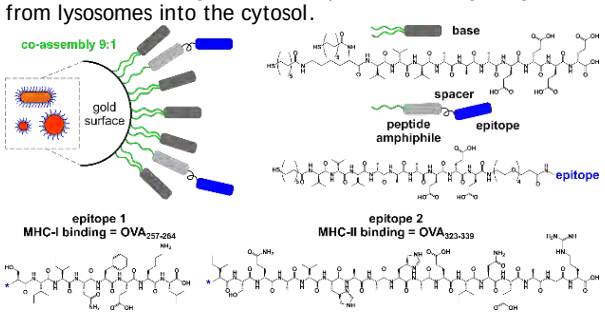
Bimal Koirala, Sean Brady
*Laboratory of Genetically Encoded Small Molecule, The Rockefeller University, New York, USA*

Synthetic-Bioinformatic Natural Product (syn-BNP) is a new small molecule discovery pipeline; it bioinformatically predicts and chemically synthesizes small molecules inspired by natural product biosynthetic gene clusters. This new approach circumvents the two main bottlenecks in traditional natural product discovery methods, i.e., culture and gene expression, and is the most efficient method to translate genetic information into molecular structures to date. We have thus far screened hundreds of non-ribosomal peptide syn-BNPs and found new antimicrobial agents against Gram-negative, Gram-positive, as well as fungal pathogens.

## P066 Gold Nanoparticles Decorated with Ovalbumin-Derived Epitopes: Effect of Shape and Size on T-Cell Immune Responses

E.A. Egorova, A.L. Boyle, B. Slütter and A. Kros
*Department of Supramolecular & Biomaterials Chemistry, Leiden Institute of Chemistry, Leiden University, 2333CC Leiden, The Netherlands*

Gold nanoparticles (GNPs) can be manufactured in various shapes, and their size is programmable, which permits the study of the effects imposed by these parameters on biological processes. However, a protective coating on the gold surface is required to maintain particle stability in biological media, to prevent aggregation, and to eliminate potential cytotoxicity. Previously, we reported the use of thiolated peptide amphiphiles as stabilizers for both GNPs and GNRs.[1,2] These coatings insulate the gold surface, prevent particle aggregation under harsh conditions (up to 3 M NaCl, or 1 M competing thiols), and provide GNPs with surface chemistry described as "protein-like".
To date, there is currently no clear evidence that a certain nanoparticle shape or size is beneficial to induce desired T-cell immune responses. Therefore, we have utilized GNPs and gold nanorods (GNRs) functionalized with model epitopes derived from chicken ovalbumin ($OVA_{257-264}$ and $OVA_{323-339}$). By using these two distinct epitopes, it was possible to draw conclusions regarding the impact of nanoparticle shape and size on different aspects of the immune response. Smaller GNPs (~15 nm in diameter) induce significantly less intense T-cell responses. Furthermore, effective antigen presentation via MHC-I was observed for larger spherical particles, and to a lesser extent for rod-like particles. We believe these findings will have implications for vaccine development, and lead to a better understanding of cellular uptake and antigen egress from lysosomes into the cytosol.




Scheme 1. Schematic representation of the shell composition**.** Base peptide and epitope-bound peptides **1** and **2**) used to stabilize GNPs and GNRs. Base peptide was mixed with either **1** or **2** at a 9:1 molar ratio to form shells around GNPs and to provide near-identical surface chemistries.

Egorova, E.A.; van Rijt, M.M.J.; Sommerdijk, N.; Gooris, G.S.; Bouwstra, J.A.; Boyle, A.L.; and Kros, A. *ACS Nano* 2020, 14, 5874–5886.
Egorova, E.; Arias Alpizar, G.; Vlieg, R.; Gooris, G.S.; Bouwstra, J.; van Noort, j.; Kros, A.; Boyle, A.L. *J.Mater.Chem. B.* 2022, https://doi.org/10.1039/D2TB00073C.

## YI-P067 A Proteomimetic Scaffold for Sequence-Specific Recognition of Duplex RNA

Jonathan G. Kwok and Paramjit S. Arora
*Department of Chemistry, New York University, NY, 10003, USA*

We describe our efforts towards the rational design, synthesis, and evaluation of a proteomimetic scaffold, referred to as the Crosslinked Helical Fork (CHF) for the sequence-specific recognition of double-stranded RNA (dsRNA). The proteomimetic scaffold, realized from the *tomato aspermy virus 2b* (TAV2b) protein and duplex siRNA complex, utilizes side chain recognition to the Hoogsteen face in the RNA major groove. The optimized mimic exhibits selectivity for the double-stranded $rCUG_{10}$ RNA versus control sequences and DNA analogues. Circular dichroism (CD) analysis supports the hypothesis that the peptide sequence becomes $\alpha$-helical upon binding RNA. We envision the proteomimetic scaffold to form the basis of a recognition code for duplex RNA.

## YI-P068 SUMO-Peptide-Intein "Sandwich": Combined Expression System for Improved Stability and Yield of Peptides

T. Lamer, M. J. van Belkum, J. C. Vederas*
*Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada*

Studies of ribosomally synthesized peptides often require recombinant production of the peptide of interest in *Escherichia coli*. To achieve this, peptides are usually cloned and over-expressed as fusion proteins. The soluble fusion protein partner usually contains an affinity tag to help with purification and a protease cleavage site that leaves behind no extra residues on the peptide of interest. However, heterologously expressed peptides can suffer from proteolytic degradation or instability in *E. coli*, which can pose a major issue for applications requiring a large amount of purified peptide, such as NMR structural assignments or biochemical assays. In this work, we initially cloned and expressed four different peptides as Small Ubiquitin-like Modifier (SUMO) fusion proteins. These peptides (lactococcin A, leucocin A, faerocin MK, neopetrosiamide A) were truncated during expression and isolation as SUMO fusions, resulting in low yields of purified peptide. To prevent this degradation and improve yield, we designed a new expression system to create a "sandwiched" fusion protein of the form: $His_6$-SUMO-Peptide-Intein, which we called SPI. These SPI fusions were more stable and protected against degradation, resulting in improved yields under a set of standard expression and isolation procedures. The SPI expression system uses only two commercially available vectors and standard protein purification techniques, and therefore may offer an economical and facile route to improve yields for peptides that undergo degradation.

**YI-P069** **Isolated Protein-protein Binding Domain for the Discovery of D- peptide Inhibitors Targeting COVID-19 Cytokine Storm**

A.J. Lander, Y. Kong, C. Wu, L.Y.P. Luk.
*School of Chemistry, Cardiff University, Cardiff,* CF10 3AT, *UK.*

The *de novo* discovery of potent, proteolysis resistant D-peptide inhibitors for a variety of druggable targets has been made possible through mirror image phage display (MIPD).[1] However, synthesis of whole protein enantiomers poses numerous challenges, such as size, post-translational modifications, and protein folding. Here, we develop a method to isolate mid-chain protein domains and prepare their enantiomers as a target for MIPD. The tumor necrosis factor receptor 1 (TNFR1) has been identified as a key drug target in the treatment of COVID-19 cytokine storm.[2-3] Though, at >15 kDa in size with challenging folding Considerations, synthetic preparation of its enantiomer is laborious and expensive. The TNFR-1 domain responsible for cytokine binding was isolated, synthesized in both enantiomers, followed by folding into the proper conformation confirmed by racemic protein crystallography. MIPD of cyclic peptide libraries against the D-TNFR1 domain yielded an enrichment factor >8000 fold, with biophysical analysis of the resulting candidates ongoing. This work overcomes a significant obstacle of target synthesis in MIPD, whilst also minimizing the probability of obtaining ligands that do not compete for cytokine binding. Collectively, we hope to use this method to develop potent, D-peptide inhibitors for a broad range of targets, including TNFR-1 in COVID-19 cytokine storm.

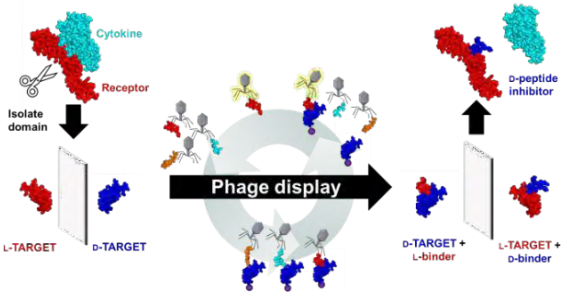



Figure 1: Project overview - From L-target domain to D-peptide inhibitor candidate

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**YI-P070** **Attenuating the *Streptococcus pneumoniae* Competence Regulon using Urea-Bridged Cyclic Dominant Negative CSP Analogs**

Muralikrishna Lella,1,# Myung Whan Oh,2,# Shanny Hsuan Kuo,2 Gee W. Lau,2,* and Yftah Tal-Gan1,*
*1Department of Chemistry, University of Nevada, Reno, 1664 North Virginia Street, Reno, NV 89557.*
*2Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL 61802*
*#Authors contributed equally. *Corresponding authors: geelau@illinois.edu or ytalgan@unr.edu.*

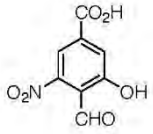
The emergence and rapid evolution of antibiotic resistance in pathogenic bacteria pose a constant threat to global public health, and *Streptococcus pneumoniae* is one such opportunistic respiratory human pathogen that rapidly acquires antibiotic resistance. Natural competence for genetic transformation in *S. pneumoniae* plays a vital role in aiding pathogenicity, and it is the best-characterized feature to acquire antimicrobial resistance genes by frequent recombination. In *S. pneumoniae*, competence and virulence factor production are controlled by a cell-density communication mechanism termed the competence regulon. This study aimed to construct stable cyclic peptide analogs using non-proteogenic amino acids to improve the high affinity and selectivity for receptor protein targets as therapeutic approaches to prevent pneumococcal infections. Here, we report the successful design and synthesis of (*i*→*i*+4) urea-bridged cyclic peptide analogs of the *S. pneumoniae* competence stimulating peptide (CSP). Structure-activity relationship (SAR) studies revealed that cyclic CSP1-E1A analogs can selectively inhibit the ComD1 receptor, with macrocycle sizes of 23-24 and 18-20 atoms being optimal for ComD1 inhibition, exhibiting IC50 values at the low nanomolar range. Surprisingly, most of the CSP1-E1A cyclic and few linear analogs were found to activate the ComD2 receptor, making them valuable tools to evaluate the effect QS modulation has on the selection between the two pneumococcal specificity groups during co-infections. This study also uncovered that the activating domain of CSP1 is present in the central region along with the known *N*-terminal activating region. Our findings highlight the possibility of a peptide-based therapeutic approach inhibiting continuous pneumococcal evolution by targeting the competence regulon.

**P071** **A Photolabile Backbone Amide Linker for the Solid-Phase Synthesis of Cyclic Peptides and Peptide Thioesters**

Matthew A. Hostetler, Miri Niedrauer and Mark A. Lipton*
Department of Chemistry, Purdue University,
West Lafayette, IN 47907-2084

Despite the immense power of Solid-Phase Peptide Synthesis (SPPS) for the routine and automated synthesis of peptides, the limitations imposed by the C-terminal anchoring of the growing peptide chain result in the well-known difficulties associated with on-resin head-to-tail cyclization and C-terminal modification of the resin-bound peptide. Among the several solutions proposed to circumvent these limitations is the concept of backbone

amide linker approach pioneered by Albericio and Barany in which growing peptide is anchored to an amide nitrogen, thus permitting modification of both the N- and C-termini of the growing peptide. We have extended this concept by incorporating photochemical, rather than acidic, cleavage of the peptide from the linker. Our new linker, 4-carboxy-2-hydroxy-6- nitrobenzaldehyde (Hcna, **1**) has been shown to afford the solid phase synthesis of N- to-C cyclized peptides and C-terminally modified peptides that can be cleaved from the resin in high yield and chemical purity. Photochemical cleavage also permits optional retention of sidechain protecting groups in the deprotection step. The utility of the Hcna linker is illustrated by the synthesis of several cyclized peptides and also a number of peptide thioesters.




### YI-P072 Development of a Cytosolic Delivery System for Therapeutic Peptides Based on CMKLR1-mediated Endocytosis

Moritz List, Annette G. Beck-Sickinger
*Institute for Biochemistry, University of Leipzig, Leipzig, Saxony, Germany*

Therapeutic peptides are promising tools to address intracellular protein-protein interactions (PPIs), which are challenging targets for conventional drug moieties. However, effective cytosolic delivery still proves to be a major problem in the development of therapeutic peptides targeting intracellular PPIs. Additionally, some common strategies to improve membrane permeation of peptides can result in off-target effects or toxicity.Therefore, selective and efficient delivery systems are highly desirable.

Here we present a shuttle system for peptide cargos based on the chemokine like receptor 1 (CMKLR1). CMKLR1 is highly expressed in a variety of tissues and an efficiently internalizing nonapeptide ligand with nanomolar affinity, chemerin-9, has been identified.[1] Further, cyclic variants of chemerin-9 have been shown to be metabolically stable and therefore are suitable for a therapeutic shuttle system.[2] Here we demonstrated that large peptide cargos can be N-terminally attached to chemerin-9 while still maintaining excellent internalization behavior. However, peptides internalized by receptor-mediated endocytosis usually become trapped in the endosome, where they are then degraded. To allow endosomal escape, we introduced activatable cell-penetrating peptides (CPPs) which are released by the endosomal protease cathepsin B after CMKLR1-mediated endocytosis. Since the CPPs are inactive in their bound state, only minor uptake into cells without CMKLR1 was observed.

Overall, our results provide a promising starting point for a cytosolic delivery system without the off-target effects that an unspecific shuttle system might exhibit.

[1] V. Wittamer et al., *J. Biol. Chem*. 2004, 279, 9956-9962
[2] T. F. Fischer et al., *Cancers* 2021, 13, 3788

### YI-P073 Enhancing the Potency of Antimicrobial Peptides through Molecular Engineering and Self-Assembly

Lucia Lombardi,[1,2,3] Yejiao Shi,[2] Annarita Falanga,[4] Emilia Galdiero,[5] Elisabetta de Alteriis,[5] Gianluigi Franci,[6] Igor Chourpa,[7] Helena S. Azevedo,[2] and Stefania Galdiero[1]
*1 Department of Pharmacy, School of Medicine, University of Naples Federico II, Via Mezzocannone 16, 80134 Naples, Italy*
*2 School of Engineering and Materials Science, Queen Mary, University of London, Mile End Road, London E1 4NS, United Kingdom*
*3 Dept. of Chemical Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ*
*4 Department of Agricultural Science, University of Naples Federico II, via Università 100, 80055 Naples, Italy*
*5 Department of Biology, University of Naples Federico II, via Cinthia, 80100 Naples, Italy*
*6 Department of Experimental Medicine, University of Campania Luigi Vanvitelli, via Costantinopoli 16, 80138 Naples, Italy*
*7 EA 6295 Nanomédicaments et Nanosondes, Université François-Rabelais de Tours, 31 avenue Monge, 37000 Tours, France*

Healthcare-associated infections resulting from bacterial attachment and biofilm formation on medical implants are posing significant challenges in particular with the emergence of bacterial resistance to antibiotics. Here, we report the design, synthesis and characterization of self-assembled nanostructures, which integrate on their surface antibacterial peptides. The antibacterial WMR peptide, which is a modification of the native sequence of the myxinidin, a marine peptide isolated from the epidermal mucus of hagfish, was used considering its enhanced activity against Gram-negative bacteria. WMR was linked to a peptide segment of aliphatic residues (AAAAAAA) containing a lipidic tail ($C_{19}H_{38}O_2$) attached to the ε-amino of a terminal lysine to generate a peptide amphiphile (WMR PA). The self-assembly of the WMR PA alone, or combined with coassembling shorter PAs, was studied using spectroscopy and microscopy techniques. The designed PAs were shown to self-assemble into stable nanofiber structures and these nanoassemblies significantly inhibit biofilm formation and eradicate the already formed biofilms of Pseudomonas aeruginosa (Gram-negative bacteria) and Candida albicans (pathogenic fungus) when compared to the native WMR peptide. Our results provide insights into the design of peptide based supramolecular assemblies with antibacterial activity, and establish an innovative strategy to develop self-assembled antimicrobial materials for biomedical applications.

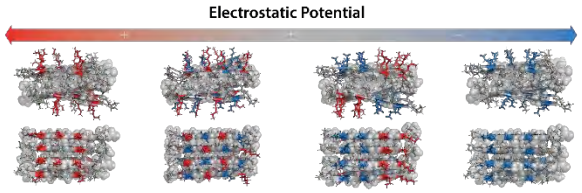
*Biomacromolecules* 2019, 20, 1362−1374
*Sci Rep* 7, 44425 (2017)

### YI-P074 The Effect of Electrostatics on the Immune Response to Peptide-based Materials

T.L. Lopez-Silva and J.P. Schneider
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick MD, 21702, USA*

Peptide hydrogels are promising materials with growing interest in their use for biomedical applications such as controlled drug delivery, regenerative medicine, and cancer

therapy. They offer multiple advantages as they are made purely of amino acids, have naturally occurring structures, and self-assemble into nanofibrous scaffolds under biocompatible conditions. For over 20 years, the peptide materials field has uncovered the chemical features and supramolecular interactions driving self-assembly, resulting in a great variety of assembled structures, and explored their utility as biomaterials. Although the immune response to biomaterials is one of the most relevant factors for their success and therapeutic efficacy, the fundamental interactions of peptide materials and the immune system are not well understood. Different material attributes can affect the elicited immune response, such as stiffness, porosity, size, morphology, and surface chemistry. Here, we determine how peptide charge and charge distribution along the peptide fibrils modulate the immune responses to ß-hairpin peptide hydrogels. For this purpose, we developed a novel family of ß-hairpin peptide gels that have a range of charges from highly positive, neutral (zwitterionic), and negative. These materials have similar viscoelastic properties and fibrous structure, which ensures that the net charge and charge distribution are the main determining factors eliciting the observed immune responses. We evaluate the host response to this family of peptide gels using a subcutaneous injection model to characterize the degradation profile, histological appearance, and the immune cell infiltrate. We observe distinct and divergent host responses elicited by the differentially charged peptides, which indicates that the electrostatic charge influences the response to the materials. This project contributes to improving and developing basic principles for peptide material design, tailoring existing peptide materials for specific applications, and developing immunomodulatory materials for cancer and autoimmune diseases.




### P075 Flip & Tag: Studying Protein with Chemistry

A. Lander, STM Tang, D Cardella and LYP. Luk
*Room 1.54, School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff, CF10 3AT*

In this presentation, Louis will present two active branches of his research, “Flip” and “Tag”:

“Flip”: Enantiomeric Miniprotein Synthesis. At the current state of the art, polypeptides entirely composed of D-amino acids must be chemically synthesized. Though their preparation can be challenging, the availability of protein enantiomers is valuable for research. In this presentation, we will report the chemical synthesis of the bacteriocins aureocin A53 and lacticin Q as well as mechanistic insights gained from the racemic protein crystallography analysis. As a result of the current pandemic, we have also designed non-proteolytic D-miniprotein binders through synthesizing enantiomers of cytokine storm targets. (Ref 1)

“Tag”: Chemo-enzymatic Protein Bioconjugation. A chemo-enzymatic labelling system that exploits the substrate promiscuity of the plant transpeptidase AEP and the facile chemical reaction between N-terminal cysteine and 2-formyl phenylboronic acid (FPBA) was developed. This work enables protein labelling at the terminus of choice for protein of different sequences with reduced equivalent of labelling agents. (Ref 2)

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### P076 A Copper Catalyzed Approach to Substituted β-Homoprolines for Studying Peptide Topology

N. Maarouf , W.D. Lubell
*Département de Chimie, Université de Montréal, Montréal, Québec, Canada*
*nassim.maarouf@umontreal.ca*

Natural proline favors specific peptide secondary structures. Proline derivatives offer tools for exploring the folding and function of biologically active peptides [1-4]. Our presentation focuses on the synthesis and application of 4-vinyl ß-homoproline analogs, which have been pursued by a route employing aspartate as chiral educt [5]. The olefin of this unique ß-amino acid is designed to serve as a handle for diversification to obtain different tools for constraining peptide backbone and side chain geometry.

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### P077 Novel Peptide Ligands Identification Directly through Cell-based Functional Assays in HTS Library

Rumit Maini[1], Sally Wang Liang[2], JD MPH
*[1]Assoc. Dir of Business Development, PepLib; [2]VP of Business Development,PepLib ,*

A novel high diversity HTS-compatible cyclic peptide library has been constructed based on the Peptide Information Compression Technology (PICT) and designed to have high accuracy and functional assay capabilities. The library consists of ~75,000 80-mer cyclic peptide constructs that contain all possible 3,200,000 (205) pentapeptide sequences, and in total half a billion unique peptide (up to 80 amino acids) sequences. The library is capable of high-sensitivity and high- specificity drug mining for a broad spectrum of targets, including membrane-proteins, such as GPCR and ion channels, and directly on cell-lines. It breaks

through traditional limitations of peptide screening platforms, extending capabilities beyond binding assays to cell-based functional assay. PepLib has validated the efficiency of the platform in over 40 successful screenings on molecular targets with binding assays and cell-lines with functional assays. A few examples of successful screening campaigns include against a receptor tyrosine kinase, a cytokine receptor, a immune check point receptor, and a GPCR target. After initial screening of the primary 80-mer library, the decompression process from the 80-mer cyclic peptide hits generated short linear/cyclic peptide leads between 10 and 40 amino acid long.


**P078** **Peptide Hydrogel with Self-healing and Redox-responsive Properties**

Areetha D'Souza; Liam Marshall; Jennifer Yoon; Alona Kulesha; Siddarth Chandrasekaran; Dona I.U. Edirisinghe; Olga Makhlynets

We have rationally designed a peptide that assembles into a redox-responsive, antimicrobial metallohydrogel. The resulting self-healing material can be rapidly reduced by ascorbate under physiological conditions and demonstrates remarkable 160-fold change in hydrogel stiffness upon reduction. Given its antimicrobial and rheological properties, the newly designed hydrogel can be used for removable wound dressing application, addressing a major unmet need in clinical care.

**YI-P079** **A Powassan Virus Domain III Nanoparticle Immunogen Elicits Neutralizing and Protective Antibodies in Mice**

Ryan J. Malonis,[1] George I. Georgiev,[1] Denise Haslwanter,[2] Laura A. VanBlargan,[3] Georgia Fallon,[1] Olivia Vergnolle,[1] Sean M. Cahill,[1] Richard Harris[1], David Cowburn[1], Kartik Chandran,[2] Michael S. Diamond,[3] and Jonathan R. Lai[1]

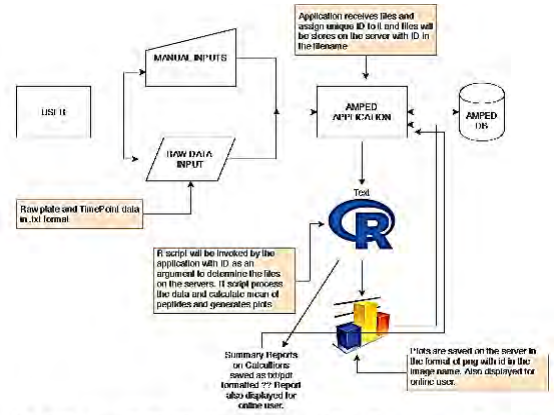
*[1]Department of Biochemistry, Albert Einstein College of Medicine, New York, NY 10461, USA*
*[2]Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY 10461, USA*
*[3]Department of Medicine, Washington University in St. Louis, School of Medicine, St. Louis, Missouri, USA*

Powassan virus (POWV) is an emerging tick borne flavivirus (TBFV) that circulates in North America and causes severe neuroinvasive disease. Currently, there are no approved treatments or vaccines to combat POWV infection. Here, we generated and characterized a novel POWV immunogen that displays the domain III (EDIII) of the POWV envelope glycoprotein on the surface of a self-assembling protein nanoparticle, *Aquifex aeolicus* lumazine synthase (LS). Although immunization of small peptide and protein antigens can result in.a weak immune response, recent work has shown that displaying such epitopes on a multivalent nanoparticle scaffold can enhance their immunogenicity. We devised an efficient strategy for the soluble bacterial expression and purification of the POWV EDIII and subsequent coupling to LS nanoparticles using SpyCatcher/SpyTag conjugation. Immunization of mice with POWV EDIII presented on LS nanoparticles resulted in significantly higher serum neutralizing titers against POWV than immunization with monomeric POWV EDIII. Furthermore, passive transfer of EDIII-reactive sera protected against POWV challenge *in vivo.* We isolated and characterized a panel of EDIII-specific monoclonal antibodies (mAbs) and identified several that potently inhibit POWV infection. Through a combination of competition, mutagenesis, and NMR studies, we find that these mAbs engaged distinct epitopes within the lateral ridge and C-C′ loop of the
EDIII. Together, we show that our subunit-based nanoparticle immunogen elicits antibodies with protective activity against POWV infection, and our findings enhance our understanding of the molecular determinants of antibody-mediated neutralization of TBFVs.

**P080** **Characterization and Identification of Antidiabetic Bioactive Peptides from Young and Mature Soybean Protein Hydrolysates**

Feyisola Fisayo Ajayi[1], Amna Alnaumi[1], Priti Mudgil[1], Hina Kamal[1], Chee Yuen Gan[2], Sajid Maqsood[1*].
*[1]Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al-Ain, UAE.*
*[2]Analytical Biochemistry Research Centre (ABrC), Universiti Sains Malaysia, 11800 USM, Penang, Malaysia*
**Sajid.m@uaeu.ac.ae*

The antidiabetic properties of hydrolysates produced from young and mature soybean proteins were investigated.

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Hydrolysates were generated separately using three different food-grade enzymes (alcalase, bromelain, and flavourzyme) from young and mature soybean proteins for 2, 4, and 6 h hydrolysis times. Young soybean protein hydrolysates (YSPHs) and mature soybean protein hydrolysates (MSPHs) produced were explored for their inhibitory properties towards antidiabetic enzymatic markers [α-amylase (AA) and dipeptidyl peptidase IV (DPP-

IV)] in relation to time of hydrolysis. The results indicated that the degree of hydrolysis (DH) of both YSPHs and MSPHs significantly increased ($p<0.05$) with increased time of hydrolysis. The highest AA and DPP-IV inhibitory activities for both YSPHs and MSPHs were demonstrated by flavourzyme at hydrolysis time of 2 h and 4 h, respectively. Overall, YSPHs exhibited higher AA and DPP-IV inhibitory activity with $IC_{50}$ values 26.57 and 29.26 µg/mL, respectively compared to MSPHs. Peptides were identified in selected hydrolysates using LC-MS-MS QToF and in-silico analysis was employed to study possible interactions of the peptides with AA and DPP-IV. Among all the peptides identified, CFAAPEGPL identified in YSPHs and AIPVNKPGRF from MSPHs possessed the maximum AA binding sites (7 and 9, respectively). While as peptides SFFFPFELPRE and FAGVVPAPLCCAT derived from YSPHs and MSPHs were predicted to be the most potent sequence binding up to 12 and 11 residues at active sites of DPP-IV, respectively. Peptide FAGVVPAPLCCAT derived from MSPHs using alcalase at 6 h were identified as potential AA and DPP-IV inhibitors. In conclusion, YSPHs and MSPHs displayed potential inhibitory properties towards enzymatic markers responsible for regulating diabetes.

### P081 Agile Interdisciplinary Design for AMPed, the Antimicrobial Peptide Editable Database

A. Singamaneni, J. Peckham and L. M. Martin
*Departments of Computer Science and Statistics, and Cell and Molecular Biology , University of Rhode Island, Kingston, RI 02881, USA*

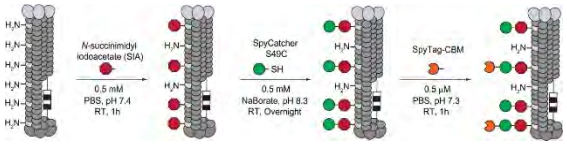
A truly interdisciplinary effort by computer scientists, and biochemists to understand how bench scientists acquire and process information may yield new insights for both fields.[1] So, if the CS part of the interdisciplinary team understands how the raw data has been generated through the experiment, producing raw data to be analyzed, they can help to identify the key characteristics in designing and implementing an algorithm which converts the raw data into required output and that output data structure should make sense to the biologists to ensure transparency. The data science problem in many peptide research labs is that the data files generated by instruments (and researchers!) are linear text files, which are not sorted and arranged in a specific manner in order for their contents to be traced or reconstructed easily. Nowadays there exist relational databases which hold much data efficiently, making vast amounts of data easily accessible through universal queries and allow all data to be easily exportable in a variety of file formats. The AMPed database is an agile editable central MySQL relational database, linked to a NoSQL raw data repository and a java-enabled online web portal, wherein values, attributes, and relationships can be updated as research projects evolve. The agile database design process described in this presentation integrates all of the relavant content and to assures that the data remains consistent and correct. Of central importance is that data manipulation through all algorithms can be accessed and monitored efficiently by multiple users coming from different disciplines.

The entity relationship diagram (ERD) design team of biologists and computer scientists collaborated for several semesters through online meetings, collected user requirements, figured out the connections and relationships between the various types of peptide research and bioinformatics datasets and finally arrived at an ERD design and process that is a model for use by interdisciplinary software development teams.

### YI-P082 Multivalent Display of Lectins on Phage for Studying Glycans on the Cell Surface

Guilherme Meira Lima[1], Alexey Atrazhev[1], Mirat Sojitra[1], Gisele Monteiro[2], Matthew S. Macauley[1,3], Lara K. Mahal[1], and Ratmir Derda[1].

*[1] Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada*
*[2] Departamento de Tecnologia Bioquímico-Farmacêutica, Universidade de São Paulo, São Paulo, SP 05508 000, Brazil*
*[3] Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB T6G 2J7, Canada*Glycans coat the surface of cells in every kingdom of life. They mediate cell-cell interactions in various physiological and pathological processes and changes in glycosylation is a hallmark of cancer and inflammation.[1, 2] Understanding the composition and function of glycans in biology is key to the development of new therapeutics but deciphering the complexity of glycans *in vivo* remains a challenge. Herein, we report the development of multivalent lectin-phage conjugates that interact and decode the complexity of glycans on cells. Synthesis of lectin-phage conjugates started from acylation of ~50% of the major coat phage protein with *N*-succinimidyl iodoacetate (SIA) followed by conjugation to SpyCatcher S49C mutant protein. Reaction of SpyCatcher-modified phages with carbohydrate-binding module (CBM)[3] fused with a SpyTag peptide at the N-terminus yielded constructs with 350-500 copies of CBM on phage. Panning of CBM-phage conjugates against sialylated U937 cells resulted in ~1000-fold increase in phage recovery compared to blank phages and 15-fold increase in phage recovery compared to panning of CBM-phage against desialylated U937 cells. Synthesis of more complex DNA-barcoded lectin-phage conjugates as well as panning of these constructs *in vivo* will be followed. We envision that this research will provide a more rapid, accurate and multiplexing technology to decipher the complexity of glycans *in vivo*.

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### YI-P083 Identification of Passive Permeability Patterns for Cyclic Heptapeptide Scaffolds Cyclized via $Cu^{2+}$ Click-Chemistry

Cristobal Morfin
*University of California, Santa Cruz*

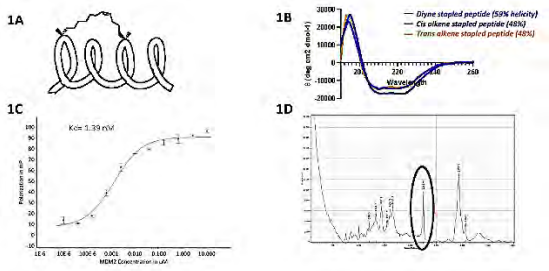
Cyclic peptide versatility allows drug targeting of protein-protein interfaces, whereas classical small molecules typically only target ligand binding sites. However, therapeutic development of cyclic peptides for intracellular targets is hindered by their inability to exhibit passive cellular permeability. The purpose of this project is to investigate structural implications on the permeability of cyclic heptapeptides cyclized via click-chemistry. Our lab has shown that strategic placement of N-methylations, stereochemistry, and inclusion of peptoids in cyclic peptides increase permeability. Strategic patterns of these structural features result in intramolecular hydrogen-bond networks that shape the cyclic peptide and separate polar and hydrophobic patches into a more permeable conformation. To identify and optimize the effects of stereochemistry and N-methylation on heptapeptides, a library of 256 compounds was designed via split-pool method. Preliminary data from shake-flask partition experiments show that heptapeptides partition well into aqueous and organic solvents, suggesting good solubility and permeability. A second-generation library will be designed that incorporates peptoids to further optimize passive permeability, while also creating a foundation for addition of drug-like moieties. By biasing a cyclic peptide library towards membrane-permeable scaffolds, the chance of finding potent library hits that exhibit membrane permeability will increase, and thus increase the chance for potent cyclic peptides to meet necessary drug properties. Furthermore, this work can inform the design of DNA-encoded libraries of click-cyclized peptides which can screen >$10^6$ compounds simultaneously for potency.

### YI-P084 Development and Application of Stapling Strategies for α-helical Peptides

*Danielle C. Morgan,* Laura McDougall,* Astrid Knuhtsen,* Lori Buetow,** Feroj Syed,** Danny Huang** and Andrew G. Jamieson.**
**School of Chemistry, University of Glasgow, Joseph Black Building, Glasgow, G12 8QQ*
***The Beatson Cancer Institute, Switchback Road, Bearsden, Glasgow G61 1BD*

Stapled peptides are an important class of alpha-helical peptides which are conformationally constrained. They are used in the regulation of Protein-Protein Interactions (PPIs), utilising their ability to bind to shallow PPI interfaces. Conformationally constraining wild type peptides using staples could pay the entropic penalty of folding, resulting in favourable binding affinities and improved selectivity's. Earlier stapling techniques used by the group include an all-hydrocarbon alkene staple (Figure 1A), achieved from ring closing metathesis reactions using unnatural amino acids. Installing an alkene staple into the peptide affords cis and trans isomers, ultimately lowering the yield and making the purification of the peptides challenging. Previous work in the group has been to develop a new technique to achieve i, i + 7 stapled peptides using a novel constraint, attaining a more rigid staple with no isomers. Additionally, the novel constraint has Raman activity, with a significant peak in the cell-silent region allowing for bi-functional properties. We propose that the staple can act as an alpha-helical constraint and enable visualisation of peptides in cells without the need for a fluorophore.

The project aims included selecting a well known stapled peptide and installing the novel staple into the sequence and comparing the properties. A well discussed PPI in literature is that between p53 and MDM2. This interaction is well acclaimed as a cancer therapeutic and led to the discovery of ALRN-6924 (Aileron therapeutics), a stapled peptide in clinical trials. The sequence of ALRN-6924 has not been disclosed for patenting reasons and therefore its precursor stapled peptide drug, ATSP-7041, was selected for comparison. A stapled analogue with the novel constraint was synthesised and compared to ATSP-7041 using (Figure 1B) circular dichroism (CD), (Figure 1C) fluorescence polarisation (FP) and (Figure 1D) Raman spectroscopy. The novel surrogate showed comparable binding properties, improved alpha-helicity and was Raman active.




### YI-P085 A Comparative Investigation into the Peptidomic Profile of Anti-Obesity Peptides Obtained via Probiotic Fermentation of Camel, Bovine, Sheep, and Goat Milk

Priti Mudgil[1], Chee-Yuen Gan[2] and Sajid Maqsood[1]
[1]*Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates*
[2]*Analytical Biochemistry Research Centre (ABrC), Universiti Sains Malaysia, 11800 USM, Penang, Malaysia.*

Peptides obtained after fermentation of milk from four different domestic animals were studied for their potential anti-obesity effects *in vitro* through inhibition of pancreatic lipase (PL). Furthermore, effect of storage and fermentation carried out by five different probiotic was assessed. Overall, *Pediococcus pentosaceus* driven fermentation of bovine, camel and sheep milk depicted maximum inhibition of PL. For goat milk however, *Lactobacillus fermentum* demonstrated the maximum PL inhibition. Further, storage of fermented milk samples at 4°C for 7 and 14 days showed significant increase in PL inhibition suggesting further release of peptides with PL inhibitory properties. Most significant decline in PL-$IC_{50}$ values was observed for camel milk followed by sheep milk. PL inhibitory peptides were identified from *Pediococcus pentosaceus* fermented bovine, camel, goat and sheep milk for interpretation of their inhibitory mechanisms. Peptides, RELEELNVPGE, PIGSENSEKTT, SDIPNPIGSENSEKTTMPLW, NRAM, RFNH and DLNPAR from fermented bovine milk indicating stronger interactions with PL as indicated by their HPEPDOCK Score and number of binding hotspots. Among camel milk derived peptides, IMEQQQTEDEQQDK, YDLF and RTPLDELKDTR indicating stronger interactions

with active sites residues of PL. For goat milk obtained peptides VPQRDMPIQA, IHPFAQAQS, PETPLT, NLRL, YLSH and MTPY were detected with significant interactions with PL. However, peptides HQTEDELQDK and YSGH obtained from sheep milk showed the most significant HPEPDOCK score and affinity for binding towards PL. In conclusion, although this study lays the foundation for identification of antiobesity peptides from fermented milk from different animal species, further studies under *in-vivo* conditions are warranted to establish their physiological significances.

### YI-P086 Immune-responsive Peptide Hydrogels for Controlled Delivery of Enhanced Co-stimulation Blockade Therapy to Prolong Vascular Allograft Survival

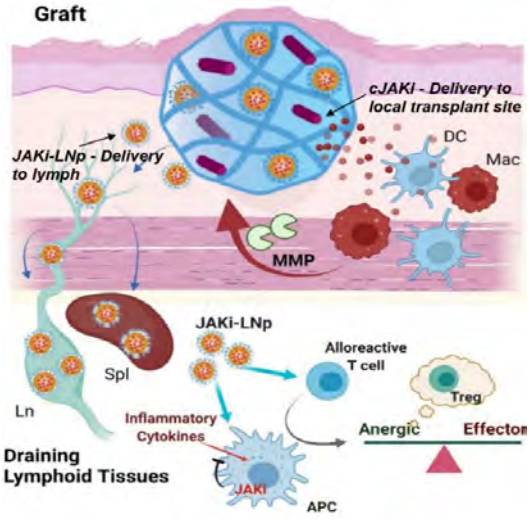
Monessha Nambiar,[1] Chen Liang,[1] Xiomara Calderon-Colon,[2] Olivia Tiburzi,[2] Alexander Komin,[3] Julia Patrone,[2] Giorgio Raimondi,[3] and Joel Schneider[1]

[1]*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA*

[2]*The Johns Hopkins Applied Physics Laboratory, Laurel, MD 20723, USA.*

[3]*Vascularized Composite Allotransplantation Laboratory, Department of Plastic and Reconstructive Surgery, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA.*

Vascularized composite allotransplantation (VCA) provides a restorative option for patients requiring the transplantation of multiple tissues as a single functional unit. Further, successful patient outcome after transplantation correlates with effective life- long application of immunosuppressive drugs. However, debilitating side-effects (nephrotoxicity, cardiovascular disease, diabetes, and cancer) counterbalances the benefits of immunosuppressive therapy. This presents an imminent need for developing therapeutic methods that limit tissue rejection after VCA surgery. Typically, T-lymphocytes are at the core of transplant rejection, controlling both the cellular and humoral arms of the rejection response. As such, combination therapies that target multiple pathways involved in immune rejection of transplants hold promise for patients in need of restorative therapy. Enhanced co-stimulation blockade therapy[1] regulates alloreactive T-cell activation by targeting T-cell costimulatory receptors (CD28) with biologic CTLA4-Ig, while simultaneously inhibiting the signaling and production of inflammatory cytokines with the JAK1/3 inhibitor, Tofacitinib. Our recent work has demonstrated that local delivery of Tofacitinib from crystalline deposits encapsulated within a peptide hydrogel injected at the grafting site, along with systemically delivered CTLA4-Ig, dramatically inhibited rejection.[2] We are developing a second-generation dual component platform that provides both a localized and lymph-targeted delivery of Tofacitinib, tuned to rejection response intensity. The dual system utilizes crystalline Tofacitinib for a sustained gradient near the transplant,[2] with the addition of Tofacitinib-containing lipid nanoparticles that traffic to the lymph, in response to rejection-associated proteases. This dual platform, in combination with CTLA4-Ig, should enable long-term VCA transplant survival.

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### YI-P087 Peptide Based Delivery of Anti-miR-210 using Tachyplesin, a Cell Penetrating Antimicrobial Peptide, for Glioblastoma Treatment

Pankhuri Narula[1], Anirban Jana[2], Ritu Kulshreshtha[2*] and Archana Chugh[1*]

[1] *Kusuma School of Biological Sciences, Indian Institute of Technology- Delhi*

[2] *Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology- Delhi*

Glioblastoma is the most common and aggressive primary brain tumor in adults. Since the current GBM treatments are ineffective, the importance of exploring new therapeutics is very much needed. In an effort to find out novel approaches, studies have focused on microRNAs that are small non-coding RNAs and function as key regulators of gene expression by acting both as oncogenic and tumor suppressing in nature. However, due to their anionic charge miRNAs are unable to permeate mammalian cell membrane. miR-210 is an oncogene overexpressed in many types of carcinomas including gliomas and is known to induce cell proliferation, migration, invasion, angiogenesis and inhibits apoptosis. Therefore, delivery of anti-miR-210 can help in reducing the level of overexpressed miR-210 in GBM cells. Cell penetrating peptides are extensively employed as nanocarriers for delivery of variety of cargoes owing to their efficient cell translocation ability. In our studies, Tachyplesin (Tpl), a marine antimicrobial cell penetrating peptide exhibited excellent cell penetrating ability in GBM. Further, Tpl formed a non-covalent complex with anti-miR-210 and delivered it efficiently into GBM cells. After internalization of the complex, significant reduction in miR-

210 levels with a concomitant increase in the levels of its target genes was observed. Significant inhibition of cell proliferation, spheroid formation, migration and induction of apoptosis in GBM cells was
also observed after treatment with anti-miR-210:Tpl complex. Our findings demonstrate that efficient delivery of anti-miR-210 molecule with Tpl may hold great promise for treatment of GBM and other solid tumors with high miR-210 levels.

### P088 Losing The Turn: Design of a Minimal ß-Sheet

Alex Nazzaro[1], Nick Sawyer[2], Andrew Watkins[3]
*[1]Arora Lab at New York University*
*[2]Fordham University*
*[3]Genentech*

Peptidomimetics remain attractive therapeutic strategies to target protein-protein interactions (PPIs), as a large part of the proteome is deemed undruggable by traditional small molecules. These interfaces are populated with secondary & tertiary structures such as alpha helices, beta strands, and beta sheets. With an extensive history of developing synthetic helices and higher order helical structures such as coiled-coils for targeting PPIs, beta-strand and sheet mimics that have successfully targeted intracellular PPIs remains in its infancy. Over the past twenty years there has been abundance of strand and sheet mimics that have been limited to spectroscopic analyses, with few successful examples of targeting proteins. These ß-mimics have previously limited their applications in a biological context due to aggregation, dependence on specific turn sequences, or utilization of several side chains for stability rather than molecular recognition. Through various spectroscopic analyses, we show the successful design of a turn-free beta sheet scaffold that is soluble in aqueous media and has full retention of all side chains for planned protein targeting.

### YI-P089 Amino Acid Based Amphicharged Bolaamphiphilic Surfactants

R. Ramapanicker and Neelakshi
*Department of Chemistry, Indian Institute of Technology, Kanpur, Uttar Pradesh, Kanpur 208016, INDIA*

The noun “bola” relates to the shape of a South American missile weapon.[1] The simplest form of “bola” consists of two balls which are attached to both ends of a cord. The term “bolaform electrolyte” (short form: “bolyte” or “bolion”) was introduced by Fuoss and Edelson in 1951 for a chain of hydrophobic groups connecting two hydrophilic end groups. For less water-soluble analogues, the name “bolaform amphiphiles” (short form: “bolaamphiphiles”) is preferred.[2] The biological and biochemical applications of amino acids and synthetic peptides have evolved over the years, offering an effective means to satisfy the technological demand of modern biomaterials. Design of peptide-based bolaamphiphiles offers a simple and facile means to organize peptide and amino acid motifs with the aid of nonbiological hydrophobic centers, realizing a protein-mimetic configuration at the molecular level. Inspired by these thoughts, we have synthesized a new class of amino acid based bolaamphiphilic surfactants derived from L-Lysine and L-Glutamic acid residues and have studied their aggregation properties. The results of our studies in this direction are presented in the poster.

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### P090 Synthetic Natural Product Inspired Cyclic Peptides

Samantha Nelson,[2] Matthew A. Hostetler,[1] Chloe Smith,[1] Ian Woosley,[1] Autumn Frerk,[1] Braden Baker,[1] Jessica Gantt,[1] Elizabeth I. Parkinson[1, 2, *]
*[1]Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States*
*[2]Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana 47907, United States*

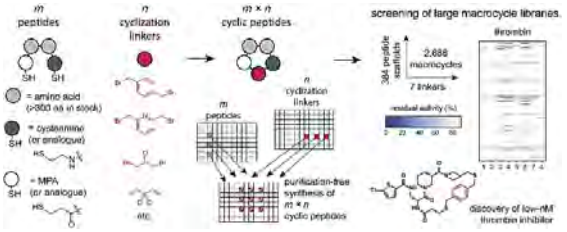
Approximately 78% of FDA approved antibiotics are derived from natural products. Additionally, natural products are traditionally better at hitting challenging targets. However, natural products tend to be difficult to discover due to cryptic biosynthetic gene clusters (BGCs). Our lab developed Synthetic Natural Product Inspired Peptides (SNaPP) to identify novel natural product inspired molecules. This method utilizes bioinformatic predictions of non-ribosomal peptide synthetases and synthesis of the predicted natural products (pNPs). Penicillin binding protein-like cyclase was used for the inspiration for our chemical library due to this class of enzymes ability to specifically catalyze head-to-tail cyclization of peptide natural products. We analyzed 500 BGCs and identified 131 novel pNPs. Utilizing SNaPP, we were able to synthesize 52 pNPs. 14 of these compounds exhibited antibiotic activity, including 8 with activity against Gram-negative bacteria. Furthermore, a structure activity relationship was developed for 2 of the most interesting pNPs. Overall, SNaPP has shown to be an efficient way to identify novel bioactive pNPs.

### YI-P091 Purification-free Synthesis and Screening of Thousands of Cyclic Peptides

Alexander L. Nielsen, Zsolt Bognár, Ganesh K. Mothukuri, Anne S. L. Zarda, Manuel Merz, Mischa Schüttel & Christian Heinis
*Institute of Chemical Sciences and Engineering, School of Basic Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.*

Macrocyclic peptides provide an attractive modality for drug development due to their ability to bind feature-less protein targets, for which small-molecule binders are extremely difficult to obtain. However, synthesis of large macrocyclic libraries is currently limited by low-yielding cyclization reactions and the need for chromatographic purification of individual peptides. Therefore, there is an unmet need for methods that provide easier and faster access to macrocycle libraries applicable for high-throughput screening campaigns against challenging targets. Herein we report a method that allows for the synthesis of thousands of diverse macrocycles based on the cyclization of dithiol peptides with bis-electrophilic linkers. Synthesis is initiated by functionalizing cysteamine derivatives onto resin immobilized via a disulfide bridge. Following automated SPPS in 4×96-well format, side-chain protecting groups are removed with TFA mixture, which leaves only unprotected dithiol-peptide left on the resin. Subjection of a volatile reducing reagent leads to reductive release of

crude dithiol peptides in excellent purity, which are cyclized with a plethora of linkers in a fully automated fashion applying automated liquid handling and acoustic droplet ejection in 384- or 1536-well plates to provide access to thousands of cyclic peptides. The strategy was utilized to create a focused library of 2,688 structurally diverse macrocycles for which one of the most potent thrombin inhibitors reported to date was identified. Conclusively, the method overcomes a major bottleneck in producing macrocyclic peptide libraries, as no purification steps are required for the synthesis of thousands of peptide macrocycles that can be directly used in screening campaigns.




## YI-P092 Thiol-Ene Mediated Peptide Macrocyclisation for the Synthesis of Neuropeptide Analogues

Mark D. Nolan, Rita Petracca and Eoin. M. Scanlan
*Trinity Biomedical Sciences Institute, Trinity College Dublin, D02 R590, Ireland*

The chemical synthesis of peptides and proteins represents an area of growing interest, in part due to the rapidly expanding applications of peptide and proteins as therapeutics and diagnostics. Peptide stapling and cyclisation has been demonstrated as a potent approach towards improved therapeutic properties of peptides, affording improved stability as well as often improving binding due to stabilisation of the overall conformation.[1]

Thiol-Ene "Click" chemistry is highly efficient for peptide macrocyclization, and has been previously applied to peptide and protein modification.[2,3] We have developed methodology for the Thiol-Ene "Click" macrocylisation of peptides through short backbone-backbone linkages, exemplified by synthesis of thioether analogues of the neuropeptide oxytocin and highly efficient synthesis of the clinically important therapeutic Carbetocin. The disulfide bridge is second only to the amide bond its prevalence as a covalent linkage between amino acids in peptides and proteins, but is readily cleaved in reducing environments. Thus, its replacement with suitable linkages enables rapid access to robust analogues suitable for biological screening.

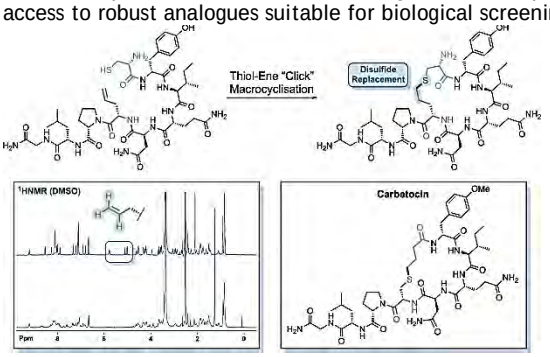



*Figure 1: Thiol-Ene "Click" macrocyclisation of oxytocin analogues.*

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## P093 Exploring the Scope of i,i+4 Thioether Staples on a ß-Catenin Peptide Inhibitor

Paula Ortet[1], Dashiell Anderson[2], Brian White[1], Sorabh Agarwal[1], Jessica Ramirez[1], Erica Visness[1], Charles Ponthier[1], Charlie Johannes[1], Martin Tremblay[1], Greg Verdine[1]
[1] *Fog Pharmaceuticals Inc, 30 Acorn Park Dr, Cambridge 02140 MA, USA*
[2] *Northeastern University, 360 Huntington Ave, Boston, MA 02115*

Peptide stapling has been shown to enhance target binding affinity, proteolytic stability, and cell permeability of α-helical peptides [(1-3)]. Several methods to stabilize peptides into α-helices via amino acid sidechain crosslinking have been developed. Arguably the most robust crosslinking strategies include ruthenium-catalyzed ring closing metathesis, lactamization, azide-alkyne cycloaddition, and thiol alkylation [(3, 4)]. More lipophilic linkages such as the all-hydrocarbon and thioether staples have been reported to exhibit superior cell permeability compared to more polar linkages namely lactam and triazole-containing crosslinks [(5)]. We have learned that appending a hydrocarbon stitch staple onto a peptide inhibitor of ß-catenin, which covered all but the C-terminus of the peptide chain, resulted in increased target binding affinity and cell activity. We postulated that introducing a C-terminal thioether staple would improve target binding affinity by further reducing the conformational entropy of the peptide inhibitor, and the lipophilic properties of the staple would help boost peptide cell uptake. In this study, a library of our lead peptide inhibitor containing a C-terminal i,i+4 thioether staple was generated. A large variety of thioether staples were synthesized by scanning four unique thiol-containing amino acids at the i and i+4 positions and testing a diverse set of thiol-reactive linkers. This work led to the discovery of several thioether stapled peptides with similar cell activity profile as the lead peptide inhibitor and increased our understanding of the effect of thioether linkages on peptide conformational stability.

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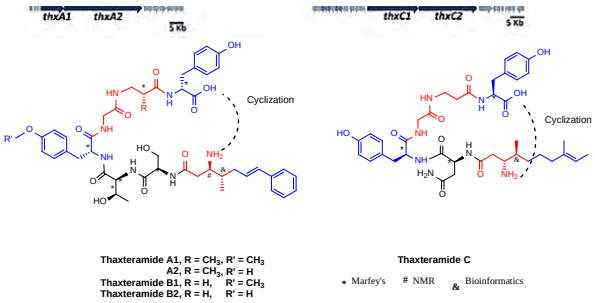
**P094** **Thaxteramide Lipopeptides: Inactive by Nature, Bioactive by Cyclization**

Emilia Oueis,[1] Thorsten Klefisch,[2] Nestor Zaburannyi,[2] Ronald Garcia, [2] Alberto Plaza, [2] and Rolf Müller[2]
[1]*Department of Chemistry, Khalifa University, Abu Dhabi, UAE*
[2]*Department of microbial natural products, Helmholtz-institute for pharmaceutical research Saarland (HIPS),Saarbrücken, Germany*

Nature-derived or inspired drugs have proven their worth over the years despite a recent decline in their pursuit by pharmaceutical companies. Nonetheless, advances in analytical techniques and bioengineering strategies are overcoming the known hurdles of natural products (NPs) discovery. The huge molecular and structural diversity introduced by NPs extends to natural peptide families. Herein, we report the discovery of the linear lipopetides thaxteramides produced by *Jahnella thaxteri*, a soil dwelling mixobacterial strain. Structure elucidation revealed a common *C*-terminal tetrapeptide Tyr-Gly-ß-Ala-Tyr core, while differing in the stereochemistry of the tyrosine units, methylations, the sequence, and the *N*-terminal polyketide. Two related but distinct hybrid PKS/NRPS gene clusters were revealed for the biosynthesis of thaxteramides in the genome sequence analysis. The linear natural derivatives lacked any biological activity in the assays performed. On the other hand, the chemically macrocyclized compounds were found to inhibit the growth of Gram-positive bacteria with promising results against *S. aureus* resistant strains.[1]




**P095** **Peptide-Guided Delivery Improves the Therapeutic Efficacy and Safety of Glucocorticoid Drugs for Treating Inflammatory Diseases**

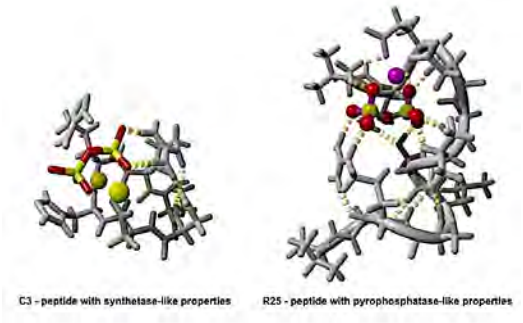
Hong Guo, Xian Wu, Yue-Xuan Li, Yushuang Wei, and Hong-Bo Pang
*Department of Pharmaceutics, University of Minnesota, Minneapolis, MN 55455, USA*

Glucocorticoid drugs are widely used to manage a variety of acute and chronic inflammation, but their clinical applications are often limited by adverse effects due to nonspecific accumulation in healthy organs and thus the impairment of normal immunity. To tackle this problem, we have covalently conjugated two model glucocorticoid drugs, prednisolone (PSL) and dexamethasone (DEX), with a targeting peptide, termed CRV. Murine models of acute lung injury (ALI) and rheumatoid arthritis (RA) were established as examples of acute and chronic inflammatory diseases, respectively. We showed that CRV peptide, upon systemic administration, can selectively home to the inflamed lung of ALI model, but not healthy organs or the lung of healthy mice. Similar result was seen in RA model as well. The expression of CRV receptor, retinoid X receptor beta (RXRB), was elevated in these inflammatory sites of ALI and RA mice, as well as in relevant patient samples, which may be the basis of CRV targeting. CRV-PSL and CRV-DEX conjugates remain intact in the circulation and get cleaved to release free PSL and DEX only after they enter the cells. In vivo biodistribution studies showed that CRV conjugation increases the amount of PSL or DEX in the inflamed lung (or joints) but reduces it in healthy organs. Accordingly, CRV-PSL and CRV-DEX showed a stronger activity to reduce the inflammation in the ALI lungs and RA joints, while reduced immune-related side effects elsewhere. Taken together, we provide a peptide-based delivery strategy to improve the therapeutic efficacy and safety of glucocorticoid drugs for treating inflammation.

**YI-P096** **Obtaining Minimal Phosphate Transferases**

Radoslaw Piast, Aleksandra Misicka, Maciej Garstka
*Warsaw University*

Phosphate transferases are key enzymes for life as they incorporate phosphate ions into organic molecules which in turn constitute energy metabolism, build information storing molecules, activate anabolic substrates, and are a cell signaling pathway. In our study, we were trying to obtain minimal versions of phosphate-transferring peptides - a few amino acid-long peptides able to mimic the catalytic abilities of a whole enzyme. This work explores our attempts to obtain a minimal peptide synthetase to be used as a catalyst in technological processes (peptide C3) and a minimal inorganic pyrophosphatase able to dissolve crystals of calcium pyrophosphate - the etiological factor of chondrocalcinosis (peptide R25).



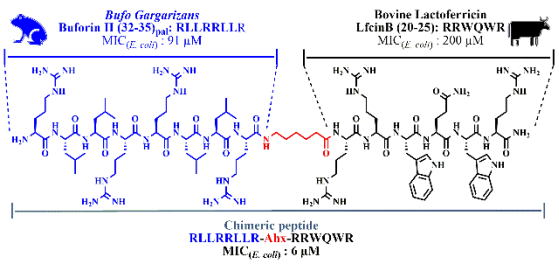

**YI-P097** **CHIMERIC PEPTIDES CONTAINING SEQUENCES DERIVED FROM BOVINE LACTOFERRICIN AND BUFORIN WITH ENHANCED ANTIBACTERIAL ACTIVITY**

H.M. Pineda-Castañeda, J.E. Garcia-Castañeda and Z.J. Rivera-Monroy
*Department of Chemistry, Universidad Nacional de Colombia, Bogotá, Colombia*

The indiscriminate use of antibiotics has generated increase in bacteria resistance and it is considered as a growing threat to health worldwide[1]. Developing new therapeutic potential for treatment of infections caused by resistant strains is mandatory. In this context, antimicrobial peptides (AMPs) arise as candidates for the design and development

of new therapeutic agents since AMPs activity is based on action mechanisms different from those exhibited by conventional antibiotics.

The design of chimeric peptides today is a of the strategies that seeks to enhance the activity of synthetic AMPs. For this study, two AMPs were selected Buforin II (BFII) and Bovine Lactoferricin (LfcinB). LfcinB corresponds to the N-terminal region of Bovine Lactoferrin (BLF), an important regulatory antimicrobial and immunological protein. On the other hand, the BFII corresponds to the N-terminal region of the H2A histone; it is present in the Asian toad species Bufo Gargarizans, whose functions are mainly associated with the packaging and regulation of genes in eukaryotic cells. LfcinB and BFII have presented broad spectrum of antimicrobial activity. Here in, chimeric peptides were obtained which combines the antimicrobial minimal motifs of both AMPs through SPPS and Click-chemistry. The antibacterial activity of synthetic chimeras was evaluated strains of *E. coli*, *S. aureus*, *E. faecalis* and *P. aeruginosa*, it was found chimeras with activity against the evaluated strains whose minimal inhibitory concentrations (MICs) ranged from 6 µM to 131 µM. The chimeric peptides that presented the best activity contain in their structure the RRWQWR and RLLRRLLR motifs (BFII(32-35)/Ahx/LfcinB(20-25): RLLRRLLR-Ahx-RRWQWR and LfcinB(20-25)/Ahx/BFII(32-35): RLLRRLLR-Ahx-RRWQWR), and exhibited bactericidal and bacteriostatic activity in the evaluated strains. The results of this work suggest that the design of chimeras derived from LfcinB and BFII is a viable alternative to generate new synthetic AMPs, promising for the development of new therapeutic agents.

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**YI-P098** **Genetically Encoded Fragment-Based Discovery of Covalent Peptide Macrocycle Inhibitors of PKM2 with Extended *in-vivo* Circulation**

Arunika I. Ekanayake†, Ryan Qiu† and Ratmir Derda†
†Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada

Peptides and peptide-derived therapeutics (PDT) have the potential to engage targets that cannot be addressed by small-molecule therapeutics (aka "undruggable targets"). PDTs can provide highly selective interactions with drug targets, rendering them favorable binders for therapeutically important proteins. The high specificity of peptides can be combined with electrophilic chemical fragments to generate irreversibly reacting PDTs that can interact with active and allosteric site nucleophilic amino acid residues such as cysteines. Using a methodology developed in our group[1], we can create libraries of covalently reactive ligands that can provide highly specific interactions with the target by grafting electrophilic compounds onto genetically encoded phage-displayed macrocyclic libraries. This serves as a method to identify novel PDTs bearing electrophilic modalities. Pyruvate kinase muscle isozyme 2 (PKM2) exhibits two catalytic cysteine sites addressable by electrophilic fragments and binding pockets targetable by peptide sequences.

The metabolic role of PKM2 is applied when cancer cells switch to a glycolytic phenotype known as the Warburg effect. Therefore, PKM2 is an interesting biomarker for cancer and a therapeutic target for tumorigenesis. While PDTs provide many desirable properties as drugs, they present several caveats, such as short half-life in the bloodstream and poor kidney retention. The binding of peptides to abundant serum proteins such as human serum albumin (HSA) can aid in addressing these drawbacks, increasing the circulation of PDTs by delaying renal excretion from the bloodstream. Previously, we discovered macrocyclization with a perfluorinated aromatic linchpin that provides an amphiphilic scaffold recognizable by the hydrophobic binding site of HSA *in-vivo.*[2] By linking both peptides together, creating a small macrocyclic antibody-like scaffold (SMALS), we can add circulation extension properties to our therapeutic peptide macrocycle.

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**P099** **From Small to Large Scale: Optimizing and Upscaling the Synthesis of Melanotan II (MT-II) - Melanocortin Receptor Agonist, on PurePep® Chorus and PurePep® Sonata+**

Colton Quick, Lukasz Frankiewicz, Luisa Aguiar, Juan Bello, Victor Nikolaev
*Gyros Protein Technologies, 4675 South Coach Drive, Tucson, Arizona, 85714, U.S.A.*

α-Melanocyte Stimulating Hormone (α-MSH) is responsible for triggering the production of melatonin – body's natural protection against ultraviolet (UV) radiation. α-MSH production is prompted, in itself, by the exposure of the skin to UV rays; however, stimulating the biosynthesis of melatonin prior to UV exposure could possibly prevent UV-induced skin cancer. [1]

Considering that the native form of α-MSH was too unstable in vivo to be administered as a therapeutic agent, a wide range of analogues were synthesized; [2-4] one of them is MT-II (Figure 1), a cyclic pseudopeptide with high resistance to enzymatic degradation and an extraordinary potency.

Given the relevance of MT-II and the need for its production at a larger scale, our work focused on the optimization of a fully automated synthetic pathway, i.e., synthesis of the linear sequence and on-resin
lactamization of the final peptide. In this work, we report the fully automated synthesis of MT-II on PurePep® Chorus using different coupling conditions and orthogonal protection schemes, for different cyclization strategies. Furthermore, we describe the upscale of MT-II synthesis, using the new PurePep® Sonata+ large scale peptide synthesizer.

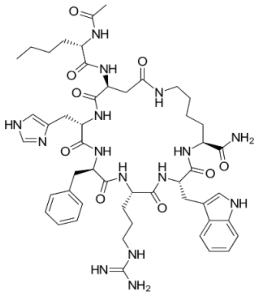

Figure 1. Structure of pseudopeptide MT-II.

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### P100 Proteomimetic Models and Inhibitors of Disease-Associated Tau Assembly

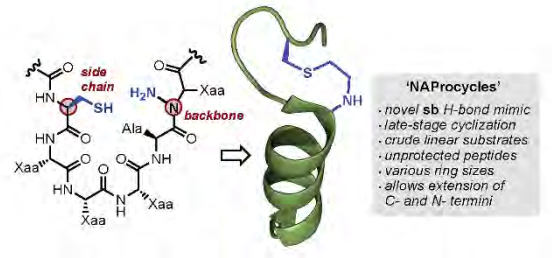
B. H. Rajewski, J. R. Del Valle*
*Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA*

The formation and spread of neurofibrillary tangles comprised of aggregated tau protein is central to the progression of Alzheimer's Disease and other tauopathies. Recent cryo-EM data suggest that fibrillar tau adopts unique folds in various diseases, resulting in the aggregation-prone VQIVYK sequence (PHF6) engaging in a cross-ß interaction with an opposing hexapeptide b-strand depending on the tauopathy. Aggregation model systems based on truncated tau variants do not account for these interactions, and often rely on cofactors which may not be biologically relevant. The development of synthetic mimics of pathogenic tau folds could provide valuable insight into the role of cross-ß interactions in fibrilization. Such compounds would also enable the development of assays to identify selective chemical probes and serve as a template for the design of potent inhibitors of tau aggregation. In this work, macrocyclic epitope mimics of conformational strains of tau, dubbed 'ß-bracelets,' were prepared by solid-phase synthesis followed Cys bis-alkylation. Our designs incorporate the PHF6 sequence and unique cross-ß interacting strands from specific tau conformations. ß-Bracelets aggregate rapidly without the need for additional cofactors and form fibrillar structures with unique morphologies when visualized by transmission electron microscopy (TEM). Furthermore, backbone N-amination of parent b-bracelets affords macrocyclic N-amino peptide (NAP) inhibitors of tau aggregation. These NAPs block the fibrilization of recombinant tau P301L in vitro as well as the seeding of endogenous tau in a cell-based reporter assay. This proteomimetic strategy thus enables the rational design of ligands that may be able to discriminate between closely related amyloid folds.

### YI-P101 A Novel Covalent Surrogate for Macrocyclic Sidechain-to-backbone H-bonds in Proteins

B.M. Rathman and J.R. Del Valle*
*Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46637, USA*

Overcoming the loss or lack of secondary structure in excised portions of proteins is a common challenge in developing peptide-based inhibitors of protein-protein interactions (PPIs). Macrocyclization is a key strategy in developing peptidomimetic drugs used to target PPIs involved in a variety of biologically significant processes that could lead to useful treatments of diseases. Several contemporary strategies for peptide macrocyclization exist, however most involve head-to-tail, sidechain-to-sidechain, or sidechain-to-head/tail connectivity. Sidechain-to-backbone (sb) tethering is less common, presumably due to the challenge of installing a reactive handle onto an internal amide. Our laboratory is addressing this through the synthesis of backbone N- aminated peptides (NAPs) that feature handles for further chemical modification. Here, we demonstrate a late-stage NAP bis-alkylation reaction with haloaldehydes to afford ethylene bridged N-amino peptide macrocycles (NAProcycles). Using this approach, we prepared a series of macrocycles with varying ring size from crude, unprotected linear substrates under aqueous conditions. Our approach features a novel covalent H-bond surrogate well-suited for mimicry of sb H- bonds that are prevalent in proteins. We incorporated our covalent constraint into a model loop-helix motif derived from the viral matrix protein VP40. We demonstrate that NAProcycle constraint significantly enhances helicity relative to linear analogues. Application of this tethering strategy to the stabilization of tertiary folds will also be discussed.




### YI-P102 Fluorogenic Smartprobes for the Detection of Activated Neutrophils and Neutrophil Extracellular Traps in Inflammation

M. R. Rios[a], G. Garoffolo[b], G. Rinaldi[c], A. Megia-Fernandez[a], S. Ferrari[b], C. T. Robb[c], A. G. Rossi[c], M. Pesce[b], and M. Bradley*[a]

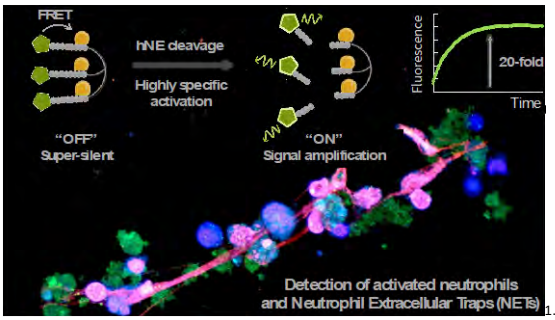
[a] EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK.

[b] Tissue Engineering Research Unit, IRCCS, Milan, Italy
[c]Queen's Medical Research Institute, University of Edinburgh, UK

Human neutrophil elastase (hNE) is a serine protease implicated in the pathogenesis of acute and chronic inflammatory diseases and is secreted by inflammatory neutrophils to destroy pathogens. Activated neutrophils are pivotal actors in many diseases. Herein we report a series of sensors providing an OFF/ON fluorescent signal targeting HNE in activated neutrophils and the detection of Neutrophil Extracellular Traps (NETs).

The first generation probe was green-emitting based on a multivalent scaffold combined with three copies of a FRET-labelled peptide1 using Fluorescein and Methyl-Red as fluorophores and quenchers, respectively. The multivalent scaffold2 approach enables "self-quenching", making the probe super-silent under physiological conditions and offering excellent signal amplification upon substrate cleavage (>20-fold). The probe was found to be specifically cleaved by HNE, with no cleavage by related proteases. Activated human neutrophils and NETs were successfully labelled with the probe under inflammation-induced conditions, allowing visualisation of different stages of NETosis and activation could be monitored by flow cytometry.

Current efforts are focused on synthesising a NIR-I variant of the probe using the fluorophore sulfo-Cy5 and the quencher QSY21 as a FRET pair. Due to the size and limited stability of the dyes, the solid phase approach developed for the "green variant" proved to be unsuccessful, and therefore, a "click" chemistry construction was developed. This involved the synthesis of two building blocks: an azide functionalized fluorescent peptide (synthesized using conventional SPPS) and a tribranched scaffold that contains three terminal alkyne groups and the quencher such that the two building blocks can be "clicked" together to give the desired tribranched compound.




1. M. R. Rios et al., *Chemical Communications*, 2021, 57, 97-100
2. N. Avlonitis et al., *Org Biomol Chem*, 2013, 11, 4414-4418.

## P103 D-peptide Inhibitors of Uropathogenic *E. coli* Adhesion Proteins to Treat Urinary Tract Infections

M. M. Robins, S. E. Apple, G. G. Quichocho, S. R. E. Draper, and M. S. Kay
*Department of Biochemistry, University of Utah, 15 North Medical Drive East, Room 4100, Salt Lake City, Utah 84112, United States*

Approximately 80% of urinary tract infections (UTIs) are caused by Uropathogenic *E. coli* (UPEC). Adhesion proteins on the pilus-covered surface of UPEC are required for attachment to host cells for colonization and disease progression. The primary adhesion protein associated with UPEC virulence is FimH, which binds D-mannose on uroepithelial cells. Our goal is to develop UTI treatments that prevent the attachment and subsequent internalization of UPEC in uroepithelial cells, eliminating bacteria from the urinary tract. However, it remains challenging to design long-lived inhibitors that reach the urinary tract without disrupting adhesion in commensal gut bacteria. We are using mirror-image phage display to identify D-peptide inhibitors of UPEC adhesion. Towards this goal, we have synthesized FimH in four peptide segments and used native chemical ligation to assemble them. FimH is densely populated with hydrophobic and negatively charged amino acids, making its synthesis incredibly difficult without the use of removable, solubilizing "Helping Hand" (HH) tags developed by our lab that can be added at Lys or Glu positions. Additionally, the use of a newly developed Asp HH proved critical for solubilizing one of the FimH segments. Using recombinant protein, we have validated a refolding protocol and confirmed that refolded FimH retains the same structure and function using circular dichroism and mannose binding assays. We are now synthesizing FimH using D-amino acids to provide the D-target to screen in mirror-image phage display and identify D-peptide inhibitors to prevent and treat UTIs.

## YI-P104 Antifreeze Activity of Peptides and Synthetic Dimers of Sequential Motives of Antifreeze Proteins

R.Rojas[12], Aróstica M.[23], Marshall S.[2], Aguilar L[3] ,F. Albericio[4] ,P. Carvajal-Rondanelli[5], Guzmán F.[2].
*1Universidad de las Americas (UDLA), Viña del Mar, Chile.*
*2 Núcleo de Biotecnología Curauma, PUCV, Valparaíso, Chile.*
*3Doctorate Program in Biotechnology. Universidad Técnica Federico Santa María and Pontificia Universidad Católica de Valparaíso (PUCV), Valparaíso, Chile.*
*3 Institute of Chemistry, PUCV.*
*4Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1-11, Barcelona 08028, Spain*
*5School of Food, PUCV.*
*rrojasg@udla.cl , r.rojas@outlook.cl*

Antifreeze proteins (AFP) and antifreeze glycoproteins (AFGP) are molecules with a known anti-freeze effect [1]. Peptides with the alanine-alanine-threonine sequence (AATAATAATAA) that belongs to the AFGP are good models for studying the importance of both the order and the key extension in an antifreeze process. Another modification for appreciating possible changes in the properties of the peptide was the substitution of threonine for lysine and proline residues (AAKAAKAAKAA and AAPAAPAAPAA).

Additionally, a comparison was made with peptides of 12 residues with cysteine at both amino and carboxy terminal (CAAKAAKAAKAAC, CAATAATAATAAC and CAAPAAPAAPAAC).

The secondary structure determined by circular dichroism (CD) between 5°C and 50°C, showed that the peptides had a random coil structure, but the addition of cysteine increased the propensity to alpha helix structure.

All the peptides were synthesized by solid phase using Fmoc methodology [2], purified and characterized by mass spectroscopy and HPLC. The secondary structure was determined by CD Jasco J-815. The antifreeze activity was measured by differential scanning calorimetry (DSC).

Peptides without cysteine have a low thermal hysteresis in DSC, whereas peptides with cysteine have a higher thermal hysteresis at the same molar concentration; additionally, the latter showed a increase in their antifreezing activity as their concentration increases.

This work generates guidelines to create structures analogous to AFGP using a simple and low-cost procedure of synthesis.

Acknowledgement: Fondecyt Grant Nr 1140926; Internal proyect UDLA DI- 11/21 of Dr. R. Rojas; CONICYT-PFCHA/National Doctorate/2021-21191654 of Ms M.Aróstica

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### P105 How to Tackle Aspartimide Formation - A Systematic Comparison of Different Methods

Kai Holland-Nell, Jacqueline Dalski, Thomas Bruckdorfer, Raimund Maier, Karin Rustler, Markus Weishaupt, Rudolf Dölling, Stephan Pritz
*Iris Biotech GmbH*

Aspartimide formation is still a serious challenge in peptide synthesis. This side reaction is strongly sequence dependent and preferably occurs at Asp-Aaa motifs (Aaa = Gly, Asp, Asn, Gln or Arg). In a first step, the cyclic aspartimide is formed, which can re-open in a second reaction leading to (epimerized) alpha- and beta-Asp peptides and corresponding piperidides. Over the last decades, several approaches to solve this problem have been developed.

In this work, we systematically compared the combination of different strategies on a model peptide. The steric effect of Asp side chain protecting groups was investigated (OBut, OEpe, OBno).[1] The influence of various Fmoc-cleavage reagents was studied, including acidic additives.[2] Furthermore, these results were compared with the application of dimethoxybenzyl (Dmb) as amide backbone protection and cyanosulfurylide (CSY)[3] as side chain protection.

Finally, our identified optimal conditions were tested in the synthesis of other peptide sequences prone to aspartimide formation.

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### YI-P106 Phage-Displayed Macrocyclic Libraries with Installed Bio-imaging Handles

G. A. Sable, C. Peng, and R. Derda*
*Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada*

Phage display is a revolutionary technique that won the 2018 Nobel Prize in chemistry for its ability to rapidly generate peptide based ligands against various therapeutic targets. Over time, phage display with natural amino acids has been expanded through adapting strategies to incorporate unnatural building blocks including pharmacophores and therapeutically valuable chemical fragments. These post-translational chemical modifications have been utilized to create phage-displayed macrocyclic libraries as a strategy to improve the pharmacokinetic properties of displayed peptides.

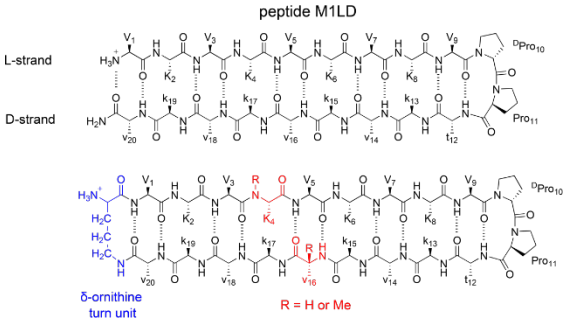
Previously, we developed a late-stage modification strategy to append various chemical fragments to phage-displayed peptides in a convenient and straightforward fashion[1]. Although our reported method is robust and efficient, monitoring modifications on phage can be cumbersome and time-consuming. Herein, we explored this previously described strategy to introduce bio-imaging agents directly on phage through post-translational chemical modification. Introducing such imaging agents shall have dual benefits of detecting phage modification in real-time and obtaining high-affinity ligands with existing imaging handles useful for further *in-vivo* studies. We demonstrate the efficient synthesis of such 'hydrazine functionalized imaging agent-linker conjugates.' Reactivity kinetics between synthesized hydrazine conjugates and the model peptides were evaluated to replicate it on phage for chemical modification. Our preliminary results show that the phage modification using model imaging agents was consistent with the previously reported results. Therefore, we envision testing a series of dyes and metal chelating agents for the post-translational phage modification and utilizing it to quantify the progress of modification and to discover lead peptides with a pre-installed imaging agent for rapid drug discovery.

[1] Ekanayake, A. I. *et al.* Genetically Encoded Fragment-Based Discovery from Phage-Displayed Macrocyclic Libraries with Genetically Encoded Unnatural Pharmacophores. *Am. Chem. Soc.* 2021, 143, *14,* 5497–5507.

### YI-P107 Progress Toward Understanding the Structural Determinants of Rippled β-sheets in Peptide-based Materials

T.D. Samdin, G.Fichman, J.P. Schneider
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA*

Rippled β-sheets are a unique and thus far underutilized structural motif in the design of peptide- based materials. First described by Pauling and Corey in 1953, empirical observations of rippled β-sheet interactions have only been made in the last decade by the Raskatov, Nilsson, and Schneider groups. The Schneider group has discovered that racemic mixtures of the hydrogel- forming β-hairpin peptide MAX1 exhibit a 4-fold greater gel stiffness over enantiopure hydrogels of either L- or D-MAX1. Diffusing wave spectroscopy reveals that these racemic gels are more mechanically rigid because the fibrils that comprise the gel are, themselves, more rigid. Molecular modeling of a rippled β-sheet comprising L- and D-MAX1 shows how the intermolecular nesting of hydrophobic valine side chains facilitates a tighter packing of fibrils and thus their increased stiffness. Inspired by these results, we designed stereo-mixed peptide M1LD, a β-hairpin that bears enantiopure L- and D-strands connected by a canonical type II' turn. Remarkably, peptide M1LD forms a gel that is nearly two orders of magnitude more rigid than gels formed by pure L- or D- MAX1. To facilitate X-ray crystallographic studies and better understand the assembly of rippled β-sheets, we designed and prepared several macrocyclic homologues of peptides MAX1, D- MAX1, and M1LD. Influenced by the Nowick group, each homologue is constrained to a macrocycle via a δ-linked-ornithine turn unit which connects the *N*- and *C*-terminal residues and bears an N- or Cα-methylated residue. The incorporation of rippled β-sheet-interactions into peptide-based hydrogels holds the promise of developing materials with novel biophysical properties and potential therapeutic applications.




### YI-P108 Peptide-PNA Assembly

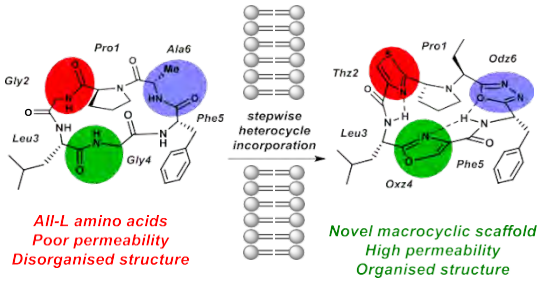
Srijani Sarkar, Joel Schneider*
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick MD, 21702, USA*

Peptide nucleic acids (PNAs) are unique materials that possess properties of both peptides and nucleic acids. Much is known concerning their gene targeting ability, however their use in materials development is little explored. We have conjugated PNAs to gelating peptides and studied the self-assembly properties of the resulting Peptide-PNA conjugates. We have designed two conjugates called Pep_P1 and Pep_cP1, where Pep is a hydrogel-forming peptide and P1 and cP1 are 10-mer self-complementary PNAs. The PNA and peptide are separated by a $PEG_5$ linker. The P1 and cP1 components are designed to form a thermally stable PNA-PNA duplex. We first optimized the synthesis of Peptide-PNA conjugates to address difficult coupling reactions, which ultimately led to their successful synthesis as determined by HPLC and MALDI. We determined the thermal stability of the P1-cP1 duplex alone by UV melting analysis, which showed the duplex melting temperature to be 68.8 ± 3.7 °C in nano-pure water. However, UV melting analysis on an equimolar mixture of Pep_P1 and Pep_cP1 showed the duplex melting temperature dropped to 60.5 ± 2.2 °C. Thus, the presence of peptide affects the thermal stability of PNA-PNA duplex. Further, temperature dependent CD at different conditions showed that the presence of PNA also influences the self-assembly properties of the peptide. Thus, careful design of both PNA and peptide conjugates is necessary to construct conjugates useful for material development. Lastly, rheology and TEM data will be discussed with respect to peptide self-assembly leading to hydrogel formation.

### YI-P109 Property-Driven Development of Privileged Macrocyclic Scaffolds using Heterocycles

George J. Saunders and Andrei K. Yudin
*Davenport Research Laboratories, University of Toronto, Toronto, Ontario, M5S 3H6, Canada*

Passive membrane permeability is a fundamental challenge in the development of bioactive macrocycles. To achieve this objective, chemists have resorted to various strategies, the most common of which is deployment of *N*-methylated amino acids and/or D-amino acids. Here we investigate the effect of heterocyclic grafts on the passive membrane permeability of macrocycles and report the structural consequences of iterative amino acid replacement by azole rings.[1] Through stepwise substitution of amino acid residues for heterocycles, we show that lipophilicity and PAMPA permeability of a macrocycle can be vastly improved. Overall, changes in permeability do not scale linearly as more heterocycles are incorporated, underscoring the subtleties of conformation-property relationships in this class of molecule. NMR analysis and molecular dynamics simulations provide insights into the structural consequences of the added heterocycles and foreshadow the emergence of privileged macrocyclic scaffolds.

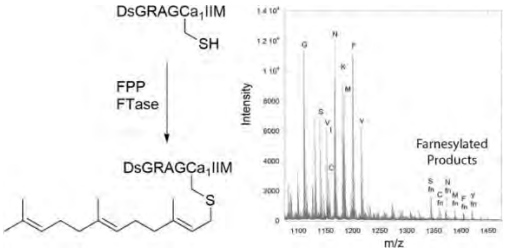



1 G. J. Saunders, A. K. Yudin, *ChemRxiv*, 2022, DOI: 10.26434/chemrxiv-2022-nq98h.

**P110** **Expanding the Scope of Extended Farnesyltransferase Substrates with a MALDI/MS Screening Approach**

G.L. Schey[1], P.H. Buttery[1], E.R. Hildebrandt[2], H.A. Passetti[1], S.X.H. Novak[3], J.L. Hougland[3], W.K. Schmidt[2], and M.D. Distefano[1] *[1]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota USA. [2]Department of Cellular Biology, University of Georgia, Athens, Georgia USA. [3]Department of Chemistry, Syracuse University, Syracuse, New York USA.*

Protein prenylation is a post-translational modification where a 15 carbon farnesyl or 20 carbon geranylgeranyl isoprenoid is appended to the C terminal end of a protein by either farnesyltransferase (FTase) or geranylgeranyl transferase type 1, respectively. In the canonical understanding of FTase, the isoprenoids are attached to the Cysteine residue of a four amino acid CaaX box sequence. However, recent work has shown that five amino acid sequences can be recognized, such as the pentapeptide CMIIM. This new discovery greatly increases the number of potential FTase substrates, as the enzyme is already known to tolerate a wide variety of amino acids in the canonical CaaX box. With the goal of developing a more rapid and methodical method to evaluate potential substrates, we envisioned using MALDI to assay libraries of 10 peptides at a time, varying one amino acid in the CaaaX box to all 20 canonical amino acids over two libraries, utilizing both yeast and rat FTase. Through this method we observed over 30 hits in the mass spectrum and chose eleven for further evaluation. Nine of these sequences are novel substrates for FTase, with several meeting or surpassing the *in vitro* efficiency of the benchmark sequence CMIIM. Additionally, *in vivo* experiments in yeast demonstrate that proteins bearing these sequences can be efficiently prenylated in a biological context. Searching the human genome for pentapeptide CaaaX sequences found several hits that prenylated with similar efficiency to a native CaaX sequence, raising the possibility of relevance of these sequences in humans.

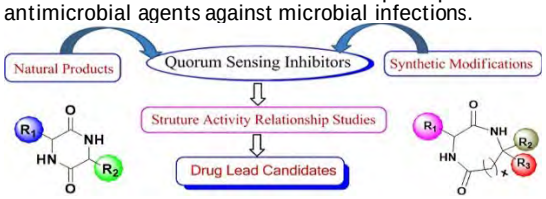



**P111** **Cyclic Peptide Containing Disubstituted β- and γ-amino Acid as Antimicrobial Agents**

S. Shankar and A. Jamieson
*School of Chemistry, University of Glasgow, Scotland, G128QQ, UK*

At this time when we are going through an unprecedented global pandemic, it is an unarguable fact that new and advanced drugs are of paramount importance. A pressing issue is the development of new medicines that treat microbial infections to prevent the growing health threat posed by resistant pathogenic microorganisms. Being able to control drug-resistant strains of gram-negative bacteria, without prolonging the course of treatment is even more challenging. Amongst the naturally occurring antimicrobial peptides, cyclic peptides have exhibited traits of potential antibiotics due to their diverse biological properties and metabolic stability compared to linear peptides. Their peculiar heterocyclic system i.e. 2,5-diketopiperazine found in several natural products constitutes a rich source of new biologically active compounds. Naturally occurring cyclopeptides isolated from fungi, plants and bacteria have been reported to target and disrupt the functions of microbial cell membranes. Major problems associated with such natural peptides are their low bioavailability, low metabolic, proteolytic stability, complex structures and high cost of goods.

To overcome the inherent limitation, these peptides can be modified using the peptidomimetic approach by introducing synthetic β- and/or γ-amino acid to develop short and potent peptide therapeutic agents against microbial infections. The present work aims to the develop novel cyclic peptides incorporating non-proteinogenic β- and γ-amino acids having non-native diketopiperazine with different side-chain modifications to explore potent antimicrobial agents against microbial infections.



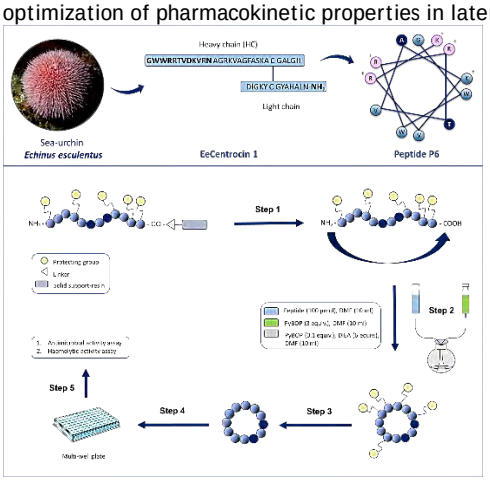

**P112** **Investigation of the Antimicrobial Activity and Proteolytic Stability of the Short Analogues of the Marine Peptide *EeCentrocin 1***

Danijela Simonovic*[1], Hymonti Dey*[2], Natascha Johansen[1], Trude Anderssen[1], Terje Vasskog[1], Elizabeth G. Aarag Fredheim[1], Tor Haug [2], Morten B. Strøm[1]
*[1] Department of Pharmacy, Faculty of Health Sciences, UiT – The Arctic University of Norway, NO-9037 Tromsø, NORWAY.*
*[2] The Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries and Economics, UiT – The Arctic University of Norway, NO-9037 Tromsø, NORWAY*
** Shared 1st authorship.*

The antimicrobial peptidkknerre *EeCentrocin 1*, isolated from the marine sea urchin *Echinus esculentus,* consists of a hetero-dimeric structure with a heavy chain (HC) essential for antimicrobial activity. In a recent study the *EeCentrocin 1* HC was successfully truncated and modified, resulting in the highly potent peptide P6. [1,2] We have synthesized a series of short analogues of a lead peptide P6 developed from the heavy chain (HC) of the marine antimicrobial peptide EeCentrocin 1. We have furthermore explored ways to optimize the lead peptide P6 by increasing its net positive charge, its lipophilicity through N-terminal fatty acid acylation and by addition of a Trp residue, and by synthesizing head-to-tail cyclic peptides using a pseudo dilution cyclization method.[3] A highly potent linear peptide P6-W6R8 (MIC 0.8 – 1.6 µM) was synthesized with improved antimicrobial activity against S. aureus and S. epidermidis compared to P6. Among the synthesized lipopeptides, C8-P6-R8 (lipopeptide with the shortest acyl chain), was the most promising by displaying high antimicrobial activity (MIC 1.6 – 6.6 µM) and no haemolytic toxicity. Successful head-to-tail cyclization gave the promising peptide cP6-W6R8 (MIC 0.8 – 6.7 µM). No peptide dimerization was observed using the pseudo dilution head-to-tail cyclization method. All synthesized peptides displayed antifungal

activity (MIC 3.2 – 26 µM), but with little variation in activity following the various peptide modifications.

Our study demonstrates that different peptide modifications can be successfully used to fine-tune antimicrobial and haemolytic properties. Furthermore, these modifications can serve as a useful tool for optimization of pharmacokinetic properties in later studies.

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## YI-P113 Myristic Acid and Trans-Activator of Transcription Conjugation of Protein Kinase C Epsilon Peptide Inhibitor Improves Left Ventricular Function and Reduces Infarct Size in an *in vivo* Porcine Study of Myocardial Ischemia-Reperfusion Injury

Sunit G. Singh [1], *Tameka C. Dean [1,2], Desmond Boakye Tanoh [1,2], Kayla Harrell [1], Arjun Nair[1], Cameron Stinson [1], Annam Humayun[1], Qian Chen[1], Robert Barsotti [1], Lindon H. Young [1,2]*

*[1] Philadelphia College of Osteopathic Medicine, Department of Biomedical Sciences, Philadelphia, PA; [2]Young Therapeutics, Philadelphia, PA.*

Protein Kinase C epsilon (PKCε) signaling is known to mediate superoxide production from mitochondrial and uncoupled endothelial nitric oxide synthase sources in myocardial ischemia-reperfusion (I/R) injury. Previously, PKCε peptide inhibitor conjugated with myristic acid and trans-activator of transcription (*N*-Myr-Tat-CC-EAVSLKPT [PKCε-]; Myr-Tat-PKCε-) exhibited profound reduction in infarct size compared to Myr-PKCε- or Tat-PKCε- in *ex vivo* rat hearts†. This study aims to evaluate the effects of Myr-Tat-PKCε- in porcine myocardial I/R *in vivo* compared to a scrambled control peptide.

Male Yorkshire castrated pigs (38-54kg) were subjected to regional I(1hr)/R(3hrs) via catheter-balloon in the left anterior descending coronary artery (LAD) at the location of the second LAD branch. Myr-Tat-PKCε- or Myr-Tat-PKCε-scrambled control peptide (*N*-Myr-Tat-CC-LSETKPAV [PKCε-scram]; Myr-Tat-PKCε-scram) bolus (0.2 mg/kg) was administered into the LAD at reperfusion. Echocardiography was used to determine ejection fraction (EF). Following reperfusion, hearts were excised and stained. The area at risk (AR) and area of necrosis (AN) were identified with 1% Evans Blue dye and 1% triphenyltetrazolium chloride respectively. Infarct size (AN/AR) and EF were analyzed with unpaired Student's t-test.

Myr-Tat-PKCε-scram exhibited a reduced final EF compared to baseline (55±1 vs 62±1%, n=3). Myr-Tat-PKCε- significantly increased final EF back to baseline (59±1 vs 59±1%, n=5; $p<0.05$). Myr-Tat-PKCε- exhibited a reduction in infarct size (10±2%, n=4; $p<0.01$) compared to Myr-Tat-PKCε-scram (29±7%, n=3). Results suggest that Myr-Tat-PKCε- mitigates myocardial I/R injury when administered during reperfusion. Future studies will test the effects of Myr-Tat-PKCε- in an 8-week porcine myocardial I/R survival study to determine its therapeutic potential for heart attack patients.

† Details of the ex *vivo* data were presented at the 15th Annual Peptide Therapeutics Symposium (2020).
This research was supported by the Division of Research, Department of Biomedical Sciences, Center for Chronic Disorders of Aging at Philadelphia College of Osteopathic Medicine, and Young Therapeutics, LLC.

## P114 Heterotrimer Collagen-Cell Penetration Hybrid Peptides for Synchronized Combination Therapy

T. Perez, E. Ashrafichoobdar and K. Slowinska
*Department of Chemistry and Biochemistry, California State University, Long Beach. Long Beach, CA 90840, USA*

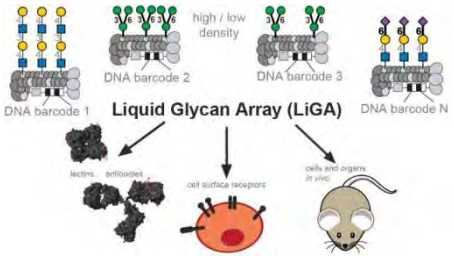
The ability of collagen peptides to self-assembly proved to be a useful tool in the formation of functional materials. The assembled collagen peptide in triple helix conformation can be viewed as fully organic nanoparticle with high aspect ratio and platform for the development of drug delivery systems. Here we present the collagen peptide heterotrimer assembly (each peptide strand has a different sequence) designed to carry the small molecule cargo. The cargo are three chemically different drugs conjugated to N-terminus of the peptide. The drugs used for proof of concept were Paclitaxel, Doxorubicin, and 5-Fluorouracil; the combination used in treatment of (ER)-positive and (PR)-positive breast cancer. In addition, the heterotrimer collagen peptides were designed as hybrid with cell penetrating peptides to improve cellular internalization. The proposed peptide drug carrier allows not only to control delivery ratio of drugs, but also to enhance cellular uptake and improve solubility of cancer drugs. The structural characterization was performed spectroscopically, and the functional characterization was performed in-vitro with the MCF-7 metastatic adenocarcinoma breast cancer cells.

### YI-P115 Genetically Encoded Multivalent Liquid Glycan Array Displayed on M13 Bacteriophage

Mirat Sojitra[1], Susmita Sarkar[1], Jasmine Maghera[1], Edward N. Schmidt[1], Emily Rodrigues[1], Eric J. Carpenter[1], Shaurya Seth[1], Daniel Ferrer Vinals[1], Nicholas J. Bennett[1], Revathi Reddy[1], Amira Khalil[1], Xiaochao Xue[1], Michael R. Bell[1], Ruixiang Blake Zheng[1], Ping Zhang[2], Corwin Nycholat[3], Justin J. Bailey[1], Chang-Chun Ling[2], Todd L. Lowary[1,4,5], James C. Paulson[3], Matthew S. Macauley[1,6], Ratmir Derda[1]

*[1]University of Alberta, [2]University of Calgary, [3]The Scripps Research Institute, [4]Institute of Biological Chemistry, Academia Sinica, [5]Institute of Biochemical Sciences, National Taiwan University, [6]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB T6G 2G2, Canada*

Cell surface proteins are often post-transitionally modified with glycans, and therefore abnormal cell surface glycosylation is a hallmark for diseased cell. However, the underlying role of information encoded by glycans is not fully understood. Binding information obtained from glycan arrays provides a critical starting point for downstream applications such as carbohydrate-based inhibitors, vaccines, and other therapeutics. However, using powerful techniques like DNA deep sequencing to analyze glycan recognition is challenging due to the lack of 1:1 correspondence between DNA and glycan structures. Therefore, we have developed **Li**quid **G**lycan **A**rray (LiGA). This technology provides 1:1 correspondence between the glycan displayed in multiple copies on a M13 bacteriophage carrier and the DNA barcode within the phage. LiGA is generated by acylation of phage pVIII protein with dibenzocyclooctyne, followed by ligation of azido-modified glycans. The display of glycans on each phage virion can be controlled from 30- 1500 copies to incorporate the critical variables in glycan recognition: valency and density. A simple pulldown of the LiGA and lectins followed by deep sequencing of the DNA in the bound phage decodes the recognized glycans. LiGA is target agnostic and measures the binding profile of lectins expressed on intact cells, such as Siglec-1, Siglec-2, Siglec-7 and DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin), and in live mice.[1] From a library of 50-100 multivalent glycan probes, LiGA identifies the glycan-phage conjugates with optimal valency and density for binding to antibodies and lectins on cells *in vitro* and *in vivo*.

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### YI-P116 Multi-segment Templated Native Chemical Ligations

P. Spaltenstein, P. W. Erickson, and M. S. Kay
*Department of Biochemistry, University of Utah, 15 North Medical Drive East, Room 4100, Salt Lake City, Utah 84112, United States*

Chemical protein synthesis (CPS) enables site-specific modifications of proteins and peptides that would otherwise be difficult or impossible to access via recombinant expression. Peptides containing ~50 AAs are routinely obtained by solid-phase peptide synthesis (SPPS) and can be joined together by native chemical ligation (NCL) to produce larger proteins. NCL is highly chemoselective, but can suffer from slow kinetics and typically requires mM concentrations that are difficult to attain for hydrophobic peptides. We developed Click-Assisted NCL (CAN) to overcome slow NCL kinetics by "clicking" two peptide segments together to increase their effective concentrations.

Here, we expand on the CAN methodology to perform templated NCL at multiple junctions simultaneously. To do so, we established a second conjugation reaction that is compatible with NCL conditions and can be used in tandem with CAN. In addition, we demonstrated selective traceless modifications of peptides at two distinct sites to enable multi-segment templated ligations. The chemistry established in this work provides the foundation for TEMplated Peptide-Oligo (TEMPO) NCL, in which peptides functionalized with traceless "helping hand" linkers are conjugated to oligos. Peptide-oligo conjugates hybridizing to a complementary DNA template position the peptide segments in close proximity for a one-pot templated NCL. TEMPO NCL is anticipated to greatly facilitate syntheses of proteins that are currently unattainable by CPS. To complement the chemical advancements described above, we have updated our Automated Ligator (Aligator) program to predict optimal synthetic strategies that reflect templated NCL methods.

### P117 Exploring the Use of Boronic Ester Crosslinks in Peptide Hydrogels

Kevon Stanford and Joel Schneider
*Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick MD, 21702, USA*

Peptide hydrogels have been attractive tools for biomedical applications owing to their ability to facilitate drug delivery. Amphiphilic peptides can assemble into fibrils, which can undergo higher-order assembly to form physically crosslinked gel networks. Typically, fibril crosslink density and the bending modulus of the fibrils define material stiffness. One way to modulate gel rigidity is to introduce chemical crosslinks into the fibril network. Herein, we explore the use of boronic esters to crosslink peptide fibers. We have designed and synthesized boronic acid-containing peptide sequences by incorporating different numbers of 3-aminophenylboronic acids (3-APBA) at varying positions designed to interact with peptides displaying diol moieties, such as L-DOPA to form intermolecular crosslinks. Boronic ester formation was studied using a fluorescence assay by utilizing the boronic acid-diol sensor dye, Alizarin Red S, which confirms the formation of diol-esters. Further, rheological studies suggest that the mechanical rigidity of corresponding gels increased by boronic ester formation.

Lastly, peptide structural changes that occur during self-assembly and the morphology of resulting fibrils was studied by temperature-dependent CD and transmission electron microscopy (TEM), respectively.

### P118 Investigating Cross-ß Peptidomimetics as Inhibitors of Tau Aggregation and Propagation

S. K. Starnes, B. H. Rajewski, K. M. Makwana, J. R. Del Valle*
*Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA*

The misfolding and ordered assembly of amyloidogenic tau into cytotoxic filaments is a hallmark of neurodegenerative tauopathies. Recent cryo-electron microscopy structures of tau filaments derived from patient samples indicate that fibril morphology is distinct between different tauopathies. Within these filaments, two aggregation-driving hexamer sequences ($_{275}$VQIINK and $_{306}$VQIVYK) engage in face-to-face packing with disease-specific complementary (cross-ß) sequences. Despite the demonstrated need for selective tau aggregation inhibitors, ß-sheet-rich fibril assemblies are notoriously difficult to target with small molecules. Our previous results demonstrated that N-amination of aggregation-prone hexapeptide sequences affords proteolytically stable and soluble ligands of tau. Here, we investigate the cross-ß hexapeptide segments within tau as lead sequences for inhibitor development. Starting from $_{373}$THKLTF, the opposing ß-strand module from Alzheimer's Disease tau, we synthesized a library of N-amino peptides (NAPs) derivatives on solid support. These peptidomimetics were evaluated for their effects on recombinant tau fibrillization and propagation in cellular assays. Several NAPs show the ability to reduce tau aggregation in vitro by ThT fluorescence and prevent the seeding of endogenous tau by extracellular aggregates in biosensor cells. These studies suggest that cross-ß sequences are viable starting points for the development of polymorph-specific tau ligands.

### YI-P119 Electrophilic Ethynyl-phosphinates and –phosphine Oxides for Cysteine Selective Bioconjugation

Christian E. Stieger[1], Luise Franz[1], Yerin Park[2], Mark A.R. de Geus[1], Mu-Hyun Baik[2], Christian P.R. Hackenberger[1]
[1] *Chemical Biology Department, Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) Berlin, Germany*
[2] *Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST) Daejeon, Republic of Korea*

Cysteine bioconjugation is one of the most convenient strategies for the site-selective modification of proteins. Due to its highly nucleophilic properties under physiological conditions and low natural abundance. Despite the development of a plethora of cysteine selective labelling reagents[1] highly Cys-selective labelling reagents that can be modularly accessed and produce stable bioconjugates are still in demand.

Previously our group has introduced unsaturated phosphonamidates[2,3] and phosphonothiolates[4] as cysteine directed electrophiles for protein modification. These functional molecules could be successfully employed in the generation of antibody-drug-conjugates (ADCs) as well as in protein-protein conjugation.

Here we present ethynyl-phosphinates and –phosphine oxides as alternative building blocks for cysteine selective electrophiles. Diethynyl-P(V) compounds can undergo two subsequent thiol additions, one on each alkyne moiety. Thereby one can use these molecules not only for protein double-conjugation but also for disulfide rebridging as exemplified on the therapeutic monoclonal antibody Trastuzumab.[5] Moreover, we show that by altering one of the ethynyl-moieties the reactivity of the compound can be significantly altered. For instance, one of the alkynes can be conjugated to any azide-containing molecule in aqueous media via copper-catalyzed azide alkyne cycloaddition. The formed triazolyl-phosphinate shows a tenfold increase in reactivity towards thiols. By using this approach, we could modularly access various functional electrophiles that were used to generate peptide-, protein- and antibody-conjugates. Additionally, we demonstrate that this class of electrophiles is suitable for proteome-wide cysteine profiling.

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### P120 Peptide Nucleic Acids as an Investigational Genetic Therapy for Myotonic Dystrophy Type 1

Dani M. Stoltzfus, Valentina Di Caro, William Riedl, Noel R. Monks, Nathan Tavenor, Barry Badeau, Jeremy Gleaton, Ramesh Batwal, Sandra Rojas-Caro, Anthony Rossomando, William Mann, Dietrich A. Stephan
*NeuBase Therapeutics Inc. 350 Technology Drive, Pittsburgh, PA, 15219, U.S.A*

Many diseases remain undruggable with current therapeutic technologies, which leaves millions of patients with limited treatment options. Most diseases are genetic, in whole or in part, which underscores the criticality of developing new medicines that can modulate genetic drivers of disease for future human health. NeuBase's PATrOLTM platform is based upon the ability to design, synthesize, and formulate peptide nucleic acids (PNAs) to Drug the GenomeTM. We have developed ultra-high binding affinity PNAs that can target the genome with allele selectivity.

Myotonic dystrophy type 1 is one such genetic disease with no cure. Patients with this disease suffer from cognitive

defects and muscle pathology which is caused by a trinucleotide repeat in the DMPK gene. In this presentation, we will demonstrate that a PATrOLTM -enabled PNA is able to correct the DM1 phenotype in vitro and will present our latest preclinical in vivo data to support the potential for our DM1 lead candidate to be developed as a potential therapy for DM1 patients.

### YI-P121 Oleyl-Histidine-Arginine Peptides as Efficient Sirna Delivery Carriers to Silence the Stat-3 Gene in the Breast Cancer Cells

Muhammad Imran Sajid and Rakesh K. Tiwari
*Center for Targeted Drug Delivery, Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Harry and Diane Rinker Health Science Campus, Irvine, California 92618, United States*

Nucleic acid therapeutics require a suitable, efficient, and safer delivery system for their clinical application. Peptides containing cell-penetrating or molecular transporter properties offer a unique opportunity to explore their application in nucleic acid delivery. Herein we hypothesized to synthesize a series of oleyl conjugated histidine(H) arginine (R) peptides, X-$(R)_n$-$(HR)_4$, (where X is a flexible hydrophobic chain of olely and n = 0-5) and $(HR)_4$ is a cell-penetrating peptide (CPP)) which contain cationic and hydrophobic residues to encapsulate siRNA and deliver it to the cells. Oleyl conjugated peptides were synthesized using fmoc solid-phase peptide synthesis, followed by purification and characterization. The peptide-siRNA complexes were evaluated for cytotoxicity using a cell viability assay, which demonstrated 100% cell viability (up to 20 µM) in the selected breast cancer cell lines (MCF-7 and MDA-MB-231) and normal breast cells (MCF-10A). Most of the peptides bind completely with siRNA at N/P ratio 5 and above, as indicated by gel retardation assay. Peptides protect siRNA at N/P ratio 20 and above when incubated with 25% FBS for 24 h. Significant uptake of Alexa-488 labeled siRNA into the cytoplasm of breast cancer cells (MDA-MB-231 and MCF-7) using Oleyl-$R_5$-$(HR)_4$ peptide was demonstrated in flow cytometry and confocal microscopy studies. Preliminary western blotting data with Oleyl-$R_3$-$(HR)_4$ and Oleyl-$R_5$-$(HR)_4$ shows that the peptides-siRNA delivery system can silence STAT-3 for up to 70 %. The study reveals that oleyl conjugated histidine arginine peptides serve as effective siRNA carriers in breast cancer cell lines.

### YI-P122 Biocompatible Peptide Bicyclisation using Dicyanopyridine Amino Acids

Sven Ullrich, Josemon George, Christoph Nitsche
*Research School of Chemistry, Australian National University, Canberra, ACT 2601, Australia*

Bicyclic peptides are becoming an increasingly prominent class of potential next-generation therapeutics. Owing to their unique properties, they are capable of bridging the gap between small molecules and antibodies.[1, 2]
The most frequently used synthetic procedures to generate bicyclic peptides rely on the formation of disulfide or thioether bonds and often require special reagents or catalysts.[2, 3] We present a substantially different bicyclisation strategy, based on the catalyst-free, biocompatible 'click' reaction between cyanopyridine and 1,2-aminothiol, previously developed in the Nitsche group.[4, 5]
A novel reagent enables the synthesis of unnatural amino acids through its reaction with the nucleophilic side chains of cysteine and lysine-like amino acids. These dicyanopyridine-featured amino acids can be generated as Fmoc derivatives for incorporation into standard solid phase peptide synthesis (SPPS). Alternatively, dicyanopyridine amino acids can be synthesised on the solid support during SPPS *via* the use of inexpensive orthogonally protected amino acids. The bicyclic peptide is subsequently formed within minutes through a spontaneous intramolecular reaction in aqueous solution at physiological pH. The process is orthogonal to all canonical amino acids and fully amenable to automation.To demonstrate the great potential for drug discovery, we generated a bicyclic inhibitor of the Zika virus protease NS2B-NS3, which is derived from the protease's recognition sequence. Bicycles can also be further modified post-synthetically, for example, to introduce labels or even generate tetracyclic peptides.

This methodology pushes the boundaries of bicyclic peptide synthesis, informs next-generation drug design and is likely to be compatible with genetically encoded peptide libraries.

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### YI-P123 Myristoylated Protein Kinase C Epsilon Inhibitor Improves Renal Function in a Mouse Model of Acute Kidney Injury

Alexis B. Verwoert[1], Sunit G. Singh[1], Tameka C. Dean[1], Devani Johnson[1], Lisa Shah[1], Qian Chen[1], Robert Barsotti[1], Yanlin Jiang[2], James George[2], Anupam Agarwal[2], and Lindon Young[1,3]
*[1] Philadelphia College of Osteopathic Medicine, Philadelphia, PA; [2] University of Alabama at Birmingham School of Medicine, Birmingham, AL; [3] Young Therapeutics, LLC, Philadelphia, PA*

Delayed graft function (DGF) is a post-transplant acute kidney injury that is caused by prolonged ischemia resulting in oxidative stress-mediated damage during reperfusion. Previously,
myr-PKCε- reduced infarct size and serum $H_2O_2$ when administered upon reperfusion in an *ex vivo* rat heart ischemia-reperfusion (I/R) model and *in vivo* hindlimb I/R model, respectively. Myristoylated protein kinase C epsilon peptide inhibitor (N-myr-EAVSLKPT; myr-PKCε-) is known to confer protection by inhibiting superoxide production from uncoupled endothelial nitric oxide synthase and mitochondrial ATP-sensitive $K^+$ channels. We hypothesized

myr-PKCε- would attenuate renal injury, characterized by elevated serum creatinine (Cr) and decreased glomerular filtration rate (GFR), compared to a scrambled control peptide (N-myr-LSETKPAV; myr-PKCε-scram).

Renal pedicles of male C57BL/6J mice (25–30g) were clamped bilaterally for 19 min.
Myr-PKCε- or myr-PKCε-scram (1.6 mg/kg; 20 µM blood) were administered into the tail vein 1 min before unclamping. Cr was measured at baseline, 24h, 72h, and 96h post-injury. GFR was determined with fluorescein-isothiocyanate (FITC)-Sinistrin renal clearance. Data were evaluated by unpaired Student's t-test.

This bilateral 19 min renal ischemia resulted in significant GFR reduction (Fig. 1) and Cr elevation (Fig. 2) throughout the post-ischemic time-course. Myr-PKCε- (n=6) significantly improved both GFR and Cr at 72h and 96h compared to myr-PKCε-scram control (n=4, p<0.05).

Results support the hypothesis that Myr-PKCε- improves kidney function after 19-min warm ischemic injury. Immunolabeling of PKCε, Kim-1, and NGAL biomarkers will be used to further evaluate PKCε localization and the extent of Myr-PKCε- protection against renal tubular damage.

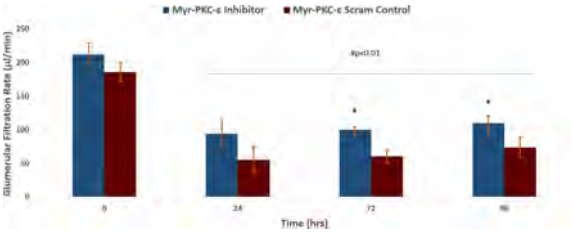


Figure 1. Myr-PKCε Inhibitor Improves GFR in a 19-min Murine Bilateral Renal I/R Model. GFR levels were calculated using FITC-Sinistrin renal clearance. #p<0.01 baseline (0 h) vs. 24h, 72h, and 96h post-ischemia; *p<0.05, Myr-PKCε- vs. Myr-PKCε-scram control at 72h and 96h post-ischemia..

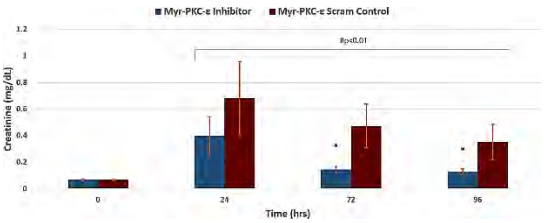


Figure 2. Myr-PKCε Inhibitor Improves Serum Cr in a 19-min Murine Bilateral Renal I/R Model. Cr was measured at baseline, 24h, 72h, and 96h post-injury. #p<0.01 baseline (0 h) vs. 24h, 72h, and 96h post-ischemia; *p<0.05, Myr-PKCε- vs. Myr-PKCε-scram control at 72h and 96h post-ischemia..

Support or Funding Information:
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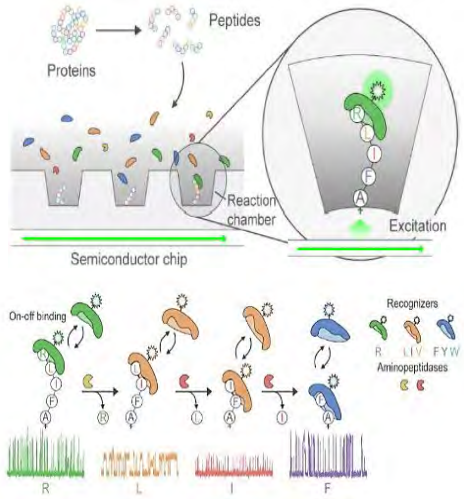
**P124 Next-Generation Protein Sequencing on an Integrated Semiconductor Device**

Brian D. Reed, Omer Ad, Robert Boer, Haidong Huang and Y.-C. Wang
*Quantum-Si, Inc., CT 06437, USA*

Advancing sequencing techniques to analyze complex protein mixtures is essential to detect aberrant proteome in a disease state. A novel parallel fluorescence-based single-molecule detection technology was developed and applied to protein sequencing by Quantum-Si. In our method, protein mixtures were digested into smaller peptides. Peptides were then conjugated to macromolecular linkers. The whole complexes were loaded onto our proprietary semiconductor chips. Each well on the chips contains single peptide with N-terminus exposed in the solution. During sequencing, different N-terminal residues were recognized by corresponding binders to generate signals characteristic to the N-terminal amino acids. Proteases kept exposing the subsequent amino acids to the N-termini to be detected until the last residues. Much information from peptide sequences can be obtained in one experiment. A brief workflow, sequencing of peptides from recombinant human proteins and detection of point mutations between peptides are presented. Different from conventional mass spectrometry-based sequencing method, our method is one of the emerging next-generation protein sequencing technologies




**YI-P125 Constrained Tryptophan Peptide Synthesis**

Xiaozheng Wei and William D. Lubell
*Département de chimie Université de Montréal Complexe des Sciences, B-3015 1375 Avenue Thérèse-Lavoie-Roux Montréal, Québec H2V 0B3 Canada*

1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (Tic) and 1,2,3,4-tetrahydro-carboline-3-carboxylic acid (Tcc) are commonly used as constrained phenylalanine and tryptophan derivatives in the study of structure-activity relationships in peptide-based drug discovery (Figure) [1,2]. On the contrary, their aza-counterparts has received less attention due in great part to synthetic challenges [3,4]. Efforts to synthesize peptides bearing aza-iso-Tcc will be presented with the interest to utilize such analogs to stabilize cis-amide bonds and type VI beta-turns.

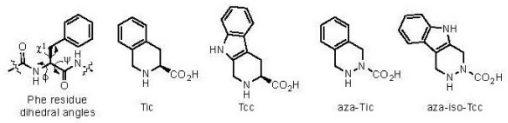



Figure. Conformational constraint of aromatic amino acids using cyclic and aza-cyclo counterparts

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### YI-P126 Site-Selective Synthesis of Sulfated CCR5 N-Terminal Peptides with Pico and 2-Cl-Trityl Protection Groups

H. Weyrick, A. Rediger, and H. J. Zhu
*Departments of Chemistry and Biology, Dordt University, Sioux Center, IA 51250, USA*

C-C chemokine receptor type 5 (CCR5) plays critical roles in directing immune cells to target inflammatory sites.[1] Binding with C-C chemokine ligands (CCLs) initiates downstream signal transduction cascades. Sulfation on N-terminal peptide of CCR5 is essential for the interactions between CCR5 and its ligands, which participate in immunological responses and pathological pathways.[2-4] Tyr sulfation of CCR5 N-terminal is highly variable. There are four potentially sulfated tyrosine residues on CCR5 N-terminal peptide presenting a synthetic challenge for investigating the roles of sulfation in the binding processes. While site-selective solid-phase sulfation has been demonstrated as an efficient process for the synthesis of sulfated peptides, the options for Tyr side chain protecting groups are limited. Compatibility issues arise when solid-phase deprotection and sulfation are applied multiple times. In our work, we used two new protection strategies for Tyr side chain protection: the Picoloyl ester (Pico) group and the acid-sensitive group, 2-Cl-Trityl. In our previous work, Pico protected Fmoc-Tyr have been successfully synthesized; the Pico group smoothly removed in our test reactions of Pico protected Fmoc-Tyr monomer using $Cu(OAc)_2$ as a catalyst and in methanol-containing solvent. Our recent study indicates the Pico group of Pico-containing CCR5 N-terminal peptide can be removed on solid-phase and followed by solid-phase sulfation. After removal of the protection group of the newly installed sulfation group with Pd/C catalyzed hydrogenation reaction, a sulfated CCR5 N-terminal peptide was synthesized. The current research is focusing on incorporating both Pico and 2-Cl-Trityl protected Tyrs for site-selective sulfation of CCR5 N-terminal peptide.

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### P127 Synthesis, Characterization, Biological Analysis, and Molecular Docking Studies of Opioid Agonist and Antagonist Derivatives Containing a Sonogashira Linkage

K.R. Wilson[1], M.G. Goertzen[1], J.C. Ouellette[1], T.L. McGomery[1], A. W. Johnson[1], K.R. Boykin[1], A. Hunkele[2], B. Paul[2], and S. Majumdar[2]
*[1]Department of Chemistry, Wingate University, Wingate, NC 28174, USA. [2]Center for Clinical Pharmacology, Washington University in St. Louis, St. Louis, MO 63130.*

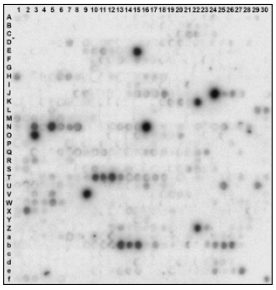
Opioid drugs have been utilized for centuries for their pain-relieving properties. A large number of opioid peptides have been discovered over the years which act as agonists and antagonists of the human opioid receptors. Some of these peptides are cyclic peptides, which have been proven to be flexible, allowing for various conformations in solution. A rigid cyclic linkage could help prevent enzymatic degradation, as well as limit free rotation of the compound, “locking” it into its bioactive conformation. Both of these factors may help to increase the amount of bioavailable peptide at the receptor site, by increasing both the potency and stability of the drug. The Sonogashira reaction is a palladium-mediated transmetallation reaction that takes place between an aryl halide and an alkyne, creating a unique rigid aryl-triple bond linkage. Incorporation of this type of bond into cyclic peptides could, therefore, make the peptides more resistant to degradation and limit free rotation. Microwave-assisted solid phase peptide synthesis was investigated as a potential means of Sonogashira peptide cyclization, uniquely under atmospheric conditions. This linkage has been incorporated into three opioid peptides: DPDPE, TAPP, and CTP; creating novel cyclic Sonogashira peptides, each with nanomolar binding affinity for at least one of the three human opioid receptors, making them receptor-selective. These peptides were tested via binding assay and functional assay, and then molecular modeling and receptor docking studies were performed to visualize the 3-dimensional conformations of these Sonogashira peptides.

**P128** **Investigation into the Antibody Responses of COVID-19 Positive Individuals**

D.F.H. Winkler[1], A. Atrey[1], J.C. Kraft[1], J. Wang[2], J.Z. Zhao[1] and S. Pelech[1,3]
[1] *Kinexus Bioinformatics Corporation, Vancouver, BC V6P 6T3, Canada*
[2] *Department of Nursing, Vancouver General Hospital, Vancouver, BC V5Z 1M9, Canada*
[3] *Department of Medicine, Division of Neurology, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC V6T 1Z3, Canada*

The Coronavirus Disease of 2019 (COVID-19) represents the most severe global health crisis since the Spanish Flu around 100 years ago. It is caused by a virus that is related to the virus that was responsible for the Severe Acute Respiratory Syndrome (SARS) that appeared in 2002.

To investigate the binding patterns of antibodies in the serum of recovered patient, we systematically synthesized peptides covering the entire genome of SARS-CoV-2. This investigation was carried out by peptide scans generated by SPOT synthesis.
An investigation of SARS-CoV patients using the same approach showed the existence of relatively few, defined epitopes. In contrary to these results, we found the antibody response of patients with SARS-CoV-2 to be far more complex. In fact, in most cases the binding patterns of all tested samples showed little similarities, even between relatives. Nevertheless, we were able to identify several epitopes that were significantly more immunogenic than others, and may be useful for diagnostic purposes and vaccine development.



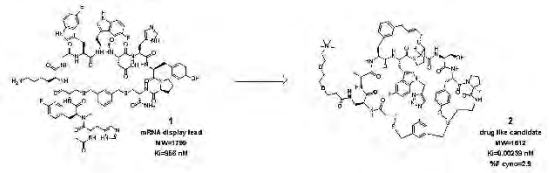
*Example of a peptide scan of the spike, nucleocapsid and membrane proteins probed with serum from a COVID-19 positive individual.*

**P129** **From mRNA Lead to Drug Candidate-Discovery of a Novel, Potent, and Orally Bioavailable, Tricyclic Peptide PCSK9 Inhibitor**

Chengwei Wu, Thomas J.Tucker, Abbas Walji, Mark W. Embrey, Candice Alleyne, Rupesh P. Amin, Alan Bass, Bhavana Bhatt, Elisabetta Bianchi, J. Craig Blain, Nicolas Boyer, Danila Branca, Tjerk Bueters, Nicole Buist, Sookhee N. Ha, Mike Hafey, Huaibing He, John Higgins, Douglas G. Johns, Kelli Jette, Angela D. Kerekes, Kenneth A Koeplinger, Jeffrey T. Kuethe, Derek LaPlaca, Nianyu Li, BethAnn Murphy, Peter Orth, Alonso Ricardo, Scott Salowe, Kathleen Seyb, Aurash Shahripour, Joseph R Stringer, Yili Sun, Rodger Tracy, Weixun Wang, Yusheng Xiong, Hyewon Youm, Hratch J. Zokian, Harold B. Wood.
*Merck & Co. Inc., Kenilworth, NJ 07033, USA*

Proprotein convertase subtilisn-like/kexin type 9 (PCSK9) is a clinically validated drug target related to hypercholesterolemia and coronary artery disease. PCSK9 inhibitors that bind directly to the large flat LDL receptor (LDLR) binding surface on the PCSK9 protein can block the interaction between the two proteins, thus preventing LDLR degradation, thereby enhancing LDL-cholesterol clearance. Our goal is to identify an oral cyclic peptide PCSK9 inhibitor for the treatment of hypercholesterolemia.

From a cyclic peptide hit **(1)** generated from mRNA display screening for cyclic peptides, we employed structure-based drug design to obtain structurally novel tricyclic peptide drug like candidates with excellent potency, good metabolic stability, and oral bioavailability using an enabled formulation. This report will highlight the discovery efforts leading tricyclic peptide inhibitor (**2**) which demonstrated its pharmacodynamic effects in cynomolgus monkeys similar to the FDA approved, parenterally dosed anti- PCSK9 mAb, with the advantage of oral administration using lipidic dosing vehicle Labrasol®.



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**YI-P130** **Genetically Encoded Libraries of Macrocyclic Covalent Inhibitors of Glutaminolysis: Enabling Metabolic Engineering in Cancer**

Kejia Yan, Ratmir Derda
*Department of Chemistry, University of Alberta, Edmonton, AB T6E 2G2*

Glutaminase 1 (GLS1) is a mitochondrial enzyme that hydrolyzes glutamine into glutamate. It is noteworthy that glutaminase also fuels rapid proliferation of cancer cells. Therefore, GLS1 might be a target for inhibiting tumorigenesis and progression of cancers. A previously described glutaminase antagonist, 6- diazo-5-oxo-l-norleucine (DON) (Fig. 1) forms covalent interactions with glutaminase in the glutamine binding site. Due to its similarity to glutamine, DON is highly promiscuous leading to low specificity and high toxicity. There is currently no reported research on chemically modified DON to increase the selectivity and specificity towards glutaminase.

Genetically encoded fragment-based discovery (GE-FBD) is a powerful method for the development of peptide drugs. In this project, I employed GE-FBD to discover selective inhibitors for glutaminase. As previously reported, a 1,5-dichloropentane-2,4-dione linchpin can convert peptide libraries to form 1,3- diketone bearing macrocyclic peptides

(DKMP)[1]. A hydrazine functionalized DON fragment was inserted onto these DKMP libraries through a well-established Knorr pyrazole synthesis reaction. The diazo reactive handle on DON enables covalent interactions with glutaminase 1. I utilized these DON functionalized macrocyclic libraries to select ligands that can specifically and irreversibly bind to glutaminase 1. I applied the established three-round screening, deep sequencing and analysis pipeline developed in the Derda research group, followed by glutaminase activity assay to test the inhibitory properties of the selected ligands.

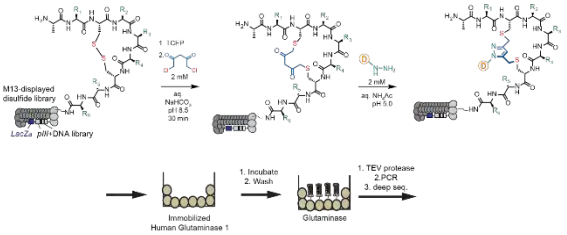


**Fig 1.** Genetically encoded fragment-based discovery on Glutaminase 1.

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### YI-P131 Influence of Environment on the Assembly of Mixtures of Enantiomeric Peptides

Francine E. Yanchik-Slade*, Julian E. vonHofe*, and Bradley L. Nilsson*
* *Department of Chemistry, University of Rochester, Rochester, NY 14627-0216, USA*

In 1953, Pauling and Corey predicted that enantiomeric mixtures of ß-sheet forming peptides would coassemble to form a new motif termed a "rippled" ß-sheets. Rippled ß-sheets are comprised of alternating L- and D- peptides to create a "rippled" surface distinct from the pleated surface observed in ß-sheets of stereo-uniform peptides. Recent work has provided data consistent with the formation of rippled beta-sheets from various mixtures of enantiomeric peptides (Raskatov et al., Acc Chem Res 2021;54:2488-2501). In this work, coassembly of L- and D-peptides into putative rippled ß-sheets has been shown to be energetically favorable compared to pleated beta-sheet assembly of the individual enantiomers. We have recently shown that enantiomers of a fragment of the amyloid-beta peptide assemble into materials that are consistent with rippled-beta sheets (Nilsson et al., Molecules 2019;24:1983). Herein, we present additional studies that describe the influence of changes in environmental solvent and pH influence the assembly behavior of enantiomeric mixtures of this amyloid-beta fragment.

### P132 Structure-driven Machine Learning Platform for Therapeutic Peptide Design

Tracy Stone*, Ozge Yoluk*, Chris Ing, David White (*equal contributors, alphabetical)
*ProteinQure Inc., 119 Spadina Ave Suite 304, M5V 2L1, Toronto, Canada*

Peptides are a unique drug modality, well-suited for targeting protein-protein interactions, with demonstrated capability for high potency, selectivity and minimal toxicity. However, obtaining these properties and overcoming known limitations continues to impede therapeutic applications.

From hit identification to lead optimization, ProteinQure balances the use of novel computational technologies to broaden chemical search space *in silico* with hypothesis-driven workflows to enrich successful compounds at the bench. Using a cross-disciplinary design strategy, we enable the discovery of novel linear and cyclic peptides against challenging targets (i.e. GPCRs) using limited structural/functional information, simultaneously optimized for a range of desired drug-like properties.

Here, we present example applications of the ProteinQure platform, in which we have designed competitive binders to two challenging targets using a massively scalable computing platform. We review multiple competing protocols for peptide design, ranging from structure-based drug design using a known motif or template to de novo protein design and deep learning-based binder hallucination. All methods integrate machine learning models for drug-like property predictions to prioritize developable peptides which enhance or preserve strong binding affinity to their desired targets.This work demonstrates that a structure-based machine learning platform for peptide design is an effective strategy to overcome drug development bottlenecks that are specific to this therapeutic class and to drive the creation of novel peptides for unmet clinical needs.

### YI-P133 Chemoselective Functionalization af N-Arylglycinyl Peptides with Boronic Acid Derivatives on Solid Support

H.A. Young, and C. Proulx
*Department of Chemistry, North Carolina State University, Raleigh, North Carolina, 27695*

Chemoselective late-stage functionalization of peptides have been reported and subsequently used in structure-activity relationship (SAR) studies of therapeutically relevant peptide sequences. While there are examples of peptide backbone modifications, they often require harsh conditions (e.g. metal catalyst, chemical oxidant, high temperature). Specifically, N-aryl glycine derivatives have been shown to oxidize to an α-imino amide intermediate, which allows for coupling to a variety of nucleophiles at the α-carbon. However, examples of such oxidative couplings on longer functionally dense peptide sequences remain scarce. We previously demonstrated that electron-rich N-aryl peptides undergo oxime ligation reactions with aminooxy nucleophiles under mild aqueous, catalyst-free conditions.[1-3] Here, we describe the late-stage α-C-H functionalization reaction of resin-bound N-aryl peptides with boronic acid nucleophiles in organic solvent under catalyst-free conditions. We explore the effect of the electronics of the N-aryl ring on reactivity and present a scope of the optimized reaction where both the peptide sequence and nature of boronic acid derivatives are varied.

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2 Guthrie, Q. A. E.; Young, H. A.; Proulx, C. Chem. Sci. 2019, 10, 9506–9512.
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**YI-P134 Improving Metabolite Identification for Complex Peptides using MassMetaSite.**

Tatiana Radchenko, Fabien Fontaine, Ismael Zamora

Introduction
It is a commonly used strategy in the peptide therapeutics field to introduce chemical modifications such as cyclisation, changing the stereochemistry of an amino acid, substitution of natural amino acids to chemically modified ones and others to improve their efficacy and ADME profile. The study of the metabolic degradation products for synthetically modified therapeutic peptides using LC-MS is a challenging issue due to complex fragmentation and the need to evaluate many nonstandard metabolites compared to natural peptides. Nowadays different chemoinformatics approaches can be used for automated metabolite identification. These tools can propose metabolite structures based on the combination of metabolite prediction and analysis of mass spectrometry data. Especially in the case of chemical modified peptides, they undergo a higher variety of metabolic reactions depending on the modification applied. This makes building the virtual set of all potential metabolites time and computational resource intensive. In addition, fragmentation analysis requires even more computation time due to some modifications introduced such cyclization, incorporation of the modified amino acids or conjugation. Finally, there is a third challenge related to the depiction of the parent and the metabolites as atoms/bonds in a manner suitable for an algorithm to predict where in the molecule the metabolic reaction occurs. These tasks were solved in the new version of the MassMetaSite (4.2) developed specifically for the analysis and the interpretation of the high-resolution LC/MS data for peptides and oligonucleotides.

Methods
New algorithms that address the challenges in highly modified therapeutic peptide structure elucidation have been developed. The peak detection algorithm was improved to use the Most Abundant Isotope for parent and potential metabolites. A new algorithm is producing all virtual metabolites applying the library of chemical reactions to each monomer while maintaining the connection to the atoms. A third improvement is the fragmentation algorithm that has two layers of analysis, one at the monomer level and the other one at the bond level. Finally, we will also show the results of the implemented algorithm for the analysis results visualization.

Preliminary data
Using MassMetaSite we analyzed a collection of experimental data for a set of peptides where the data was collected on a Q-Exactive Thermo instrument.
The metabolite identification study was performed using a peptide set that included eight compounds: somatostatin and its seven synthetic analogues. All test compounds were incubated in serum. These peptides are all cyclic peptides and seven of them had unnatural amino acids. The structural assignments were performed for 17 degradation products with high mass accuracy (ppm<3). Most of the metabolites resulting from one or two metabolic modifications were produced by amide hydrolysis and were detected by the new algorithm. All the metabolites were checked manually including a review of the assigned fragments. Metabolites were considered as reliable because the fragmentation was adequate, isotope pattern was as expected, the small differences between the m/z of observed and theoretical, and the mass score was high. As previously reported in the literature, the first two most abundant metabolites with assigned structure correspond to cleavage of the linear part of the somatostatin. Finally, we analyzed experimental data for the insulin and semaglutide data where the data was collected on a Waters QToF. Insulin is a cyclic peptide that contains 3 disulfide bridges and semaglutide is a linear peptide that contains linkages and fatty acid. The visualization algorithm developed allows to show results for these complex structures in a such a manner that it is easy to interpret due to the constraint structure alignment between the substrate and the metabolite (always keeping the same orientation) and a possibility to combine monomer and atom/bond notation. Therefore, the metabolic changes in the structure can be easily seen by the User.

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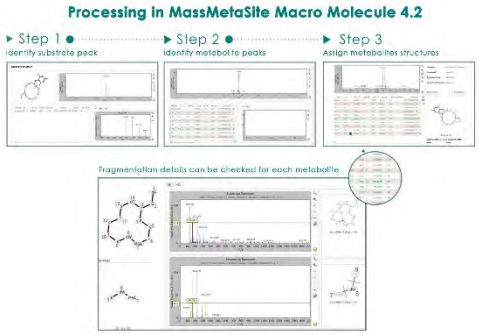


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