AMERICAN PEPTIDE SYMPOSIUM

Waiting on Lars to send new cover. I can't fix this as I don't have the original file or font to fix.

Co Chairs:

JONATHAN LAI, PHD, JON VEDERAS, PHD

NEW HEIGHTS IN PEPTIDE RESEARCH

WHISTLER | BC | CANADA | 17-22 JUNE | 2017

Barefoot Patio

Village stroll

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THE 25TH AMERICAN PEPTIDE SYMPOSIUM

25th American Peptide Symposium

WELCOME!

Welcome to Whistler BC, Canada and the 25th American Peptide Symposium, which is being held in conjunction with the 9th International Peptide Symposium. The meeting theme ***New Heights in Peptide Research*** will present important new developments from a broad range of disciplines on these fascinating molecules in the environment of "*Supernatural British Columbia*." The Whistler environment also offers numerous opportunities for mountain sports, wildlife watching and hiking.

The 2017 APS program will have two keynote lectures from renowned scientists. Professor David Baker of the University of Washington will open the symposium at 6 PM on Saturday, June 17, with his lecture on *"De Novo Peptide Design,"* followed by a reception in the Grand Foyer of the Whistler Conference Centre. Exhibits will be open during this social event. The closing talk on Thursday, June 22, will be by Professor Stephen Kent of the University of Chicago on *"Inventing Synthetic Peptide & Protein Chemistries to Reveal How Enzymes Work."* The closing banquet will start that evening at 6:30 PM in the Sea to Sky Ballroom B & C.

During the conference, a number of distinguished scientists will present award talks. Merrifield Award Lectures will be presented on Sunday June 18 by Robert Hodges of the University of Colorado and by Charles Deber of the University of Toronto. On Monday June 19 Paul Alewood of the University of Queensland will deliver the Goodman Lecture. The first of two du Vigneaud Award lectures will be on Tuesday June 20 by Ronald Raines of the University of Wisconsin, whereas the second one will be on Wednesday June 21 by Wilfred van der Donk of the University of Illinois. Thomas Kodadek of the Scripps Research Institute will give the Makineni Award lecture on Tuesday June 20.

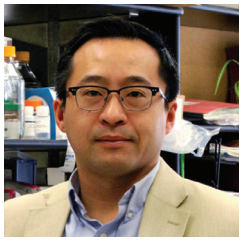
In addition to the keynote and award talks, presentations covering a broad range of peptide science will be presented. We are delighted to welcome many new participants to the program this year, with more than two-thrids of the speakers presenting at APS for the first time!

Sunday:	Peptides and Enzymes Membrane Active Peptides Peptide Synthesis, Structure and Function
Monday:	Post-Translational and Chemical Modifications Computational Design and Recognition Peptidomimetics and Non-Natural Backbones Peptide Therapeutics and Diagnostics I
Tuesday:	Glycopeptides Synthetic Methods
Wednesday:	Peptide Therapeutics and Diagnostics II Peptide Libraries Constrained Peptides and Macrocycles
Thursday:	Natural Product Biosynthesis Peptide Materials and Nanomedicine Peptide Assemblies and Complexes

Of course 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.

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. 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 conference! We hope that you will have a wonderful time both scientifically and socially !



Jonathan Lai, Co-chair

John Vederas, Co-Chair

25th American Peptide Symposium

A Message from the President of the American Peptide Society

On behalf of the American Peptide Society, welcome to the 25th American Peptide Symposium! 2017 finds Peptide Science at an exciting era in which the essential nature of peptides in all biological processes fuels advances in basic, translational and pharmaceutical research. These efforts are propelled by innovations in the chemical synthesis and the analysis of ever more complex peptide and protein analogs and their manufacture at laboratory and large-scale GMP facilities. One of the defining features of the APS symposia is that it attracts colleagues representing all areas of peptide research and industry. In addition, this biennial congregation welcomes the 8th International Peptide Symposium and I welcome our colleagues from around the world who have joined us here this week.

The American Peptide Society was founded in 1990 to support the rapidly growing research community focused on peptide chemistry and biology, as well as their manufacture and clinical development. The Society is a member of the Federation of American Societies for Experimental Biology (FASEB), which has a mission of advancing health and welfare by promoting progress and education in biological and biomedical sciences through collaborative advocacy. I would like to remind all attendees that membership in the APS is complementary to all colleagues active in the area of peptide science, I urge you to register on our website: www.americanpeptidesociety.org. The APS also partners with Wiley to support our society journal, Peptide Science that publishes both original articles and reviews that cover all aspects of peptide science. The editors welcome your manuscript submissions!

The APS is excited to return to Canada, in the beautiful mountains of British Columbia, following previous stops in Toronto, Edmonton and Montreal. I hope that you will enjoy the village of Whistler and take advantage of its sights and activities. Canada is a fitting locale to recognize the winners of our highest honor, the Merrifield Award, both of whom have strong ties to this country. I look forward to celebrating the careers and contributions of Charles Deber and Robert Hodges on Sunday afternoon. I would also like to personally congratulate the recipients of the duVigneaud Award, Ronald Raines and Wilfred van der Donk, the Makineni Award, Thomas Kodadek and the Goodman Award, Paul Alewood.

Our co-chairs Jonathan Lai and John Vederas have assembled an outstanding program covering a breath of exiting topics. I congratulate them on their successful efforts to include both established Society members and an abundance of new faces. Indeed, a significant majority of lecturers this week have not presented at our symposium within the last ten years, and many will be making their first trip to the podium! Chairing the symposium requires a tremendous amount of time dedication so, on behalf of the entire membership, I thank Jonathan Lai and John Vederas for their outstanding service to the Society. The conference could not take place without considerable effort from our colleagues who have been involved in the planning and execution of the Symposium as well as those presenting lectures and posters and hosting the exhibits. I look forward to a stimulating week and hope everyone enjoys the symposium.

Philip Dawson
The Scripps Research Institute
San Diego, California

25th American Peptide Symposium

APS SYMPOSIA CHRONOLOGY

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

Symposium	Year	Chair(s)	Location
Fifteenth	1997	**James P. Tam** Vanderbilt University	Nashville Convention Center Nashville, TN
Sixteenth	1999	**George Barany** University of Minnesota-Minneapolis **Gregg B. Fields** Florida Atlantic University	Minneapolis Convention Center Minneapolis, MN
Seventeenth	2001	**Richard A. Houghten** Torrey Pines Institute for Molecular Studies, CA **Michal Lebl** Illumina, Inc., CA	Town and Country Resort Hotel San Diego, CA
Eighteenth	2003	**Michael Chorev** Beth Israel Deaconess Medical & Harvard Medical School, MA **Tomi K. Sawyer** ARIAD Pharmaceuticals Inc., MA	Boston Marriott Copley Place Boston, MA
Nineteenth	2005	**Jeffery W. Kelly** The Scripps Research Institute, CA **Tom W. Muir** Rockefeller University, NY	Town and Country Resort Hotel San Diego, CA
Twentieth	2007	**William D. Lubell** University of Montreal **Emanuel H. F. Escher** University of Sherbrooke	Palais des Congres Montreal, Canada
Twenty-first	2009	**Richard DiMarchi** Indiana University **Hank Mosberg** University of Michigan	Indiana University Bloomington, Indiana
Twenty-second	2011	**Philip Dawson** The Scripps Research Institute **Joel Schneider** National Cancer Institute	Sheraton San Diego San Diego, CA
Twenty-third	2013	**David Lawrence** UNC Chapel Hill **Marcey Waters** UNC Chapel Hill	Hilton Waikoloa Village Waikoloa, Hawai'i
Twenty-fourth	2015	**Ved Srivastava** GlaxoSmithKline **Andrei Yudin** University of Toronto	Hyatt Regency Grand Cypress Orlando, FL
Twenty-fifth	2017	**Jonathan Lai** Albert Einstein College of Medicine **John Vederas** University of Alberta	Whistler Conference Centre Whistler, BC, Canada

25th American Peptide Symposium

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SYMPOSIUM-AT-A-GLANCE

Saturday, June 17, 2017

01:00 pm – 06:00 pm	Registration..... Grand Foyer
02:00 pm – 05:30 pm	APS Council Meeting..... Wedgemount
04:00 pm – 05:30 pm	**WORKSHOP**..... Fitzsimmons
06:00 pm – 06:15 pm	**PRESIDENTS'S WELCOME**.....Sea to Sky Ballroom A
06:15 pm – 07:00 pm	**OPENING LECTURE** .....Sea to Sky Ballroom A
07:00 pm – 10:00 pm	Opening Reception ..... Grand Foyer

Sunday, June 18, 2017

07:30 am – 04:30 pm	Registration..... ..... Grand Foyer
08:00 pm	Group 1 Posters set up..... Grand Foyer
08:00 am – 08:15 am	Opening Remarks: Co-Chairs .....Sea to Sky Ballroom A
08:15 am – 10:05 am	**SESSION 1: PEPTIDES & ENZYMES**.....Sea to Sky Ballroom A
10:05 am – 10:30 am	*Coffee with Exhibitors & Posters* .....Grand Foyer & Sea to Sky Ballroom B&C
10:30 am – 12:20 pm	**SESSION 2: MEMBRANE ACTIVE PEPTIDES**.....Sea to Sky Ballroom A
12:20 pm – 01:45 pm	*Lunch (provided)* .....Grand Foyer & Sea to Sky Ballroom B&C
12:15 pm – 01:45 pm	International Liaison Meeting..... Wedgemount
01:50 pm – 03:50 pm	**SESSION 3: PEPTIDE SYNTHESIS, STRUCTURE & FUNCTION** ....Sea to Sky Ballroom A
04:00 pm – 05:30 pm	**MERRIFIELD AWARD LECTURES**.....Sea to Sky Ballroom A
07:00 pm – 09:00 pm	Student Mixer.....Giribaldi

Monday, June 19, 2017

07:30 am – 04:30 pm	Registration..... ..... Grand Foyer
08:00 am – 10:10 am	**SESSION 4: POST-TRANSLATIONAL & CHEMICAL MODIFICATIONS**..... .....Sea to Sky Ballroom A
10:10 am – 10:30 am	*Coffee with Exhibitors & Posters* .....Grand Foyer & Sea to Sky Ballroom B&C
10:30 am – 12:10 pm	**SESSION 5: COMPUTATIONAL DESIGN & RECOGNITION** ...Sea to Sky Ballroom A
12:20 pm – 01:45 pm	*Lunch (provided)* .....Grand Foyer & Sea to Sky Ballroom B&C
01:50 pm – 03:30 pm	**SESSION 6: PEPTIDOMIMETICS & NON-NATURAL BACKBONES**..... .....Sea to Sky Ballroom A
03:40 pm – 04:05 pm	**GOODMAN LECTURE**.....Sea to Sky Ballroom A
04:05 pm – 05:35 pm	**SESSION 7: PEPTIDE THERAPEUTICS & DIAGNOSTICS I**..... .....Sea to Sky Ballroom A
05:35 pm – 08:00 pm	Group 1 Poster Session & Reception .....Grand Foyer & Sea to Sky Ballroom B&C

Tuesday, June 20, 2017

07:30 am – 12:30 pm	Registration..... Grand Foyer
08:00 am	Group 2 Posters Set Up..... Grand Foyer
08:00 am – 12:30 pm	**DU VIGNEAUD LECTURE I**.....Sea to Sky Ballroom A
08:35 am – 10:05 am	**SESSION 8: GLYCOPEPTIDES**.....Sea to Sky Ballroom A
10:05 am – 10:30 am	*Coffee with Exhibitors & Posters* .....Grand Foyer & Sea to Sky Ballroom B&C
10:30 am – 11.00 am	**MAKINENI LECTURE**.....Sea to Sky Ballroom A
11:00 am – 12:45 pm	**SESSION 9: SYNTHETIC METHODS** .....Sea to Sky Ballroom A
12:50 pm – 02:50 pm	Dr. Bert L. Schram Young Scientists' Lunch & Mixer .....Garibaldi *(graduate students & post-docs only)*
12:50 pm – 02:00 pm	Biopolymers-Peptide Science .....Spearhead Editorial Board Lunch & Meeting (Editors and Editorial Advisory Board)
03:00 pm – 05:00 pm	**WORKSHOP ON CAREER DEVELOPMENT:** **JOB SEARCH STRATEGY & PREPARATION**..... Fitzsimmons

Wednesday, June 21, 2017

07:30 am – 04:00 pm	Registration..... Grand Foyer
08:00 am – 08:35 am	**DU VIGNEAUD LECTURE II**.....Sea to Sky Ballroom A
08:35 am – 10:10 am	**SESSION 10: PEPTIDE THERAPEUTICS & DIAGNOSTICS II**..... .....Sea to Sky Ballroom A
10:10 am – 10:35 am	*Coffee with Exhibitors & Posters* .....Grand Foyer & Sea to Sky Ballroom B&C
10:35 am – 12:05 pm	**SESSION 11: PEPTIDE LIBRARIES** .....Sea to Sky Ballroom A
12:10 pm – 12:20 pm	Announcement of 26th APS .....Sea to Sky Ballroom A
12:20 pm – 01:45 pm	*Lunch (provided)* .....Grand Foyer & Sea to Sky Ballroom B&C Vincent du Vigneaud Award Lunch *(by invitation)* .....Giribaldi
03:40 pm – 05:35 pm	**SESSION 12: CONSTRAINED PEPTIDES & MACROCYLCES** ....Sea to Sky Ballroom A
05:35 pm – 08:00 pm	Group 2 Poster Session & Reception .....Grand Foyer & Sea to Sky Ballroom B&C

Thursday, June 22, 2017

07:30 am – 10:30 am	Registration..... Grand Foyer
08:15 am – 10:10 am	**SESSION 13: NATURAL PRODUCT BIOSYNTHESIS**.....Sea to Sky Ballroom A
10:10 am – 10:35 am	Coffee Break..... Grand Foyer
10:35 am – 12:10 pm	**SESSION 14: PEPTIDE MATERIALS & NANOMEDICINE**.....Sea to Sky Ballroom A
12:25 p.m – 01:40 pm	*Lunch (on your own)*
01:40 pm – 03:15 pm	**SESSION 15: PEPTIDE ASSEMBLIES & COMPLEXES**.....Sea to Sky Ballroom A
03:15 pm – 03:55 pm	**CLOSING LECTURE**.....Sea to Sky Ballroom A
03:55 pm – 04:05 pm	**CLOSING REMARKS**.....Sea to Sky Ballroom A
06:30 p.m – 10:00 pm	Closing Banquet.....Sea to Sky Ballroom A

25th American Peptide Symposium

SCHEDULE OF EVENTS

Saturday, June 17, 2017

01:00 pm – 06:00 pm	Registration..... Grand Foyer
02:00 pm – 05:30 pm	APS Council Meeting..... Wedgemount
04:00 pm – 05:30 pm	**WORKSHOP:** Elizabeth Denton, PhD, *Biotage* "How to Break Bottlenecks in Your Peptide Synthesis Workflow" ..... Fitzsimmons
06:00 pm – 06:15 pm	**PRESIDENT'S MESSAGE:** Philip Dawson, *The Scripps Research Institute*..... Sea to Sky Ballroom A
06:15 pm – 07:00 pm	**Opening Plenary Keynote:** Introduction by Jonathan Lai, *Albert Einstein College of Medicine* **L01** David Baker, *University of Washington* "De Novo Peptide Design" ..... Sea to Sky Ballroom A
07:00 pm – 10:00 pm	Opening Reception..... Grand Foyer Sea to Sky Ballroom B & C

Sunday, June 18, 2017

07:30 am – 04:30 pm	Registration..... Grand Foyer
08:00 am	Group 1 Posters Set Up..... Grand Foyer
08:00 am – 08:15 am	Opening Remarks: Co-Chairs John Vederas, *University of Alberta* Jonathan Lai, *Albert Einstein College of Medicine*..... Sea to Sky Ballroom A
08:15 am – 10:05 am	**SESSION 1: PEPTIDES & ENZYMES** **SESSION CHAIRS:** Dewey McCafferty, *Duke University and* Fa Liu, *Novo Nordisk*..... Sea to Sky Ballroom A
08:15 am – 08:40 am	**L02** Dewey McCafferty, *Duke University* "Peptide Probes of Epigenetic Demethylation Complex Protein-Protein Interactions"
08:40 am – 09:00 am	**L03** Éric Marsault, *Université de Sherbrooke* "Modulation of the Primary and Secondary Structure of Apelin to Optimize Stability, Bias Signalling and Modulate Pathophysiological Response"

SCHEDULE OF EVENTS

Sunday, June 18, 2017 continued

09:00 am – 09:25 am **L04** Sylvie Garneau-Tsodikova, *University of Kentucky*
"Understanding and Engineering Bifunctional Enzymes for Nonribosomal Peptide Synthesis"

09:25 am – 09:45 am **L05** Champak Chatterjee, *University of Washington*
"Clarifying the Complexity of Chromatin with Peptide Chemistry"

09:45 am – 10:05 am **L06** James Tam, *Nanyang Technological University*
"Peptide Ligases: Site-specific Molecular Staplers"

10:05 am – 10:30 am *Coffee with Exhibitors & Posters*..... Grand Foyer & Sea to Sky Ballroom B & C

10:30 am – 12:20 pm **SESSION 2: MEMBRANE ACTIVE PEPTIDES**
SESSION CHAIRS: Natia Tsomaia, *IPSEN* and
Charles Deber, *University of Toronto*..... Sea to Sky Ballroom A

10:30 am – 10:55 am **L07** David Craik, *The University of Queensland*
"Membrane Interactions and Cell Penetrating Properties of Cyclotides"

10:55 am – 11:20 am **L08** Gianluigi Veglia, *University of Minnesota*
"Solid-State NMR Spectroscopy Reveals the Allosteric Regulation of the Sarcoplasmic Reticulum Ca2+-ATPase by Phospholamban"

11:20 am – 11:35 am **L09-YI** Erika J. Olson, *The Scripps Research Institute*
"Structure-Guided Development of a Peptide Antagonist for EphA4, A Cell-Surface Receptor Linked to Neurodegeneration"

11:35 am – 12:00 pm **L10** Bob Hancock, *University of British Columbia*
"Peptides as Alternatives to Antibiotics"

12:00 pm – 12:20 pm **L11** Joshua Kritzer, *Tufts University*
"A New, Quantitative Assay for Cytosolic Penetration of Peptides and other Molecules"

12:20 pm – 01:45 pm *Lunch (provided)*..... Grand Foyer & Sea to Sky Ballroom B & C

12:15 pm – 01:45 pm International Liaison Meeting .....Wedgemount

01:50 pm – 03:30 pm **SESSION 3: PEPTIDE SYNTHESIS, STRUCTURE & FUNCTION**
SESSION CHAIRS: David Craik, *The University of Queensland* and
Michael Kay, *University of Utah* ..... Sea to Sky Ballroom A

01:50 pm – 02:15 pm **L12** Amy Keating, *Massachusetts Institute of Technology*
"Protein-peptide Interactions that Control Apoptosis"

02:15 pm – 02:40 pm	**L13**	Akira Otaka, *Tokushima University* "Application of N-Sulfanylethylanilide (SEAlide) Unit to Protein Chemical Synthesis and Protein Enrichment"
02:40 pm – 03:05 pm	**L14**	David Chenowith, *University of Pennsylvania* "Rational Redesign of the Collagen Triple Helix Interface"
03:05 pm – 03:30 pm	**L15**	William Lubell, *Université de Montréal* "Linear and Cyclic Azapeptide Strategies for Conceiving Cluster of Differentiation-36 Scavenger Receptor Modulators"
03:30 pm – 03:50 pm	**L16**	Steven Castle, *Brigham Young University* "Bulky Dehydroamino Acids Impart Proteolytic Stability to Peptides"
04:00 pm – 05:30 pm	**MERRIFIELD AWARD LECTURES** ..... Sea to Sky Ballroom A	
04:00 pm – 04:05 pm	Introduction by Philip Dawson, *The Scripps Research Institute*	
04:05 pm – 04:45 pm	**L17-AW**	Robert Hodges, *University of Colorado School of Medicine* "The Use of Synthetic Peptides to Probe Complex Interactions in Proteins and the Development of Synthetic Peptide Drugs and Vaccines"
04:45 pm – 04:50 pm	Introduction by Philip Dawson, *The Scripps Research Institute*	
04:50 pm – 05:30 pm	**L18-AW**	Charles Deber, *University of Toronto* "Peptides in Membranes: From Structure to Drug Design"
07:00 pm – 09:00 pm	Student Mixer.... ..... Garibaldi	

Monday, June 19, 2017

07:30 pm – 04:30 pm	Registration..... ..... Grand Foyer
08:00 pm – 10:10 am	**SESSION 4: POST-TRANSLATIONAL & CHEMICAL MODIFICATIONS** **SESSION CHAIRS:** Michael Burkart, *University of California, San Diego* and Justin Holub, *Ohio University*..... Sea to Sky Ballroom A

08:00 am – 08:25 am	**L19**	Michael Burkart, *University of California, San Diego* "Engineering Protein Interactions in Type II Non-Ribosomal Peptide Synthetases"
08:25 am – 08:50 am	**L20**	Danica Fujimori, *University of California, San Francisco* "Cross-Talk Between Chromatin Recognition and Modification in Histone Demethylases"
08:50 am – 09:05 am	**L21-YI**	Amanda Whiting, *National Cancer Institute* "Affecting Activity of the Linear Ubiquitin Chain Assembly Complex, LUBAC, with Stapled Alpha-Helical Peptides"

SCHEDULE OF EVENTS

Monday, June 19, 2017 continued

09:05 am – 09:30 am **L22** Ratmir Derda, *University of Alberta*
"Genetically-Encoded Chemically-Modified Peptides"

09:30 am – 09:50 am **L23** Marcey Waters, *University of North Carolina, Chapel Hill*
"Affinity Labeling of Histone Peptides Containing Trimethyllysine"

09:50 am – 10:10 am **L24** Mark DiStefano, *University of Minnesota*
"New Coumarin- and Nitrodibenzofuran-Based Photoremovable Protecting Groups for Cysteine Protection in Solid Phase Peptide Synthesis"

10:10 am – 10:30 am *Coffee with Exhibitors & Posters*..... Grand Foyer & Sea to Sky Ballroom B & C

10:30 am – 12:10 pm **SESSION 5: COMPUTATIONAL DESIGN & RECOGNITION**
SESSION CHAIRS: David Baker, *University of Washington* and Jung-Mo Ahn, *University of Texas at Dallas*..... Sea to Sky Ballroom A

10:30 am – 10:55 am **L25** Jeffery Saven, *University of Pennsylvania*
"Using Theory and Computation to Design Polypeptide Materials"

10:55 am – 11:20 am **L26** Gevorg Grigoryan, *Dartmouth College*
"Tertiary Alphabet for the Observable Structural Universe"

11:20 am – 11:45 am **L27** Ora Schueler-Furman, *Hebrew University of Jerusalem*
"Accurate Structural Modeling and High-Affinity Design of Peptide-Protein Interactions using Rosetta FlexPepDock"

11:45 am – 12:00 pm **L28-YI** Jonas Wilbs, *Ecole Polytechnique Fédérale de Lausanne*
"Development of a Peptide Macrocycle FXII Inhibitor for Safe Anticoagulation Therapy"

12:00 pm – 12:20 pm **L29** Jumi Shin, *University of Toronto*
"Minimalist Protein ME47 Targets the Myc/Max:E-box DNA Network and Decreases Tumor Xenograft Growth"

12:20 pm – 01:45 pm *Lunch (provided)*..... Grand Foyer & Sea to Sky Ballroom B & C

01:50 pm – 03:30 pm **SESSION 6: PEPTIDOMIMETICS & NON-NATURAL BACKBONES**
SESSION CHAIRS: Sam Gellman, *University of Wisconsin-Madison* and Kit Lam, *University of California, Davis*..... Sea to Sky Ballroom A

01:50 pm – 02:15 pm **L30** Sam Gellman, *University of Wisconsin-Madison*
"Impact of Backbone Modifications on Informational Properties of Peptides"

02:15 pm – 02:40 pm **L31** Gilles Guichard, *University of Bordeaux and CNRS*
"Interfacing Oligourea Foldamers with Alpha-Peptides: An Approach to Alpha-Helix Mimicry and to the Creation of Composite Protein-Like Architectures"

02:40 pm – 03:05 pm **L32** Juan Del Valle, *University of South Florida*
"Peptide N-Amination: A Versatile Backbone Modification for Constrained Proteomimetics"

03:05 pm – 03:25 pm **L33** Kristian Strømgaard, *University of Copenhagen*
"Targeting Protein-Protein Interactions with Peptide-based Inhibitors"

03:40 pm – 04:05 pm **GOODMAN LECTURE**
Introduction by Ved Srivastava, *Intarcia Therapeutics*
L34-AW Paul Alewood, *The University of Queensland*
"From Venoms to Drugs"

04:05 pm – 05:35 pm **SESSION 7: PEPTIDE THERAPEUTICS & DIAGNOSTICS I**
Session Chairs: Leslie Miranda, Amgen and Waleed Danho .... Sea to Sky Ballroom A

04:05 pm – 04:30 pm **L35** Ved Srivastava, *Intarcia Therapeutics*
"From Twice-daily Injection to Once or Twice a Yearly Dosing of GLP-1 Agonist for Potential Treatment for Type 2 Diabetes"

04:30 pm – 04:55 pm **L36** Dana Ault-Riche, *Reflexion Pharmaceuticals*
"Development of a Potent D-Protein Inhibitor of VEGF-A with Reduced Immunogenicity and a Longer Half-Life"

04:55 pm – 05:15 pm **L37** Victor Hruby, *University of Arizona*
"Conformational, Topographical, Receptor and Dynamic Consideration in the Design of Biostable, Receptor Selective Ligands for the Melanocortin Receptors"

05:15 pm – 05:35 pm **L38** Kamaljit Kaur, *Chapman University*
"Breast Cancer Targeting Peptides and Peptide-Drug Conjugates for Increased Therapeutic Efficacy of Current Chemotherapeutic Drugs against Triple Negative Breast Cancer"

05:35 pm – 08:00 pm Group 1 Poster Session ..... Grand Foyer
Reception..... Sea to Sky Ballroom B & C
Posters must be removed at 8:00 pm

Tuesday, June 20, 2017 continued

07:30 am – 12:30 pm — Registration ..... Grand Foyer

08:00 am — Group 2 Posters Set Up ..... Grand Foyer

08:00 am – 08:35 am — **DU VIGNEAUD LECTURE I** ..... Sea to Sky Ballroom A
Introduction by Robin Offord, *Mintaka Foundation*
L39-AW Ronald Raines, *University of Wisconsin*
"Chemical Forces that Stabilize Proteins"

08:35 am – 10:05 am — **SESSION 8: GLYCOPEPTIDES**
SESSION CHAIRS: James Paulson, *The Scripps Research Institute* and Fernando Albericio, *University of KwaZulu-Natal* ..... Sea to Sky Ballroom A

08:35 am – 09:00 am — **L40** James Paulson, *The Scripps Research University*
"HIV Glycosylation"

09:00 am – 09:25 am — **L41** Jon Thorson, *University of Kentucky*
"Microbial Natural Products Discovery and Diversification"

09:25 am – 09:50 am — **L42** Laura Kiessling, *University of Wisconsin*
"Elucidating the Features of Effective Vaccines"

09:50 am – 10:05 am — **L43-YI** Julia C. Frei, *Albert Einstein College of Medicine*
"Engineering Dengue EDIII Immunogens to Elicit Broadly Neutralizing Antibody Responses"

10:05 am – 10:30 am — *Coffee with Exhibitors & Posters* ..... Grand Foyer & Sea to Sky Ballroom B & C

10:30 am – 11:00 am — **MAKINENI LECTURE** ..... Sea to Sky Ballroom A
Introduction by Marcey Waters, *University of North Carolina, Chapel Hill*
L44-AW Thomas Kodadek, *The Scripps Research University*
"Novel Peptide-like Inhibitors of the Proteasome Ubiquitin Receptor Rpn13 Proteasome Inhibitors and Their Mechanism of Action: A Promising New Approach to Chemotherapy"

11:00 am – 12:45 am — **SESSION 9: SYNTHETIC METHODS**
SESSION CHAIRS: Stephen Kent, *University of Chicago* and Andrei Yudin, *University of Toronto* ..... Sea to Sky Ballroom A

11:00 am – 11:25 am — **L45** Bradley Pentelute, *Massachusetts Institute of Technology*
"Automated Flow Peptide Synthesis: Toward Amide Bonds at Nature's Pace"

11:25 am – 11:50 am **L46** David Perrin, *University of British Columbia*
"Organotrifluoroborates as Radioprosthetics that Enable Facile, User-friendly 18F-Labeling for Turning Peptides into Pet Imaging Agents"

11:50 am – 12:10 pm **L47** James Cain, *Gyros Protein Technologies*
"Peptide Therapeutics Method Development: Increasing Efficiency and Purity of SPPS using Parallel Synthesis Optimization with Induction Heating"

12:10 pm – 12:30 pm **L48** Monika Raj, *Seton Hall University*
"Cyclic Urethane Chemistry for Synthesis and Sequencing of Cyclic Peptides"

12:30 pm – 12:50 pm **L49** Jonathan Collins, *CEM Corporation*
"A New Level of Efficiency for SPPS"

12:50 pm – 02:50 pm Dr. Bert L. Schram Young Scientists' Lunch & Mixer..... Garibaldi
Graduate students & post-docs only

12:50 pm – 02:00 pm Biopolymers-Peptide Science Editorial Board Lunch & Meeting.....Spearhead AB
Editors and Editorial Advisory Board

03:00 pm – 05:00 pm **WORKSHOP ON CAREER DEVELOPMENT: JOB SEARCH**
Strategy & Preparation.....Fitzsimmons
Organized by **Natia Tsomaia**, *IPSEN*
Ellie Eckhoff, Senior Vice President, *ClearRock, Inc.*
Sponsored by **Scilligence**

Wednesday, June 21, 2017

07:30 am – 04:00 pm Registration..... .....Grand Foyer

08:00 am – 08:35 am **DU VIGNEAUD LECTURE II** ..... Sea to Sky Ballroom A
Introduction by John Vederas, *University of Alberta*
L50-AW Wilfred van der Donk, *Howard Hughes Medical Institute and University of Illinois at Urbana-Champaign*
"Combinatorial Lanthipeptide Biosynthesis"

08:35 am – 10:10 am **SESSION 10: PEPTIDE THERAPEUTICS & DIAGNOSTICS II**.....
..... Sea to Sky Ballroom A
SESSION CHAIRS: Thomas Kodadek, *The Scripps Research Institute*
and Wendy Hartsock, *Ferring Research Institute, Inc.*

08:35 am – 09:00 am **L51** Hyun-Suk Lim, *Pohang University of Science and Technology*
"Discovery of Macrocyclic Peptidomimetics Targeting Protein-Protein Interactions"

Wednesday, June 21, 2017 continued

09:00 am – 09:25 am **L52** John Mayer, *Novo Nordisk Research Center*
"Discovery of Soluble, Biophysically Stable Glucagon Analogs with Minimal Change to Native Sequence"

09:25 am – 09:50 am **L53** Kathlynn Brown, *SRI International*
"Systematic Discovery and Development of Peptidic Molecular Guidance Systems for the Diagnosis and Treatment of Cancer"

09:50 am – 10:10 am **L54** Jane Aldrich, *University of Florida*
"Sensitivity of the Opioid Activity Profile of CJ-15,208 Analogs to Ring Substitution"

10:10 am – 10:35 am *Coffee with Exhibitors & Posters*..... Grand Foyer & Sea to Sky Ballroom B & C

10:35 am – 12:05 pm **SESSION 11: PEPTIDE LIBRARIES**
SESSION CHAIRS: Hiroaki Suga, *The University of Tokyo* and Richard Houghten, *Torrey Pines Institute*..... Sea to Sky Ballroom A

10:35 am – 11:00 am **L55** Hiroaki Suga, The University of Tokyo
"The RaPID Discovery of Pseudo-Natural Peptides"

11:00 am – 11:25 am **L56** Sachdev Sidhu, *University of Toronto*
"From Natural Antibodies to Synthetic Proteins"

11:25 am – 11:40 am **L57-YI** Lara Malins, *The Scripps Research Institute*
"Peptide Macrocyclization Inspired by Non-Ribosomal Imines"

11:40 am – 12:05 pm **L58** Jennifer Cochran, *Stanford University*
"An Engineered Tumor Targeting Knottin Peptide is a Versatile Agent for Imaging, Drug Delivery, and Immunotherapy"

12:10 pm – 12:20 pm Announcement of 26th APS ..... Sea to Sky Ballroom A
Marcey Waters, *UNC Chapel Hill*

12:20 pm – 01:45 pm *Lunch (provided)*..... Grand Foyer & Sea to Sky Ballroom B & C

12:20 pm – 01:45 pm Vincent du Vigneaud Award Lunch ..... Garibaldi
Sponsored by BACHEM
By Invitation

03:40 pm – 05:35 pm **SESSION 12: CONSTRAINED PEPTIDES & MACROCYCLES**
SESSION CHAIRS: Paramjit Arora, *New York University* and Federicio Bernal, *National Cancer Institute*..... Sea to Sky Ballroom A

03:40 pm – 04:05 pm	**L59**	Paramjit Arora, *New York University* "Minimal Coiled Coils and their Potential as Protein-Protein Interaction Inhibitors"
04:05 pm – 04:30 pm	**L60**	Eileen Kennedy, *University of Georgia, College of Pharmacy* "Targeting Kinase Regulation with Constrained Peptides"
04:30 pm – 04:45 pm	**L61-YI**	Solomon Appavoo, *University of Toronto* "Oxadiazole Grafts in Peptide Macrocycles"
04:45 pm – 05:10 pm	**L62**	Dehua Pei, *The Ohio State University* "How Do Cell-Penetrating Peptides Work?"
05:10 pm – 05:35 pm	**L63**	Tomi Sawyer, *Merck & Company, Inc.* "Exploring Macrocyclic Peptide Cell Permeability: Stapled p53 Peptide Model System"
05:35 pm – 08:00 pm		Group 2 Poster Session ..... *Grand Foyer &* Sea to Sky Ballroom B & C Reception ***Posters must be removed at 8:00 pm***

Thursday, June 22, 2017

07:30 am – 10:30 am		Registration..... ..... Grand Foyer
08:15 am – 10:10 am		**SESSION 13: NATURAL PRODUCT BIOSYNTHESIS** **SESSION CHAIRS:** Wilfred van der Donk, *Howard Hughes Medical Institute and University of Illinois at Urbana-Champaign* and Yuki Goto, *The University of Tokyo* ..... Sea to Sky Ballroom A
08:15 am – 08:40 am	**L64**	Douglas Mitchell, *University of Illinois at Urbana-Champaign* "In Vitro Reconstitution and Mechanistic Insights into Thiopeptide Biosynthesis"
08:40 am – 09:05 am	**L65**	Eric Schmidt, *University of Utah* "Biosynthesis of RiPPs on the Coral Reef"
09:05 am – 09:20 am	**L66-YI**	Emilia Oueis, *University of St Andrews* "Enzymatic Macrocyclization of Non-Natural and Hybrid Polyketide Peptides"
09:20 am – 09:45 am	**L67**	A. James Link, *Princeton University* "Making and Breaking Lasso Peptides"
09:45 am – 10:10 am	**L68**	Albert Bowers, *University of North Carolina, Eshelman School of Pharmacy* "Chemoenzymatic Platforms for the Discovery of New Peptide Therapeutics"
10:10 am – 10:35 am		*Coffee Break*..... ..... Grand Foyer

Thursday, June 22, 2017 continued

10:35 am – 12:10 pm

SESSION 14: PEPTIDE MATERIALS & NANOMEDICINE
Session Chairs: Neal Zondlo, *University of Delaware* and Bradley Nilsson, *University of Rochester* ..... Sea to Sky Ballroom A

10:35 am – 11:00 am

L69 Joel Schneider, *National Cancer Institute*
"The Design Evolution of Anticancer Peptides from Self-Assembled Hydrogels and How Cancer Cells Gain Resistance to their Action"

11:00 am – 11:25 am

L70 James LaBelle, *University of Chicago*
"Intracellular Delivery of Therapeutic Peptide Using Enzymatically Cleavable Peptide Amphiphiles"

11:25 am – 11:50 am

L71 David Lynn, *Emory University*
"Protein Misfolding and Chemical Evolution"

11:50 am – 12:05 pm

L72-YI Muhammad Jbara, *Technion-Israel Institute of Technology Haifa*
"Palladium in Chemical Protein Synthesis and Manipulation"

12:05 pm – 12:25 pm

L73 Anouk Dirksen, *Pfizer Inc.*
"Peptide-CRM197 Conjugate Vaccines - Considerations for Process Development"

12:25 pm – 01:40 pm

Lunch Break (on your own)

01:40 pm – 03:15 pm

SESSION 15: PEPTIDE ASSEMBLIES & COMPLEXES
SESSION CHAIRS: James Nowick, *University of California*, Irvine and Ivan Korendovych, *Syracuse University* ..... Sea to Sky Ballroom A

01:40 pm – 02:05 pm

L74 James Nowick, *University of California, Irvine*
"Unlocking the Mysteries of Amyloid Diseases with Macrocyclic Beta-Sheet Peptides"

02:05 pm – 02:30 pm

L75 Ivan Huc, *University of Bordeaux and CNRS*
"Aromatic Foldamer-Based Protein Mimicry and Recognition"

02:30 pm – 02:55 pm

L76 E. James Petersson, *University of Pennsylvania*
"Thioamide Probes for Studying Peptide Stability *In Vitro* and *In Vivo*"

02:55 pm – 03:15 pm

L77 Brian McNaughton, *Colorado State University*
"Protein Evolution Yields Cyclic Peptide Inhibitors of HIV-1 Propagation"

03:15 pm – 03:30 pm	**CLOSING REMARKS:** John Vederas, *University of Alberta* Jonathan Lai, *Albert Einstein College of Medicine*
03:15 pm – 04:05 pm	**CLOSING LECTURE:** Introduction by Philip Dawson, The Scripps Research Institute **L78** Stephen Kent, *University of Chicago* "Inventing Synthetic Peptide & Protein Chemistries to Reveal how Enzymes Work"
06:30 pm – 10:00 pm	Closing Banquet ..... Grand Foyer
06:30 pm – 07:15 pm	Reception..... ..... Sea to Sky Ballroom B & C
07:15pm – 10:00 pm	Dinner, Awards and Dancing ..... Sea to Sky Ballroom B & C

25th American Peptide Symposium

EXHIBITOR LIST

EXHIBITOR	BOOTH
aapptec	46
Advanced ChemTech	19
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Christof Senn Laboratories AG	42
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Intavis, Inc.	21
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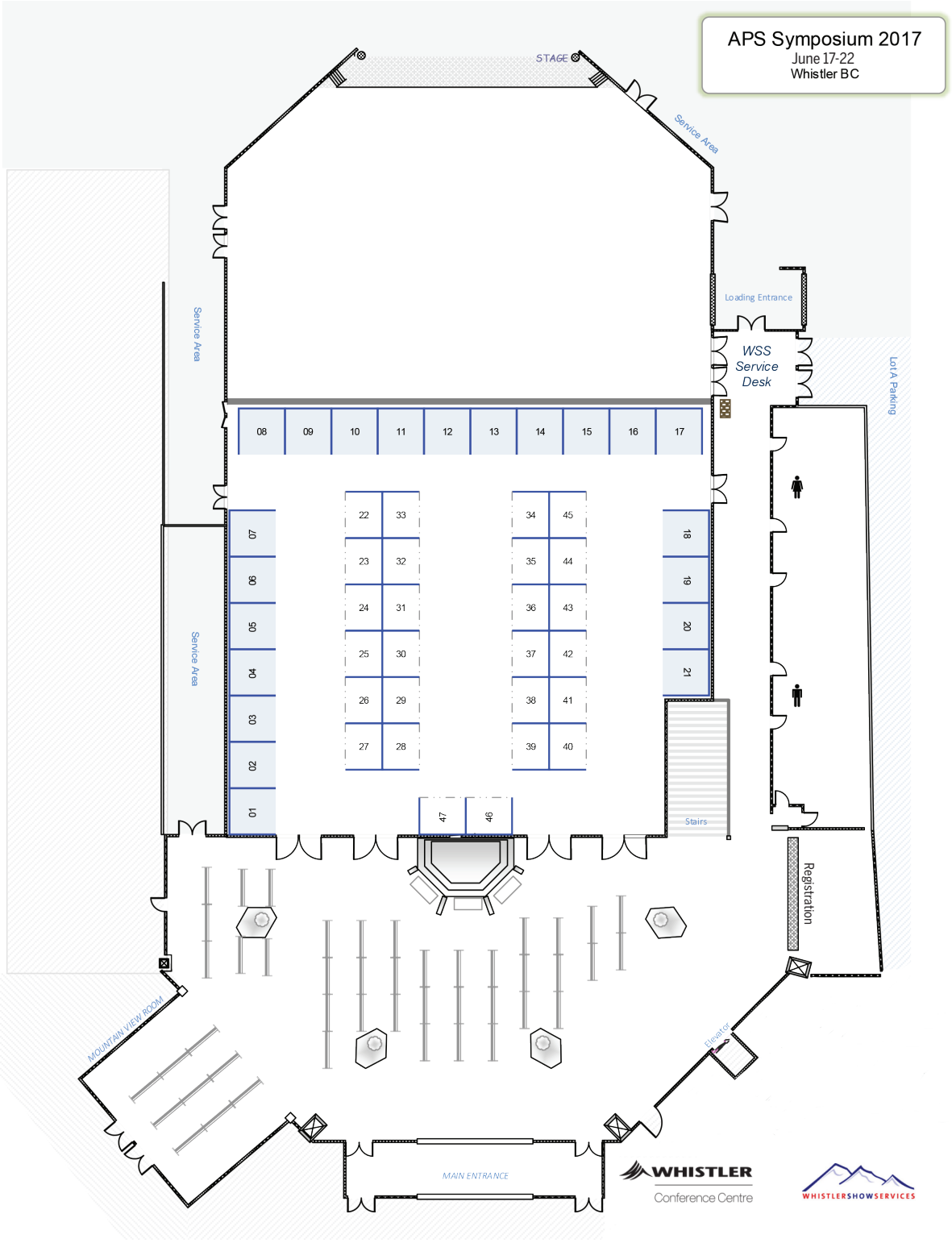
25th American Peptide Symposium

EXHIBITOR LAYOUT



25th American Peptide Symposium

The Merrifield Award Winners*

The Merrifield Award, presented at the biennial symposia, recognizes the lifetime achievement of a peptide scientist, whose work exemplifies the highest level of scientific creativity.

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.

25th American Peptide Symposium

The 2017 R. Bruce Merrifield Award

Charles M. Deber

Professor Charles M. Deber, a Senior Scientist in the Research Institute at Toronto's Hospital for Sick Children, and Professor in the Department of Biochemistry at the University of Toronto, is the co-recipient of the 2017 R. Bruce Merrifield Award of the American Peptide Society. His laboratory is in the top echelon of worldwide structural biology research in peptide-based approaches to elucidation of fundamental peptide- and protein-membrane interactions.

Charles Deber was introduced to peptide chemistry early in his career through his undergraduate research with Murray Goodman at the Polytechnic Institute of Brooklyn. Deber earned his Ph.D. in synthetic organic chemistry with Arthur Cope at the Massachusetts Institute of Technology, and carried out postdoctoral studies at Harvard Medical School with Elkan Blout. At Harvard, Deber was involved in some of the earliest applications of NMR spectroscopy to the investigation of peptide conformations, was the first to observe the presence of cis peptide bonds in proline-containing peptides, and pioneered the use of cyclic peptides as rigidifying systems to diagnose residue-dependent structural features. Deber completed his training with Henry Lardy at the Enzyme Institute at the University of Wisconsin, Madison, working on ionophore-mediated calcium transport.

As an independent investigator in Toronto, Deber became interested in fundamental and applied problems at the interface between peptide/protein chemistry and lipid/membrane biochemistry — important topics because human diseases such as multiple sclerosis, cystic fibrosis, cancer, and diabetes have been linked to critical mutations in membrane proteins. Deber developed fundamental guidelines for membrane protein engineering and identified a 'threshold hydrophobicity' concept to explain spontaneous insertion of peptides into membranes.

Among the many contributions of the Deber laboratory was a novel 'Lys-tagging' procedure that overcame, for the first time, the inherent aqueous insolubility of trans-membrane polypeptide segments, thereby enabling facile solid-phase synthesis, purification, and characterization of highly hydrophobic peptides. Deber then used membrane-spanning peptides derived from the cystic fibrosis transmembrane conductance regulator (CFTR) — the CF gene product — to work out a molecular level basis for some forms of cystic fibrosis. In other work, Deber *de novo* designed cationic antimicrobial peptides (CAPs) whose properties led to the elucidation of some factors that allow certain CAPs to select for bacterial membranes over (host) mammalian membranes. As well, the Deber lab solved a 30-year 'mystery' as to why membrane proteins migrate aberrantly on SDS-PAGE gels by discovering that SDS binds to peptide sequences differentially as a function of sequence hydrophobicity [*PNAS* (2009)].

Deber's career-long research is described in nearly 300 scientific papers and was performed by over 100 post-doctoral fellows, graduate students, and project students who were supervised and mentored by Dr. Deber. For these accomplishments, the American Peptide Society has honored Deber with the 2000 Vincent du Vigneaud Award and the 2009 Murray Goodman Award. Among other recognitions, Deber was elected in 2001 as a Fellow of the Royal Society of Canada (FRSC), and was elected in 2009 to the Board of Trustees of the Gordon Research Conferences.

With the American Peptide Society, Deber served as its first elected President (1991-1993), and was the Editor-in-Chief of the official APS journal *Peptide Science* (1998-2004). He was the co-organizer (with Ken Kopple) of the 9th American Peptide Symposium (Toronto, 1985), and co-chair (with John Smith) of the 'Chemistry & Biology of Peptides' GRC (Ventura, 1996). He served two elected terms on the APS Council (1997-2003; 2009-2015).

Professor Deber is also an outstanding teacher at the University of Toronto, receiving the W.T. Aikins Award

as the top lecturer in the Faculty of Medicine for his "Protein Structure and Function" course, and is part of the team entrusted with teaching classes of 1,200 students "Introductory Biochemistry" — the largest science course in the Faculty of Arts & Science at the University of Toronto; to-date, Deber has taught over 18,000 undergraduates in this role.

Peptides in Membranes: From Structure to Drug Design

Charles M. Deber
Division of Molecular Structure & Function, Research Institute, Hospital for Sick Children, Toronto, Ontario M5G 0A4, Canada; and Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Through synthesis and conformational analysis of model membrane-interactive peptides, our lab has been contributing to a body of work directed toward defining fundamental features of sequence, hydrophobicity, and helix-helix interactions of membrane-spanning segments in proteins. As protein–protein interactions (PPIs) within membrane proteins are of great interest as therapeutic targets, we are currently applying the resulting knowledge of these factors to facilitate the design of PPI "disruptors" — peptide reagents that target membrane-embedded protein assembly motifs. In this context, we will address bacterial multidrug resistance, which originates largely from protein pumps embedded in the bacterial cell membranes that resist the toxic effects of drugs by efficiently extruding them. We will focus on the design, synthesis, and bioactivity of novel peptide-based inhibitors that compete for — and disrupt — functional helix-helix interactions in these proteins to produce efflux pump inhibitors with significant pharmacologic properties

25th American Peptide Symposium

The 2017 R. Bruce Merrifield Award

Robert Hodges

Bob Hodges graduated with his Ph.D. in Biochemistry from the University of Alberta in 1971. He joined the laboratory of Dr. Bruce Merrifield at Rockefeller University in 1971 where he used solid- phase peptide synthesis to study the enzyme, Ribonuclease. In 1974, Bob left the Merrifield lab to accept a position as Assistant Professor of Biochemistry at the University of Alberta where he became a founding member of the famous Medical Research Council Group in Protein Structure and Function. Bob remained at the University of Alberta for more than 25 years, publishing prolifically and collaborating with scientists in many other fields, including joining two Networks of Centers of Excellence which involved outstanding researchers from across Canada to work together on research projects that bridged the gap between academia and industry. These Networks included the Canadian Bacterial Diseases Network (CBDN) and the Protein Engineering Network of Centers of Excellence (PENCE). In 1994 Dr. Hodges took over the leadership of PENCE from Michael Smith, Nobel laureate, and headed this network for 6 years. In 2000 he moved to the University of Colorado, School of Medicine to accept the position as Director of the Program in Biomolecular Structure, Professor of Biochemistry and Molecular Genetics and holder of the John Stewart Endowed Chair in Peptide Chemistry. In the U.S. he was successful at obtaining NIH R01 grants in diverse areas of science, all applying peptide chemistry, to solve questions about peptides and proteins of biological interest. Bob has won many outstanding awards in both Canada and the U.S. among which are the Distinguished Medical Research Council of Canada Scientist Award. The MRC career awards were considered the most prestigious of such awards in Canada (1995-2000). In 1995 he won the Boehringer-Mannheim Award from the Canadian Society of Biochemistry and Molecular Biology, in recognition of a record of outstanding achievements in research in the field of biochemistry undertaken in Canada by a Canadian Scientist. Recognition followed in 1995 with the Alberta Science and Technology Award for outstanding leadership in Alberta Science. In 2002, Dr Hodges won the Vincent Du Vigneaud Award from the American Peptide Society for outstanding achievements in peptide research. In 2009 he received the Inventor of the Year Award and in 2012 the Company of the Year Award (PeptiVir, Inc.) at the University of Colorado Denver. In 2013 he won the Murray Goodman Scientific Excellence and Mentorship Award from the American Peptide Society for career-long research excellence in the field of peptide science and significant mentorship and training of students, postdoctoral fellows and other co-workers. In 2017, he received the most prestigious award in peptide chemistry, the Bruce Merrifield Award, for outstanding lifetime accomplishment in peptide research, recognizing the highest level of scientific creativity.

Bob has served the peptide science community in a variety of roles. He chaired the 1993 American Peptide Symposium in Edmonton, Alberta. Interestingly, this meeting had the largest number of attendees of any American Peptide Symposium to date. From 1995-1999, Dr. Hodges served as President-elect and President of the American Peptide Society. He was Co-chair of the Gordon Research Conference on the Chemistry and Biology of Peptides in 2006. He has served on the editorial board of the Journal of Peptide Research, 5 years as associate editor and then on the editorial board of Chemical Biology and Drug Design as well as many other editorial boards.

Dr. Hodges has used peptide chemistry in an exceptionally creative and innovative manner to investigate major challenges in biomedical research. He has published over 560 publications in his career in areas highlighted below:

1. Development of amphipathic alpha-helical antimicrobial peptides as therapeutics. He discovered the concept of “specificity determinants” to remove toxicity to human cells, enhance antimicrobial activity, control gram-negative pathogen selectivity and prevent high-affinity binding to serum proteins.

2. The development of synthetic peptide anti-adhesin bacterial vaccines and antibody therapeutics for the prevention and treatment of Pseudomonas aeruginosa bacterial infections.
3. Development of synthetic peptide vaccines and peptide inhibitors to SARS-coronavirus infections. This project led to a novel templated coiled-coil system to present helical epitopes to the immune system. This technology is now being applied to develop a "universal" influenza A vaccine to highly conserved helical regions in the hemagglutinin protein.
4. He sequenced the first two-stranded coiled-coil protein, tropomyosin, during his PhD project and has used coiled-coils as a model system to study protein folding, stability and de novo protein design.
5. To understand the regulation of muscle contraction at the molecular level.
6. Development of new HPLC and capillary electrophoresis methodology.

In addition to his scientific career, Bob also had a career as an elite athlete. He had the distinct privilege of representing Canada in international speed skating. He competed in three World Championships (1968,1970 and 1971) and two Olympics (1968 Grenoble, France and 1972 Sapporo, Japan).

The Use of Synthetic Peptides to Probe Complex Interactions in Proteins and the Development of Synthetic Peptide Drugs and Vaccines

R.S. Hodges
Department of Biochemistry and Molecular Genetics, University of Colorado, School of Medicine, Anschutz Medical Campus, Aurora, CO, USA

Using a synthetic peptide approach, we were able to unravel all the complex interactions during muscle contraction and relaxation in the presence and absence of Ca^{2+}. The interactions in thin filament involve actin and the regulatory proteins, tropomyosin (Tm) and the troponin complex (TnT, TnI and TnC). During my Ph.D., I sequenced the first two-stranded α-helical coiled-coil, Tm, to identify the hydrophobic repeat responsible for the formation and stabilization of the coiled-coil structure. This hydrophobic repeat of seven amino acid residues denoted $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$ was shown to be continuous throughout the entire 284-residue polypeptide chain of Tm where positions a and d were occupied by hydrophobic residues. Next, we designed *de novo* and synthesized the first model coiled-coil protein and demonstrated its utility for studying the folding and stability of α-helical proteins. I will discuss some of the highlights of our research on coiled-coils. Most recently, we addressed the question of how stability information is transmitted along a rod-like Tm coiled-coil which is critical to under-standing signaling and function of Tm during muscle contraction. Our understanding of coiled-coils led to developing a simple, innovative and robust technology to present α-helical epitopes from native proteins to the immune system, such that the resulting conformational specific antibodies bind to the native protein target. The helical sequence of interest is inserted into a parallel two-stranded α-helical coiled-coil and disulfide-bridged template which maintains the native conformation of the helical epitope. We are using this technology to generate a universal influenza A vaccine. Our understanding of α-helical structure led to our research on α-helical antimicrobial peptides (AMPs). We developed the concept of "specificity determinants" which, when inserted in the non-polar face of amphipathic α-helical AMPs with broad-spectrum activity dramatically changed their properties: 1) reduced or eliminated eukaryotic toxicity; 2) enhanced antimicrobial activity against prokaryotic cells; 3) encoded selectivity for Gram-negative pathogens by significantly decreasing/eliminating activity against Gram-positive pathogens; 4) maintained excellent antimicrobial activity in the presence of human serum by preventing high-affinity binding to serum proteins through the non-polar face.

25th American Peptide Symposium

The Rao Makineni Lectureship*

The Makineni Lectureship recognizes an individual who has made a recent contribution (within 2 years) of unusual merit to research in the field of peptide science.

2017 – Thomas Kodadek, The Scripps Research Institute

2015 – Paramjit Arora, New York University

2013 – Samuel 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)

25th American Peptide Symposium

The 2017 Makineni Lectureship

Thomas Kodadek

Prof. Thomas Kodadek received his B.S. in Chemistry at the University of Miami (FL) in 1981 and his Ph.D. in Organic Chemistry from Stanford University in 1985. He then pursued post-doctoral studies in the laboratory of Prof. Bruce Alberts at the University of California, San Francisco Medical School from 1985-1987. In the fall of 1987 he joined the faculty of Chemistry & Biochemistry at the University of Texas at Austin, rising to the rank of full professor. In 1998, he moved to the University of Texas Southwestern Medical Center in Dallas where he served as Professor of Internal Medicine and Molecular Biology as well as the Director of the Division of Translational Research. In June, 2009, Prof. Kodadek moved to the Scripps Research Institute campus in Jupiter, FL where he is currently Chairman of Cancer Biology and Professor of Chemistry.

Prof. Kodadek works in the field of chemical biology, which involves the development of chemical tools to monitor and manipulate important processes in biology and medicine. His laboratory has also made important contributions to our understanding of how genes are rearranged and expressed. Recently, Prof. Kodadek has focused on the development of novel diagnostic and therapeutic tools for the treatment of immune diseases and cancers. This work was recognized in 2006 by a prestigious NIH Director's Pioneer Award for "exceptionally creative research". Opko, a Miami biotechnology company, has established a laboratory in Jupiter for the discovery of novel diagnostic markers for cancer, autoimmune and neurological diseases using the methods developed in the Kodadek laboratory.

Prof. Kodadek is married to Dr. Ofelia Utset, a physician. They have one child, Cristina, age 11.

Novel Peptide-like Inhibitors of the Proteasome Ubiquitin Receptor Rpn13 Proteasome Inhibitors and Their Mechanism of Action: A Promising New Approach to Chemotherapy

T. Kodadek, P. Dickson, D. Trader,
S. Simanski and P. McEnaney
Dept. of Chemistry, The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458. USA

Proteasome inhibitors such as Bortezomib have emerged as important drugs for the treatment of multiple myeloma (MM) and certain other hematological cancers, which produce prodigious amounts of protein and thus place an unusual burden on pathways to deal with mis-folded proteins, including proteasome-mediated degradation. However, they have failed in trials against solid tumors, apparently due to an insufficient therapeutic window (all cells require proteasome activity). Recently, the ubiquitin receptor Rpn13, a component of the 19S regulatory particle (RP) of the proteasome, has been shown to be non-essential in normal cells, but is highly overexpressed in a number of cancers, suggesting that Rpn13 is a "turbocharger" of the proteasome, a function that is critical in cancer cells, but not healthy cells. We developed a peptoid inhibitor of Rpn13 and found that it is toxic to a number of cancer cell lines, but displays no toxicity in normal cells.[1] We show that the peptoid abrogates Rpn13-mediated activation of the deubiquitylase (Dub) Uch37, strongly suggesting that unusually high Dub activity is critical for high-level proteasome function in cancer cells. The development of improved Rpn13 inhibitors will be also be described.

1. Trader, D.J., Simanski, S. & Kodadek, T. A reversible and highly selective inhibitor of the proteasomal ubiquitin receptor rpn13 is toxic to multiple myeloma cells. *Journal of the American Chemical Society 137*, 6312-6319 (**2015**).

25th American Peptide Symposium

The Vincent du Vigneaud Award*

The Vincent du Vigneaud Awards recognize outstanding achievement in peptide research at mid-career.

2017	**Roland T. Raines,** University of Wisconsin-Madison
	Wilfred van der Donk, University of Ilinois at Urbana-Champaign
2015	**Jean Chmielewski,** Purdue University
	David Craik, University of Queensland
2013	**Michael Chorev,** Harvard Medical School
	Kit S. Lam, University of California Davis Cancer Center
2011	**Fernando Albericio,** University of Barcelona
	Morten P. Meldal, Carlsberg Laboratory, Copenhagen
2010	**Philip Dawson,** The Scripps Research Institute
	Reza Ghadiri, The Scripps Research Institute
2008	**Tom W. Muir,** Rockefeller University
	Jeffery W. Kelly, The Scripps Research Institute
2006	**Samuel H. Gellman,** University of Wisconsin-Madison
	Barbara Imperiali, Massachusetts Institute of Technology
2004	**Steven B. H. Kent,** University of Chicago
	Dieter Seebach, ETH Zurich
2002	**Robert S. Hodges,** University of Colorado-Denver
	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.

25th American Peptide Symposium

The Vincent du Vigneaud Award*

Ronald T. Raines

Ronald T. Raines is the Henry Lardy Professor of Biochemistry, Linus Pauling Professor of Chemical Biology, and Professor of Chemistry at the University of Wisconsin–Madison. His research is focused on the chemical basis for protein structure and function. His efforts have revealed that unappreciated forces — the *n*-to-*pi** interaction and C5 hydrogen bond — stabilize all proteins, created hyperstable and human-scale synthetic collagens, led to an RNA-cleaving enzyme that is in a multi-site human clinical trial as an anti-cancer agent, and established chemical processes to synthesize proteins, catalyze their folding, and facilitate their entry into human cells, and to convert crude biomass into useful fuels and chemicals.

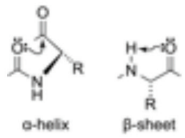
Dr. Raines received Sc.B. degrees in chemistry and biology from the Massachusetts Institute of Technology, and A.M. and Ph.D. degrees in chemistry from Harvard University under the direction of Jeremy Knowles. After studying biochemistry and biophysics as a Helen Hay Whitney postdoctoral fellow with William Rutter at the University of California, San Francisco, he joined the faculty at Wisconsin. In summer 2017, he will return to the Massachusetts Institute of Technology as the Firmenich Professor of Chemistry.

Dr. Raines has received the Pfizer Award in Enzyme Chemistry, Arthur C. Cope Scholar Award, Repligen Corporation Award in the Chemistry of Biological Processes, and Hirschmann Award in Peptide Chemistry from the American Chemical Society; the Jeremy Knowles Award from the Royal Society of Chemistry; the Emil Thomas Kaiser Award from the Protein Society; the Rao Makineni Lectureship and Vincent du Vigneaud Award from the American Peptide Society; and a Humboldt Research Award. Dr. Raines is a fellow of the J. S. Guggenheim Memorial Foundation, American Association for the Advancement of Science, Royal Society of Chemistry, and National Academy of Inventors. He has published over 300 peer-reviewed journal articles, delivered over 300 invited lectures, and supervised over 50 doctoral theses. He holds over 50 U.S. patents, and founded Quintessence Biosciences, Inc. and Hyrax Energy, Inc., which are developing inventions from his laboratory.

Chemical Forces that Stabilize Proteins

R. T. Raines
Department of Biochemistry and Department of Chemistry, University of Wisconsin–Madison, Madison, Wisconsin 53706, USA

To manifest its biological activity, the amino-acid chain of a protein must fold into a particular three-dimensional structure. In the α-helices and β-sheets that dominate protein structure, a lone pair of electrons on the oxygen atom in peptide bonds accepts a hydrogen bond. Using peptidic model systems, we discovered that the other electron pair on that oxygen atom also participates in meaningful interactions in both of these architectural elements. In an α-helix, this interaction is an O•••C=O n→π* interaction with the next carbonyl group in the main chain.[1] In a β-sheet, this interaction is an O•••H–N hydrogen bond within the residue.[2] Both of these interactions entail the formation of 5-membered rings and involve significant overlap of non-bonding and anti-bonding orbitals. Whereas the canonical hydrogen bonds engage the *s* orbital of the oxygen, the n→π* interaction and "C5" hydrogen bond engage a *p* orbital that is orthogonal to the C=O bond. These latter interactions, which are enhanced by the orbital demixing that accompanies canonical hydrogen-bond formation, have measurable effects on protein structure and implications for biology.



1. R. W. Newberry, R. T. Raines, *Accounts of Chemical Research,* **2017**, In Press.
2. R. W. Newberry, R. T. Raines, *Nature Chemical Biology,* **2016**, *12*, 1084–1088.

25th American Peptide Symposium

The Vincent du Vigneaud Award*

Wilfred van der Donk

Wilfred van der Donk was born in the Netherlands and received his B.S. and M.S. from Leiden University. He moved to the USA in 1989 to pursue his Ph.D. under Kevin Burgess at Rice University. After postdoctoral work at MIT with JoAnne Stubbe as a Jane Coffin Child fellow, he joined the faculty at the University of Illinois in 1997, where he currently holds the Richard E. Heckert Chair in Chemistry. Since 2008, he is an Investigator of the Howard Hughes Medical Institute.

Research in his laboratory focuses on using peptide chemistry, enzymology and molecular biology to gain a better understanding of the mechanisms of enzyme catalysis. His group is also exploring the utility of enzymes for synthetic purposes. Of particular interests have been enzymatic reactions in the biosynthesis of peptide antibiotics.

His work has been recognized by a number of awards including an Alfred P. Sloan Fellowship (2001), Camille Dreyfus Teacher-Scholar Award (2002), ACS Pfizer Award (2004), ACS Cope Scholar Award (2006), the Jeremy Knowles Award of the Royal Society of Chemistry (2010), and the Emil Thomas Kaiser Award of the Protein Society (2013). He is a fellow of the Royal Society of Chemistry, the American Academy of Microbiology, and the American Academy of Arts and Sciences.

Combinatorial Lanthipeptide Biosynthesis

Wilfred van der Donk
Department of Chemistry and Howard Hughes Medical Institute University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801, USA

Ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a very large class of peptide natural products.[1] These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Lanthionine-containing peptides (lanthipeptides) are examples of this growing class and many members are highly effective peptide-derived antimicrobial agents that display nanomolar minimal inhibitory concentrations (MICs) against pathogenic bacteria (termed lantibiotics). These peptides are post-translationally modified to install multiple thioether crosslinks. During their biosynthesis, a single enzyme typically breaks 8-16 chemical bonds and forms 6-10 new bonds with high control over regio- and chemoselectivity.[2] This presentation will discuss investigations of the mechanisms of these remarkable catalysts as well as their use for the generation of non-natural cyclic peptides.

1. M.A. Ortega, W.A. van der Donk, *Cell Chemical Biology,* **2016**, *23*, 31-44.
2. L.M. Repka, J.R. Chekan, S.K. Nair, W.A. van der Donk, *Chemical Reviews,* **2017**, DOI: 10.1021/acs.chemrev.6b00591.

25th American Peptide Symposium

The Murray Goodman Scientific Excellence and Mentorship Award*

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. The Awards Committee may also take into account any important contributions to the peptide science community made by the candidate, for example through leadership in the American Peptide Society and/or its journals

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)

25th American Peptide Symposium

The Murray Goodman Scientific Excellence and Mentorship Award

Paul Alewood

Paul Alewood is a Group Leader and Professor of chemistry at the Institute for Molecular Bioscience (IMB), the University of Queensland. He obtained his PhD in Organic Chemistry at the University of Calgary (Canada) and undertook postdoctoral studies at the Universities of Geneva, London and Melbourne before taking up a lectureship at the Victorian College of Pharmacy in 1985. He was a foundation staff member at Bond University (Queensland) before moving to the University of Queensland in 1990 and helped form the IMB in 2000. In 2015, he was granted a Principal Research Fellowship by the National Health and Medical Research Council, Australia.

His research encompasses the broad fields of peptide, protein and medicinal chemistry with major interests in the development of novel chemistry to modulate structure and function of cysteine-rich bioactive peptides, the design and synthesis of new peptide drugs, peptidomimetics and proteomics. Current research targets involve the discovery of novel toxins from Australia's venomous creatures, the design of mediators of neuropathic pain and ion channel therapeutics.

He was a co-founder of the Melbourne-based peptide company, Auspep, and Xenome, a spin-off biopharmaceutical company from the IMB (UQ). Through commercial partners AMRAD and Xenome, AM336 and Xen2174 entered the clinic for the treatment of neuropathic pain. He co-founded the Australian Peptide Society in 1990 and is the current co-chair. He is the chairman and founder of the Venoms to Drugs Symposium. He is author of over 300 journal articles plus 14 patents and has trained more than 50 postgraduate students

Venoms to Drugs

Paul Alewood
Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, University of Queensland, Brisbane 4072 Australia;
Email: p.alewood@imb.uq.edu.au

Many organisms including snakes, spiders, scorpions, cone snails, anemones and some mammalian species have evolved venom as either a defence mechanism or a weapon for prey capture[1]. These venoms typically contain a complex cocktail of bioactive disulfide-bond rich polypeptides called toxins that target a wide range of receptors including enzymes, ion channels, GPCRs and transporters. Of interest to drug designers is their high potency and selectivity combined with their resistance to many proteases. Their high potency and exquisite selectivity for ion channels and receptors has led to several drug candidates undergoing preclinical and clinical trials.

Of particular interest are venoms from the *Conidae*[2,3], with smaller polypeptide chains of 10-40 amino acids that are highly constrained by one to five disulfide bridges and are structurally well defined. Their high potency and *exquisite selectivity* for ion channels and receptors has led to two drug candidates[4,5] from our laboratories.

In this presentation I will outline our program of discovery, describe the amazing diversity of molecular structures being discovered and regioselective chemistry that facilitates the replacement of disulfide bonds by diselenide[6], thioether and selenoether[7] bonds. This has led to mimetics that have similar or improved potency to the native molecule plus exceptional stability when exposed to reducing environments and in plasma. Together, these results underpin the development of more stable and potent peptide mimetics suitable for new drug therapies, and highlight the application of this technology more broadly to disulfide-bonded peptides and proteins.

1. Sébastien Dutertre, Ai-Hua Jin, Irina Vetter, Brett Hamilton, Kartik Sunagar, Vincent Lavergne, Valentin Dutertre, Bryan Fry, Agostinho Antunes, Paul F. Alewood and Richard J. Lewis. *Nature Communications 5*:3521, **2014**.
2. Akondi KB, Muttenthaler M, Dutertre S, Kaas Q, Craik DJ, Lewis RJ, Alewood PF (**2014***). Chemical Reviews 114* (11) 5815.
3. Vincent Lavergne, Ivon Harliwong, Alun Jones, David Miller, Ryan J Taft, Paul F Alewood. Proceedings of the National Academy of Sciences (USA). 112 (29) E3782-E3791, **2015**.
4. RL Lewis, D Adams, I.Sharpe, M Loughnan, T Bond, L Thomas, A. Jones, J Matheson, R Drinkwater, K Nielsen, DJ Craik and PF Alewood (**2000**). *J Biol Chem, 275*(45) 35335.
5. I Sharpe, J Gehrmann, M Loughnan, L Thomas, D Adams A Atkins, DJ Craik, D Adams PF Alewood and RJ Lewis (**2001**). *Nature Neuroscience, 4*(9) 902.
6. M. Muttenthaler, S. T. Nevin, A. A. Grishin, S. T. Ngo, P. T. Choy, N. L. Daly, S-H. Hu, C. J. Armishaw, C. I. A. Wang, R. J. Lewis, J. L. Martin, P. G. Noakes, D. J. Craik, D. J. Adams, P. F. Alewood. J*ournal of the American Chemistry Society, 132* (10) 3514-3522, **2010**.
7. Aline Dantas de Araujo, Mehdi Mobli, Stuart M. Brierley, Joel Castro, Andrea M. Harrington, Irina Vetter, Zoltan Dekan, Markus Muttenthaler, Jingjing Wan, Richard J. Lewis, Glenn F. King and Paul F. Alewood. *Nature Communications 5*, 3165, **2014**.

25th American Peptide Symposium

Young Investigator Poster Competition

Sunandha Acharya, University of Rhode Island
Ahsanullah Ahsanullah, University of Montreal
Misao Akishiba, Kyoto University
Jordan Anderson, University of Washington
Alireza Bakhtiary, University of Alberta
Christian Bartling, University of Copenhagen
Jean-Louis Beaudeau, Université de Sherbrooke
Francois Bedard, Laval University
Michael Bird, The Scripps Research Institute
Reena Blade, Purdue University
Eva Brichtova, Institute of Organic Chemistry and Biochemistry of the CAS
Alba Casas Mora, CNRS, Centre de Biophysique Moléculaire
Vida Castro, Barcelona Science Park
Carmine Pasquale Cerrato, Stockholm University
Ramesh Chingle, Université de Montréal
Sorina Chiorean, University of Alberta
Philip Cistrone, The Scripps Research Institute
Christian Comeau, Université de Sherbrooke
Timothy Craven, University of Washington
Bobo Dang, University of California, San Francisco
Aline Dantas de Araujo, University of Queensland
Stepan Denisov, Maastricht university
Vahid Dianati, Universite de Sherbrooke
Rachael Dickman, University College London
Maria Disotuar, University of Utah
Tejaswi Dittakavi, Philadelphia College of Osteopathic Medicine
Antoine Douchez, Université de Montréal
Luke Dowman, The University of Sydney
Hader Elashal, Seton Hall University
Yassin Elbatrawi, University of South Florida
Patrick Erickson, University of Utah School of Medicine
Anna Escolà-Jané, Institute for Research in Biomedicine (IRB Barcelona)
Jan-Patrick Fischer, Leipzig University
Milan Fowkes, University of Oxford
Georgina Girt, University of Leicester
Bárbara Gomes, Instituto de Medicina Molecular
Andrew Gregory, Mayo Clinic Health System
Astha Gupta, Indian Institute of Technology Kanpur
Cameron Hanna, University of Sydney
Laura Hanold, University of Florida
Timothy Hill, University of Queensland
Karlijn Hollanders, Vrije Universiteit Brussel
Hanieh Hossein-Nejad-Ariani, Chapman University
Parisa Hosseinzadeh, University of Washington
Matthew Hostetler, Purdue University
Nataly Huertas Méndez, National University of Colombia
Susanne Huhmann, Freie Universität Berlin
Yahya Jad, University of KwaZulu-Natal
Josh Jesin, University of Toronto
Sangram Kale, Ecole polytechnique fédérale de Lausanne
Sergej Karel, Contipro a.s.
Alexander Kasznel, University of Pennsylvania
Hanna Kim, Philadelphia College of Osteopathic Medicine
Tove Kivijärvi, Royal Institute of Technology (KTH)
Takuya Kobayakawa, Tokyo Medical and Dental University
Sunbum Kwon, University of Bordeaux
Priyanka Lahiri, Indian Institute of Science
Tyler Lalonde, Western University
Kelsey Lamb, University of North Carolina, Chapel Hill
Tõnis Lehto, Stockholm University
Xiaocen Li, University of California, Davis
Yen-Chu Lin, A*STAR (Agency for Science, Technology and Research), Singapore
Alfredo López, University of Guanajuato
Rumit Maini, The University of Tokyo
Charlotte Martin, Vrije Universiteit Brussel
Helena Martin, Institute for Research in Biomedicine (IRB Barcelona)
Kaveh Matinkhoo, The University of British Columbia
Anahi McIntyre, Philadelphia College of Osteopathic Medicine
Barbora Mikulaskova, Institude of Organic Chemistry and Biochemistry AS CR, v.v.i.
Stephen Miller, National Cancer Institute
Kenta Mine, Faculty of Science, Hokkaido University
Chloe Mitchell, The Hospital for Sick Children
Christine Mona, University of California, Los Angeles
Takuya Morisaki, Tokushima University
Fabricio Mosquera Guagua, University of Alberta
Vikram Mulligan, University of Washington
Natsumi Nakagawa, Hokkaido University
Monessha Nambiar, Purdue University
Naoto Naruse, Tokushima University
Matthew Naylor, University of California, Santa Cruz
Vera Neves, Instituto de Medicina Molecular
Daniel Nielsen, University of Copenhagen
Roshan Xavier Norman, Indian Institute of Science
Justin Northrup, Temple University
Richard Obexer, The University of Tokyo

Paola Ojeda, University of Talca
Judith Pala-Pujadas, Institute for Research in Biomedicine Barcelona
Melek Parlak, Middle East Technical University
Aagam Patel, University of British Columbia
Hector Manuel Pineda Castañeda, Universidad Nacional de Colombia
Alla Pryyma, University of British Columbia
Tatiana Radchenko, University Pompeu Fabra, PRBB
Danielle Raymond, University of Rochester
Timothy Reichart, École polytechnique fédérale de Lausanne
Michael Remesic, University of Arizona
Cedric Rentier, Tokyo University of Pharmacy and Life Sciences
Jan Reutzel, Philipps-Universität Marburg
Gaston Richelle, University of Amsterdam
Andrea Veronica Rodriguez Mayor, Universidad Nacional de Colombia
Jorge Rodriguez, Universidad Nacional de Colombia
Joseph Rogers, University of Tokyo
Soledad Saavedra, Universidad de Buenos Aires
Norival Santos-Filho, Universidade Estadual Paulista
Matthew Sarnowski, University of South Florida
Daisuke Sato, Kyushu Institute of Technology
Jessica Sayers, University of Sydney
Vita Sereikaite, University of Copenhagen
Azar Shamloo, Universite de Sherbrooke
Anthony Silvestri, The Scripps Research Institute
Mike Smeenk, Radboud University
Mason Smith, Brigham Young University
Marc Sousbie, Université de Sherbrooke
Takuma Sueoka, The University of Tokyo
Toshiki Takei, Osaka University
George Tetley, University of Cambridge
Thibaut Thery, University College Cork
Mihajlo Todorovic, University of British Columbia
Kien Tran, Institut de Pharmacologie, Université de Sherbrooke
Phuong Dung Tran, University of Tsukuba
Kohei Tsuji, National Institutes of Health
Gerbrand van der Heden van Noort, Leiden University Medical Centre
Hitesh Verma, Indian Institute of Science
Simon Vézina-Dawod, Université Laval
Kerstin Wallraven, Vrije Universiteit Amsterdam
Louise Walport, University of Tokyo
Xiaoyi Wang, The University of Sydney
Jade Welch, University of Rochester
Samuel Whedon, University of Washington
Jessica Wickware, University of Alberta
Mareike Wiedmann, University of Tokyo
Dennis Worm, University Leipzig
Chuanliu Wu, Xiamen University
Haifan Wu, University of California, San Francisco
Yuji Yamada, National Cancer Institute-Frederick
Masafumi Yanase, The University of Tokyo
Ali Yousif, University of Texas at Dallas
Yang Zhou, University of Arizona

APS 2017 Student Activities Committee (SAC)

Philip Cistrone – The Scripps Research Institute
Maria Disotuar – University of Utah
Hader Elashal – Seton Hall University
Laura Hanold – University of Florida
Stephen Miller – National Cancer Institute
Monessha Nambiar – Purdue University
Kerstin Wallravenm – Vrije Universiteit Amsterdam

Wendy Hartsock, Ferring Research Institute, Inc.
SAC Co-Chair

Eileen Kennedy, University of Georgia, College of Pharmacy
SAC Co-Chair

Katelyn Smith, Merck & Co., Inc.
SAC Co-Chair

25th American Peptide Symposium

Travel Reward Recipients

APS Travel Awards

Jordan Anderson..... University of Washington
Solomon Appavoo ..... University of Toronto
Christian Bartling ..... University of Copenhagen
Jean-Louis Beaudeau..... Universite de Sherbrooke
Alba Casas-Mora..... CBM/CNRS
Vida Castro ..... Barcelona Science Park
Camine Cerrato..... Stockholm University
Ramesh Chingle..... Universite de Montreal
Stepan Denisov..... Maastricht University
Vahid Dianati ..... Universite de Sherbrooke
Maria Disotuar ..... University of Utah
Antoine Douchez..... Universite de Montreal
Luke Dowman..... University of Sydney
Hader Elashal ..... Seton Hall University
Anna Escola-Jane ..... Institute for Research in
..... Biomedicine of Barcelona (IRB
..... Barcelona)
Georgina Girt..... University of Leicester
Barbara Gomes..... Instituto de Medicina Molecular
Jerzy Gora..... Wroclaw University of Science
..... and Technology
Cameron Hanna..... University of Sydney
Matthew Hoestetler ..... Purdue University
Karlin Hollanders ..... Universiteit Brussel
Susanne Huhmann..... Freie Universität Berlin
Muhammad Jbara..... Technion-Israel Institute of
..... Technology
Sarabjit Kaur..... Panjab University
Kelsey Lamb..... University of North Carolina
Tonis Lehto ..... Stockholm University
Lara Malins..... Scripps Research Institute
Helena Martin..... Institute for Research in
..... Biomedicine of Barcelona (IRB
..... Barcelona)
Dominic McBrayer ..... University of Nevada, Reno
Barbora Mikulaskova..... Institute of Organic Chemistry
..... and Biochemistry
Chloe Mitchell..... Hospital for Sick Children
Monessha Nambiar..... Purdue University
Paola Ojeda ..... University of Talca
Judith Pala-Pujadas ..... Institute for Research in
..... Biomedicine of Barcelona (IRB
..... Barcelona)
Michael Remesic ..... University of Arizona
Soledad Saavedra ..... Instituto de Nanobiotecnología
..... UBA-CONICET
Norival Santo-Filho..... Sao Paulo State University
Matthew Sarnowski ..... University of South Florida
George Tetley..... University of Cambridge
Kohei Tsuji..... National Cancer Institute
Hitesh Verma..... Indian Institute of Science
Kerstin Wallraven ..... Vrije Universiteit
Jade Welch..... University of Rochester
Samuel Whedon..... University of Washington
Jonas Wilbs..... EPFL
Haifan Wu..... University of California,
..... San Francisco
Yang Zhou ..... University of Arizona

ACS Combinatorial Science Travel Award

Danielle Raymond..... University of Rochester

BC Bioconjugate Chemistry Travel Award

Tyler Lalonde ..... Western University

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.

SESSION ONE

Set-up:

Sunday	08:00 am

Posters will be on display during the hours the Exhibits are open.

Session One Posters defend

Monday	05:35 pm – 08:00 pm

Posters must be removed at 8:00pm

SESSION TWO

Set-up:

Tuesday	08:00 am

Posters will be on display during the hours the Exhibits are open.

Session Two Posters defend

Wednesday	05:35 pm – 08:00 pm

Posters must be removed at 8:00pm

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

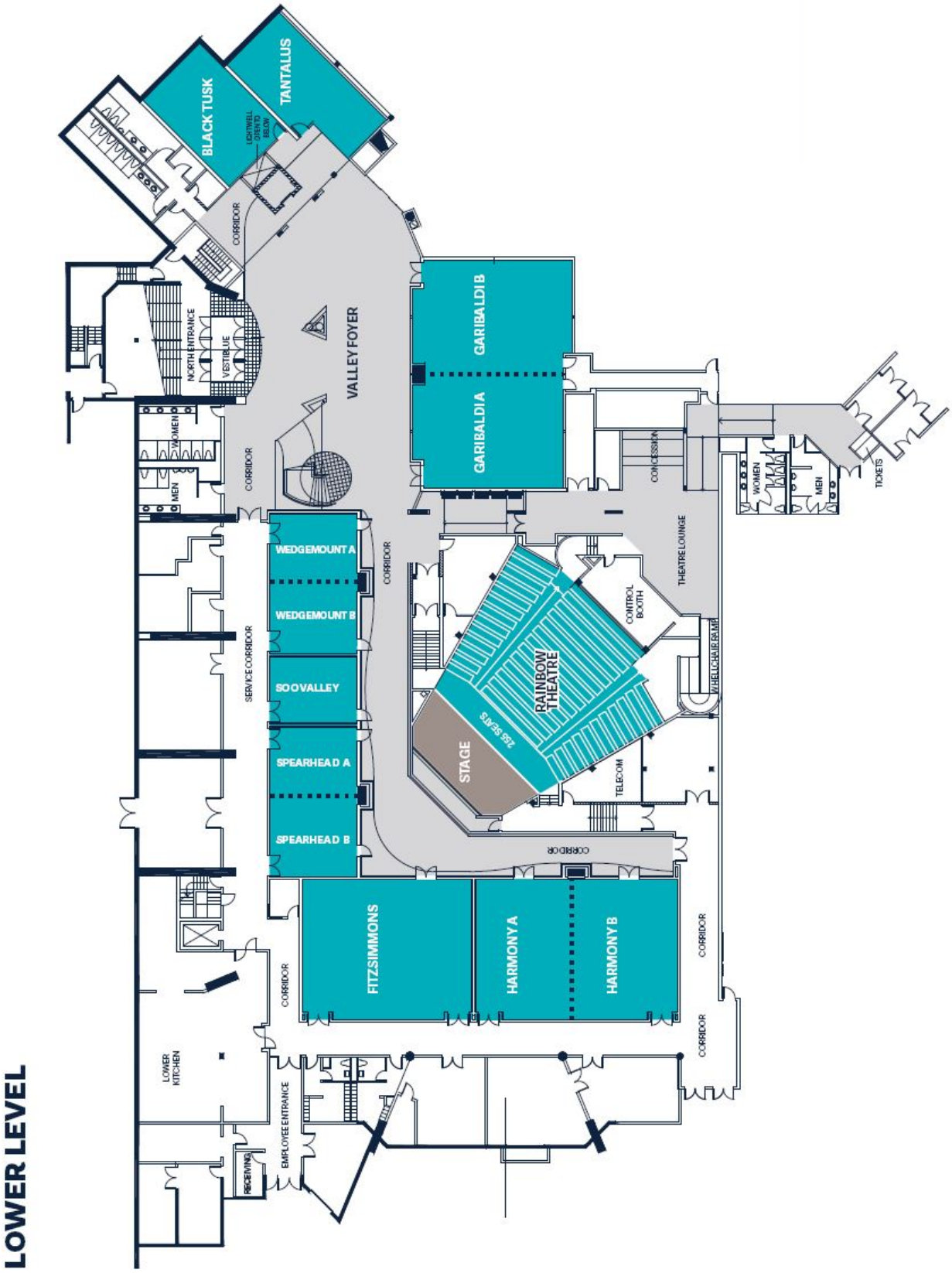
NAME BADGES

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.

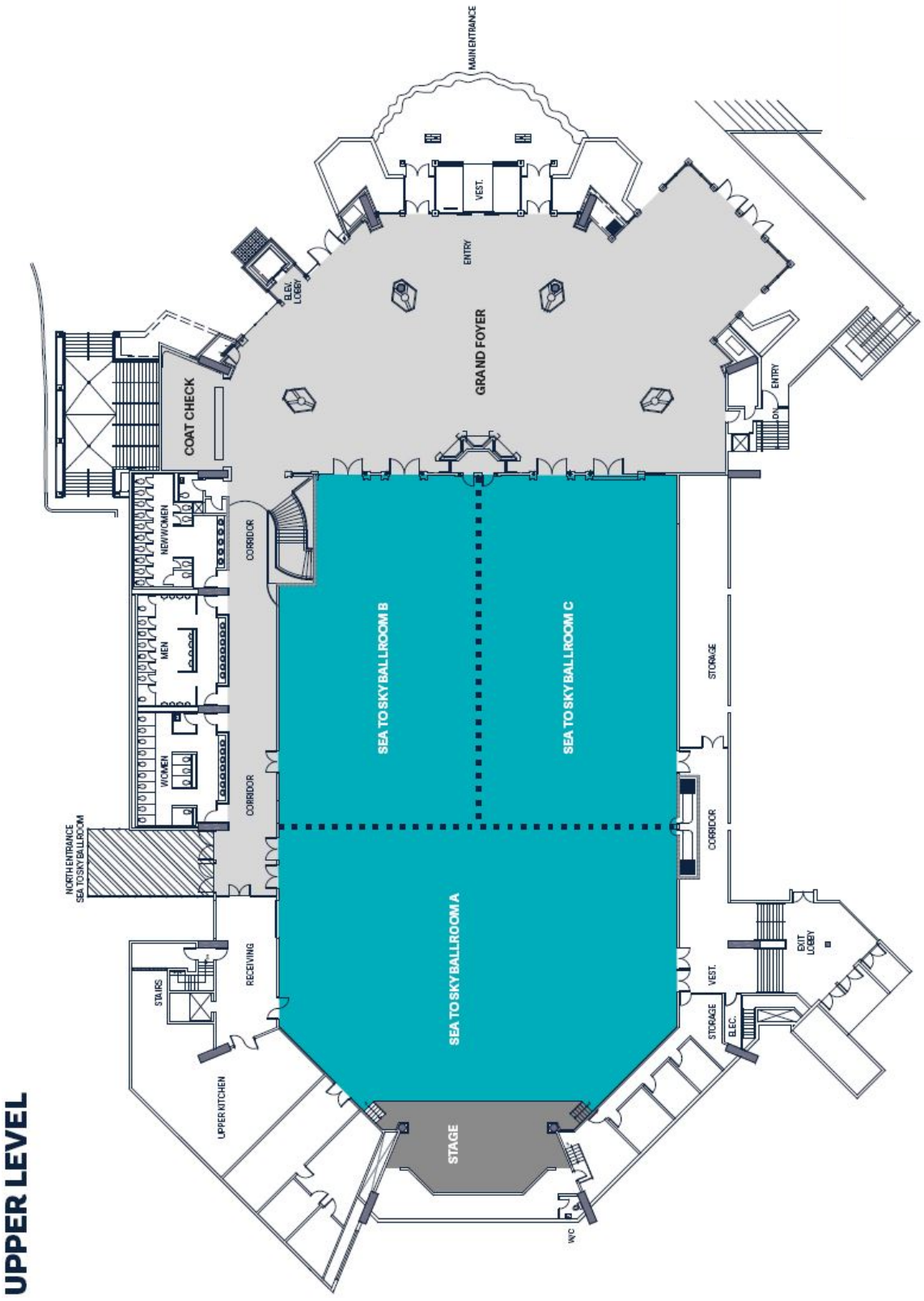
INTERNET ACCESS

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

CONFERENCE CENTER LOWER FLOOR MAP



CONFERENCE CENTER UPPER FLOOR MAP



Treetop Course

RZR 4x4

LECTURE ABSTRACTS

L01 De Novo Peptide Design

David Baker
University of Washington

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 based on Anfinsen's principle that proteins fold to their global free energy minimum. We compute amino acid sequences predicted to fold into proteins with new structures and functions, produce synthetic genes encoding these sequences or synthesize them chemically, and characterize them experimentally. I will focus on the recent application of these de novo design methods to peptides of less than 40 amino acids, including 7-14 residue cyclic peptides.

L02 Title: Peptide Probes of Epigenetic Demethylation Complex Protein-Protein Interactions

Dewey McCafferty, Jennifer Schwabe
Duke University

Lysine-specific demethylase 1 (LSD1/KDM1A) has been implicated as a regulator of breast cancer by its interaction with the estrogen receptor α (ERα). In this context, LSD1 is known to facilitate both gene activation and repression, depending on partnership with transcriptional factors and co-regulatory proteins. Given the intrinsic limitation of pan-selective, small molecules LSD1 inhibitors to distinguish between these roles, we report the development of first-generation protein-protein interaction inhibitors of LSD1 to explore the underlying mechanism governing communication between estrogen receptor-dependent signaling and recruitment of REST complex members and its impact on LSD1 recruitment, specificity, and impact on transcriptional events in breast cancers.

L03 Modulation of the Primary and Secondary Structure of Apelin to Optimize Stability, Bias Signalling and Modulate Pathophysiological Response

Éric Marsault
Institut de Pharmacologie de Sherbrooke, Université de Sherbrooke, Sherbrooke (Québec), Canada

Apelin is the endogenous ligand of the APJ receptor, a class A G protein-coupled receptor. It recently emerged as a promising target for the treatment of various pathophysiological conditions, with a particular emphasis on regulation of fluid homeostasis, cardiac and vascular functions. In an effort to decipher the structure-activity relationship of apelin-13, we implemented discrete replacements with unnatural amino acids, as well as conformational changes with the help of macrocyclization. We next analyzed how these modifications impact binding, signaling profile and plasma stability. These works have led to low pM agonists of the APJ receptor. The latter provide a better understanding of the residues critical to bias signaling of the APJ receptor toward G protein-dependent and -independent pathways, as well as factors critical for plasma stability. Finally, we explored how these modifications impact vascular pressure and cardiac performance in rats. These new ligands represent very promising pharmacological tools to link the intracellular signaling signatures to desired physiological responses.

L04 Understanding and Engineering Bifunctional Wnzymes for Nonribosomal Peptide Dynthesis

Sylvie Garneau-Tsodikova
Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY, 40536-0596, USA

Nonribosomal peptides are natural products biosynthesized by multi-modular enzymatic assembly-lines comprised of domains performing various activities. Adenylating enzymes play a critical role in dictating the identity of building blocks to be incorporated in growing peptides during nonribosomal peptide biosynthesis. To increase the structural diversity of the products it generates, Nature has evolved unique interrupted adenylating enzymes capable of performing both adenylation and methylation reactions. We will present our efforts towards understanding the mechanism by which these unique enzymes function and our biochemical and structural work towards engineering novel interrupted enzymes with adenylating and methylating activities.

L05 Clarifying the Complexity of Chromatin with Peptide Chemistry

Abhinav Dhall, Caroline E. Weller, Elizabeth L. Tyson and Champak Chatterjee
Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195

Chromatin is a large nucleoprotein complex that archives an individual's genetic code. In response to diverse cellular cues, distinct regions of chromatin undergo structural rearrangement to facilitate gene transcription, replication, and repair. Histones are the principal protein component of chromatin and their post-translational modifications (PTMs) correlate with functionally distinct chromatin states. Elucidating biochemical relationships, or crosstalk, between different histone PTMs is a gateway toward understanding complex chromatin function.[1] However, a major challenge in studying crosstalk between histone PTMs lies in their dynamic nature and heterogeneous distribution in chromatin. Therefore, histone semisynthesis is an extremely powerful strategy that yields access to homogeneously modified histones in quantities sufficient for biophysical and biochemical studies. We have developed and applied several facile strategies to synthesize human histones uniformly modified with acetylation, methylation and by the small ubiquitin-like modifier (SUMO).[2,3] Results from our detailed biochemical investigations with "designer" chromatin assembled with these semisynthetic histones will be presented, and discussed in the broader context of chromatin regulation by SUMO.[4]

1. Dhall, A.; Chatterjee, C. "Chemical approaches to understand the language of histone modifications." *ACS Chem. Biol.* **2011**, *6*, 987–999.

2. Dhall, A.; Weller, C. E.; Chatterjee, C. "Rapid semisynthesis of acetylated and sumoylated histone analogs." *Methods Enzymol.* **2016**, *574*, 149–165.
3. Weller, C. E.; Dhall, A.; Ding, F.; Linares, E.; Whedon, S. D.; Senger, N. A.; Tyson, E. A.; Bagert, J. D.; Li, X.; Augusto, O.; Chatterjee, C. "Aromatic thiol-mediated cleavage of N-O bonds enables chemical ubiquitylation of folded proteins." *Nat. Commun.* **2016**, *7*, 12979.
4. Dhall, A.; Wei, S.; Fierz, B.; Woodcock, C.; Lee, T.-H.; Chatterjee, C. "Sumoylated human histone H4 prevents chromatin compaction by inhibiting long-range internucleosomal interactions." *J. Biol. Chem.* **2014**, *289*, 33827–33837.

L06 Peptide Ligases: Site-specific Molecular Staplers

James P. Tam
Nanyang Technological University, School of Biological Sciences, 60 Nanyang Drive, Singapore 637551

Proteases are ubiquitous whereas peptide ligases, enzymes catalyzing the reverse reactions of proteases, are exceedingly rare. Thus far, only six stand-alone and ATP-independent ligases have been characterized as compared to >4200 proteases. But peptide ligases are enormously useful because they are molecular staplers which enable site-specific bonding of chemicals, polymers, peptides and proteins to form new compounds. Recently, we discovered such a "molecular stapler", a novel Asn/Asp (Asx)-specific peptide ligase named butelase 1 from butterfly pea (Bunga Telang). Butelase 1 exhibits unmatched kinetics with catalytic efficiencies of up to 1,340,000 M^{-1} s^{-1} and >10,000 times faster than other known ligases[1,2]. Our recently published work showed that butelase 1 is useful for both intra- and intermolecular ligation, cyclizing or ligating efficiently various peptides and proteins ranging in size from 8 to >300 amino acids[2-6]. Importantly, butelase 1 is C-terminus-specific for Asx, traceless, and accepts a tripeptide Asx-His-Val with the dipeptide His-Val as the leaving group. Butelase 1 accepts most N-terminal amino acids with D- or L-configuration. Thus, the high catalytic efficiency and broad substrate specificity of butelase 1 could augment new applications, both *in vitro* and *in vivo* systems for basic and translational research. Here, we will present our latest results on Asx-specific ligases and their applications to explore new frontiers in biochemical, medical and material sciences.

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L07 Membrane Interactions and Cell Penetrating Properties of Cyclotides

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Naturally occurring[1,2] as well as designer$_3$ cyclic peptides offer great potential as leads for drug design or crop protection agents in agriculture. This talk will focus on one class of cyclic peptides known as cyclotides,[4] which are topologically unique in that they have a head-to-tail cyclized peptide backbone and a cystine knotted arrangement of three conserved disulfide bonds. This makes cyclotides exceptionally resistant to chemical, thermal or enzymatic degradation and, indeed, cyclotides are amongst nature's most stable proteins. They occur in plants from the Rubiaceae (coffee), Violaceae (violet), Solanaceae (nightshade), Fabaceae (legume) and Cucurbitaceae (cucumber) families of plants where their natural function is presumed to be in host defence.[5] This presentation will describe the membrane binding properties of cyclotides and how the delineation of these properties has assisted in the understanding of their natural defense functions.[6,7] In particular, solid phase synthesis has allowed us to make a range of modified cyclotides to probe structure-activity relationships and understand how they bind to membranes representative of those in the guts of insect pests. A cyclotide-containing product was recently approved for insect control in cotton and macadamia nut crops, marking the first commercial application of cyclotides in agriculture.

Acknowledgments: Work in our laboratory is supported by the Australian Research Council and the National Health & Medical Research Council

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L08 Solid-State NMR Spectroscopy Reveals the Allosteric Regulation of the Sarcoplasmic Reticulum Ca2+-ATPase by Phospholamban

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The membrane protein complex between the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and phospholamban (PLN) controls Ca^{2+} transport in cardiomyocytes, thereby modulating cardiac muscle contractility. PLN is phosphorylated upon β-adrenergic-stimulated phosphorylation and up-regulate the ATPase via an unknown mechanism. Using solid-state NMR spectroscopy, we mapped the interactions between SERCA and PLN in membrane bilayers. We found that the allosteric regulation of the ATPase depends on the conformational equilibria of this endogenous regulator that maintain SERCA's apparent Ca^{2+} affinity within a physiological window. Here,

we present new regulatory model for PLN that represent a paradigm-shift for understanding SERCA function. Our data suggests new strategies for designing innovative therapeutic approaches to enhance cardiac muscle contractility.

YI-LO9 Structure-Guided Development of a Peptide Antagonist for EphA4, A Cell-Surface Receptor Linked to Neurodegeneration

Erika J. Olson[1], Bernhard C. Lechtenberg[2], Maricel Gomez Soler[2], Chunxia Zhao[2], Elena Rubio de la Torre[2], Ilaria Lamberto[2], Stefan J. Riedl[2], Elena B. Pasquale[2], and Philip E. Dawson[1]
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EphA4 is a cell-surface receptor tyrosine kinase primarily expressed in the central nervous system in regions of frequent remodeling such as the hippocampus and cerebral cortex. It is a member of a large family of transmembrane receptor tyrosine kinases that are critical for tissue patterning as well as neural and immune function. Acute neural trauma has been shown to increase expression levels of EphA4 in humans and disease severity correlates with increased EphA4 expression in humans afflicted by amyotrophic lateral sclerosis (ALS), while inhibition of EphA4 activation has been shown to slow neuronal death in mouse models of ALS and promote reinnervation in spinal cord injury models. Unfortunately, EphA4 cannot be inhibited by its natural ephrin ligands, as the ephrins show promiscuous binding within the family of Eph receptors. Several peptides identified by phage display were shown to bind selectively to the ligand-binding domain of EphA4 and competitively inhibit ligand binding, phosphorylation, and signalling pathway activation. Using crystallographic data to guide mutation to natural and non-natural amino acids, we have developed a peptide with IC_{50} of ~20 nM and high stability to serum proteases. We have found that this peptide inhibits the neuronal recession caused by EphA4 activation in primary culture neurons.

L10 Peptides as Alternatives to Antibiotics

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The inexorable increase in multidrug resistant infections combined with a decrease in new antibiotic discovery and a lack of compounds for chronic biofilm infections is creating a potential crisis in human medicine. Thus it is imperative to consider alternatives to conventional antibiotic strategies and particularly for infections that are recalcitrant to current therapies (e.g. sepsis and chronic biofilm infections).

Cationic host defence (antimicrobial) peptides are produced by virtually all organisms, ranging from plants and insects to humans, as a major part of their innate defences against infection. We and others have demonstrated that they are a key component of innate immunity and have multiple mechanisms that enable them to deal with infections and inflammation including an ability to favourably modulate the innate immune system, and distinct antibiotic and anti-biofilm activities.

We have defined a class of peptides that act against biofilms formed by multiple species of bacteria in a manner that is independent of activity vs. planktonic bacteria. We have now developed novel anti-biofilm peptides that (i) kill multiple species of bacteria in biofilms (MBEC <1 μg/ml), including the ESKAPE pathogens and other major clinically relevant Gram negative and Gram positive bacteria, including, (ii) work synergistically with antibiotics in multiple species, and (iii) are effective in animal models of biofilm and abscess infections. Structure activity relationships studies showed no major overlap between anti-biofilm and antimicrobial (vs. planktonic bacteria) activities, and indeed organisms completely resistant to antibiotic peptides were still able to be treated with anti-biofilm peptides. The action of such peptides is dependent on their ability to trigger the degradation of the nucleotide stress signal ppGpp.

The manipulation of natural innate immunity represents a new adjunctive therapeutic strategy against antibiotic-resistant infections. Cationic host defence peptides boost protective innate immunity while suppressing potentially harmful inflammation/sepsis, and work synergistically with conventional therapies. Using the principle of selective boosting of innate immunity we have developed novel small innate defence regulator (IDR) peptides with no direct antibacterial activity, that are nevertheless able to protect in animal models against many different microbial infections, including antibiotic resistant infection models against the superbug methicillin resistant *Staph aureus* (MRSA), *E. coli*, *P. aeruginosa*, MDR tuberculosis, as well as cerebral malaria and inflammatory diseases, providing a new concept in anti-infective therapy. Good activity in models of wound healing, pre-term birth and cystic fibrosis has also been achieved.

L11 A New, Quantitative Assay for Cytosolic Penetration for Peptides and Other Biomolecules

Joshua A. Kritzer
Department of Chemistry, Tufts University

Peptide, protein and nucleic acid therapeutics can't bind what they can't get to! However, it is challenging to compare the cytosolic penetration of these and other molecules. The most widely-used method for measuring cell penetration is conjugating molecules to fluorescent dyes, then tracking them using flow cytometry or high-content microscopy. However, these techniques are semi-quantitative at best, the dye can perturb the physicochemical properties of smaller biomolecules, and most importantly, these methods cannot completely rule out effects of material at the cell surface or trapped in endosomes. We have devised a chloroalkane penetration assay (CAPA) that exclusively measures penetration to the cytoplasm. CAPA uses a cell line with stably expressed Haloenzyme in the cytoplasm, and measures the extent of covalent reaction between this enzyme and a small chloroalkane tag appended to the molecule of interest. The CAPA readout is fast, inexpensive, and high-throughput, enabling the rapid determination of structure-activity relationships for extent of cell penetration, independent of the molecule's cellular phenotype. We are also producing CAPA cell lines for quantitating penetration into other compartments and organelles, and testing its compatibility with a wide range of bioactive molecules and drug delivery systems. With CAPA, we are making rapid progress in more specifically defining those features that promote cytosolic penetration for a variety of biomolecules.

L12 Protein-peptide Interactions that Control Apoptosis

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MIT Departments of Biology and Biological Engineering

Bcl-2 family proteins control apoptosis and are mis-regulated in many cancers and other diseases. Selective binding of short alpha helices that contain a Bcl-2 homology 3 (BH3) motif to pro- or anti-apoptotic members of the Bcl-2 family is critical for cellular life vs. death decisions. This important protein-protein interaction can be effectively mimicked using synthetic peptides. Peptides of ~23 residues with native BH3 sequences have utility for cell biology research and for diagnosing the molecular mechanisms of cancer cell survival. Short synthetic BH3-like peptides also have therapeutic potential. In my laboratory we have been exploring the landscape that describes how the sequences of helical 23-mer peptides encode specificity for one or more of five mammalian anti-apoptotic Bcl-2 paralogs. We use an integrated program that combines computational analysis, experimental screening, peptide chemistry, biophysical characterization and structure determination to discover mechanisms of binding specificity and design peptide reagents that can selectively target anti-apoptotic proteins important for cancer cell survival and chemotherapeutic resistance. I will describe our general approach along with specific examples drawn from our recent work on anti-apoptotic proteins Mcl-1 and Bfl-1.

L13 Application of N-Sulfanylethylanilide (SEAlide) Unit to Protein Chemical Synthesis and Protein Enrichment

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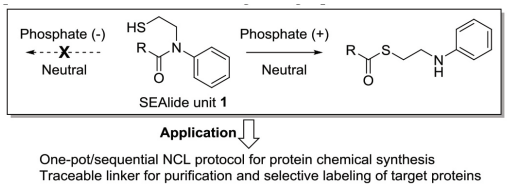
N-Sulfanylethylanilide (SEAlide) unit 1 has been developed as a chemical device for N–S acyl-transfer-mediated preparation of peptide thioesters. Although the amide-type SEAlide peptide was initially found to be converted to the corresponding thioester under acidic conditions, we recently discovered the fascinating feature that the amide-type SEAlide peptide is efficiently converted to the active thioester form in the presence of phosphate salts. The amide-type SEAlide unit remains inactive in the absence of phosphate salts, whereas the SEAlide unit functions as thioester in NCL protocol only in the presence of phosphate salts. Such features allow for the onepot/sequential NCL protocol with the use of N-terminal cysteinyl SEAlide peptide for chemical synthesis of proteins.[1] Furthermore, the SEAlide unit was applied to the development of a traceable linker for purification and selective labeling of target proteins.[2]



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L14 Rational Redesign of the Collagen Triple Helix Interface

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Collagen–the most abundant protein in mammals–has preoccupied chemists since the 1930s. Collagen is a self-assembled hierarchical peptide-based biomaterial that has found widespread use throughout history for applications ranging from modern day artificial skin to thermoplastic glues in ancient Egypt, nearly 4,000 years ago. Many debilitating diseases are defective collagen-based disorders ranging from scurvey caused by vitamin C deficiency to genetic maladies such as osteogenesis imperfecta, caused by amino acid substitutions of glycine. Glycine occupies nearly every third residue in the amino acid sequence of collagen and is crucial for maintaining strength and structural integrity. Short collagen mimetic peptides have been the topic of intense research efforts for use as new materials that mimic the higher order structure and function of natural collagen proteins and as probes for uncovering the fundamental biology and biochemistry of collagen. Recently, we reported an unnatural amino acid substitution for glycine that significantly stabilizes the triple helical structure of collagen peptides.[1-3] This simple substitution of a carbon for a nitrogen atom at the alpha position of glycine (aza-glycine) reprograms the hydrogen bonding interface of triple helical collagen peptides. The aza-glycine modification leads to triple helix hyperstability and faster folding while maintaining the natural surface topology recognized by proteins of the collagen interactome. Structural and biophysical studies of this new class of aza-amino acid containing collagen-mimetic peptide will be presented in addition to biological studies and future applications.

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L15 Linear and Cyclic Azapeptide Strategies for Conceiving Cluster of Differentiation-36 Scavenger Receptor Modulators

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The cluster of differentiation-36 scavenger receptor (CD36) plays roles in innate immunity, and pathologies such as atherosclerosis.[1] Among various ligands, CD36 binds oxidized low-density lipoproteins, thrombospondin and growth hormone releasing hormone-6 (GHRP-6, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2). Using semicarbazides as amino amide surrogates, we have pursued azapeptide GHRP-6 analogs to develop cardiovascular protective agents with therapeutic potential: e.g., [aza-Tyr[4]]-GHRP-6 has CD36 selectivity and anti-angiogenesis activity, likely because of its preferred turn conformation.[2] Targeting macrocyclic aza-GHRP-6 analogs to stabilize turn structure and enhance biological activity, we developed a novel route to cyclic peptides by a multiple component 'A^3-macrocyclization'.[3] Employing A^3-macrocyclization, aza-GHRP-6 analogs were obtained exhibiting unprecedented affinity for CD36, and capacity to modulate Toll-like receptor agonist-induced overproduction of nitric oxide, and to reduce

pro-inflammatory cytokine and chemokine production in macrophages. Recent research will be presented on the synthesis and biomedical applications of linear and cyclic azapeptide modulators of the CD36 receptor.

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L16 Bulky Dehydroamino Acids Impart Proteolytic Stability to Peptides

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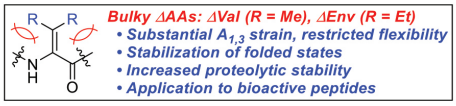

Although several strategies for stabilizing peptides to proteolysis have been devised, new approaches are required if an increase in the number of FDA-approved peptide drugs is to be realized. Bulky α,β-dehydroamino acids (bulky ΔAAs) such as ΔVal and ΔEnv have great potential to protect peptides from proteolytic degradation, as they rigidify backbones due to their high levels of A1,3 strain. However, synthetic challenges have precluded detailed investigations of the impact of bulky ΔAAs on peptide structure and stability. New methodology from our laboratory has now enabled such studies, and this presentation will describe our recent results in this area. We have shown that inclusion of ΔVal orΔEnv in the turn regions of modelβ-hairpins can substantially increase the stability of these peptides to degradation by Pronase, an aggressive mixture of nonspecific proteases. A summary of these findings and a discussion of the impact of bulkyΔAAs on β-turn structure will be presented. Extension of these studies to other secondary structures and to bioactive peptides will also be described. Ultimately, we envision a host of important future applications for ΔVal, ΔEnv, and other bulky ΔAAs as stabilizing components of peptides.

L17 The Use of Synthetic Peptides to Probe Complex Interactions in Proteins and the Development of Synthetic Peptide Drugs and Vaccines

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Using a synthetic peptide approach, we were able to unravel all the complex interactions during muscle contraction and relaxation in the presence and absence of Ca^{2+}. The interactions in thin filament involve actin and the regulatory proteins, tropomyosin (Tm) and the troponin complex (TnT, TnI and TnC). During my Ph.D., I sequenced the first two-stranded α-helical coiled-coil, Tm, to identify the hydrophobic repeat responsible for the formation and stabilization of the coiled-coil structure. This hydrophobic repeat of seven amino acid residues denoted $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$ was shown to be continuous throughout the entire 284-residue polypeptide chain of Tm where positions a and d were occupied by hydrophobic residues. Next, we designed *de novo* and synthesized the first model coiled-coil protein and demonstrated its utility for studying the folding and stability of α-helical proteins. I will discuss some of the highlights of our research on coiled-coils. Most recently, we addressed the question of how stability information is transmitted along a rod-like Tm coiled-coil which is critical to under-standing signaling and function of Tm during muscle contraction. Our understanding of coiled-coils led to developing a simple, innovative and robust technology to present α-helical epitopes from native proteins to the immune system, such that the resulting conformational specific antibodies bind to the native protein target. The helical sequence of interest is inserted into a parallel two-stranded α-helical coiled-coil and disulfide-bridged template which maintains the native conformation of the helical epitope. We are using this technology to generate a universal influenza A vaccine. Our understanding of α-helical structure led to our research on α-helical antimicrobial peptides (AMPs). We developed the concept of "specificity determinants" which, when inserted in the non-polar face of amphipathic α-helical AMPs with broad-spectrum activity dramatically changed their properties: 1) reduced or eliminated eukaryotic toxicity; 2) enhanced antimicrobial activity against prokaryotic cells; 3) encoded selectivity for Gram-negative pathogens by significantly decreasing/eliminating activity against Gram-positive pathogens; 4) maintained excellent antimicrobial activity in the presence of human serum by preventing high-affinity binding to serum proteins through the non-polar face.

L18 Peptides in Membranes: From Structure to Drug Design

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Through synthesis and conformational analysis of model membrane-interactive peptides, our lab has been contributing to a body of work directed toward defining fundamental features of sequence, hydrophobicity, and helix-helix interactions of membrane-spanning segments in proteins. As protein–protein interactions (PPIs) within membrane proteins are of great interest as therapeutic targets, we are currently applying the resulting knowledge of these factors to facilitate the design of PPI "disruptors" — peptide reagents that target membrane-embedded protein assembly motifs. In this context, we will address bacterial multidrug resistance, which originates largely from protein pumps embedded in the bacterial cell membranes that resist the toxic effects of drugs by efficiently extruding them. We will focus on the design, synthesis, and bioactivity of novel peptide-based inhibitors that compete for — and disrupt — functional helix-helix interactions in these proteins to produce efflux pump inhibitors with significant pharmacologic properties

L19 Engineering Protein Interactions in Type II Non-ribosomal Peptide Synthetases

Michael Burkart

University of California, San Diego

Type II non-ribosomal peptide synthetases (NRPSs) participate in hybrid biosynthetic pathways with fatty acid synthases, polyketide synthases, and type I NRPSs. These hybrid systems generate complex natural products that often demonstrate valuable bioactivities, and type II NRPSs typically modify

amino acids to generate unique precursors for downstream incorporation into a larger, more complex products such as oxidations, hydroxylations and chlorinations. In recent years we have developed a suite of tools to modify carrier proteins from these pathways with substrate analogs and reactive probes. These conjugates are used to study the structure of these proteins using NMR and x-ray crystallography. We have used these tools to capture both the dynamic and static interactions between carrier proteins and catalytic partners in a variety of biosynthetic systems to learn about the mechanisms of substrate binding and protein-protein interactions. These phenomena are key to understanding enzyme timing and processivity in these pathways and their understanding will be essential to future engineering efforts. Given their makeup of primarily stand-alone proteins, type II NRPSs are ideal targets for metabolic engineering efforts, however the ability to design even simple NRPS systems remains challenging. In an effort to elucidate and engineer adenylation (A)/peptidyl carrier protein (PCP) domain interactions in type II NRPS pathways, we analyzed the solution-phase NMR behaviors of proteins involved identical adenylation reactions from homologous pathways. Full solution-phase structural characterization and molecular dynamic simulations informed PCP mutagenesis engineering for gain of function. Here we demonstrate the successful engineering of a type II PCP with altered binding specificity towards homologous A domains and discovered an important recognition site in type II NRPS pathways.

L20 Cross-talk between Chromatin Recognition and Modification in Histone Demethylases

Danica Fujimori
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Post-translational modifications of histone proteins modulate chromatin structure and accessibility, and as a consequence regulate many nuclear processes. Lysine methylation is one of the most functionally diverse chromatin modifications. This modification has a critical regulatory role in a range of processes, such as heterochromatin formation and regulation of transcription. Both the position of the methylated lysine residue and the extent of lysine methylation (mono-, di-, or trimethylation) contribute to the functional effects by recruiting different effector proteins. Methylation is enzymatically controlled by opposing activities of histone methyltransferases and demethylases. Demethylases, a class of epigenetic erasers, are multidomain proteins that in addition to their catalytic domain also contain a series of chromatin reader domains. By recognizing specific chromatin marks, these reader domains both direct and regulate activity of demethylases. This talk will describe discovery and implications of several mechanisms that we recently uncovered by which chromatin reader domains regulate catalytic activities of histone demethylases.

YI-L21 Affecting Activity of the Linear Ubiquitin Chain Assembly Complex (LUBAC) with Stapled Alpha-helical Peptides

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The linear ubiquitin chain assembly complex (LUBAC) is the only E3 ubiquitin ligase known to generate linear polyubiquitin chains by linking ubiquitin units head-to-tail. LUBAC is composed of three proteins — HOIL-1L, HOIP and SHARPIN — of which the interaction between HOIL-1L and HOIP is critical for LUBAC assembly and function. One known substrate of LUBAC is the regulatory subunit NEMO (NF kappa B essential modulator), part of the IKK complex, which results in activation of NF-kB. We hypothesize that disruption of the HOIL-1L-HOIP interaction would prevent LUBAC ubiquitylation activity and result in down-regulation of NF-kB activity. The key protein-protein interaction that allows for LUBAC activity occurs between the ubiquitin-like (UBL) domain of HOIL-1L and the ubiquitin-associated (UBA) domain of HOIP. To study the effects of disrupting this complex, we synthesized a family of HOIP-based peptide inhibitors designed to mimic aspects of its unique bent alpha-helical interface and utilized "hydrocarbon stapling" to reinforce the alpha-helical structure. We determined HOIP peptide binding constants to HOIL-UBL using surface plasmon resonance (SPR), examined structural aspects of inhibitor binding via NMR spectroscopy, evaluated peptide ability to disrupt LUBAC activity using a recombinant HOIL-HOIP in vitro ubiquitination assay, and assessed peptide effect on NF-kB activity in B cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL) cells. Our findings continue to validate inhibition of LUBAC via disruption of the HOIL-1L-HOIP interaction as a potential therapeutic target against LUBAC-dependent cellular pathways, including ABC-DLBCL, the DLBCL subtype that is most resistant to current therapies.

L22 Genetically-Encoded Chemically-Modified Peptide

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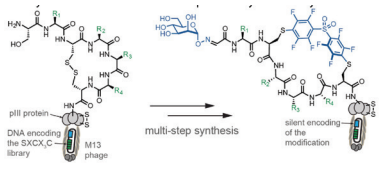
Genetically-encoded (GE) libraries of proteins and peptides are the major source of discovery of biological drugs and development of ligands. Selection of peptide and protein sequences from GE-libraries of billion-scale diversity is routine both in academia and industry. These techniques, however, have been limited to handling of structures made of 20 natural amino acids. Our group uses GE-libraries of peptides as a starting material for multi-step organic synthesis to produce GE-libraries of peptide derivatives. We developed the methodology for quantification of yield, purity and kinetics of reactions on phage-displayed peptide libraries.[2b] Examples are N-terminal conjugation[1] and cyclization of linear peptides2 with simultaneous introduction of glycan entities. These chemical modifications allowed us to develop Genetically-Encoded Fragment-Based Discovery (GE-FBD) platform,[3] which combines >10^8 peptide fragments with variable, silently-encoded modifications.[4] The talk will highlight the advances in application of GE-FBD platform to challenging targets such as carbohydrate binding proteins and disease-specific antibodies. I will also share new unpublished technologies we developed to accelerate discovery within any genetically encoded library framework and maximize the reproducibility of discovery.



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L23 Affinity Labeling of Peptides Containing Trimethyllysine

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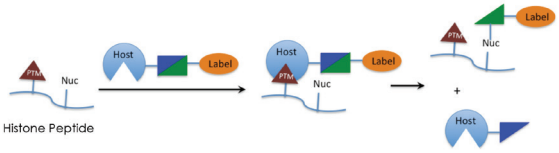

Lysine and arginine methylation are common post-translational modifications (PTMs) of histone proteins that regulate gene transcription. Moreover, dysregulation of these PTMs has been implicated in many types of cancer. However, detection and characterization of these PTMs is still a challenge. While antibodies are available for detection of these PTMs at specific sites, pan-antibodies have not been very effective. Mass spectrometry is also commonly used to detect these PTMs but this is labor intensive and low throughput. We aimed to develop a method to rapidly label histone tail peptides containing methylated lysine. Affinity labeling is a wellestablished approach to labeling proteins that bind to a ligand of interest. Our group has turned this approach on its head by using a synthetic receptor to bind a peptide "ligand" containing trimethyllysine and covalently label it, resulting in turn-on fluorescence for detection of lysine methylation. We have demonstrated the feasibility of this approach applied to characterization of enzyme activity. Details of this work will be described.

L24 New Coumarin- and Nitrodibenzofuran-Based Photoremovable Protecting Groups for Cysteine Protection in Solid Phase Peptide Synthesis

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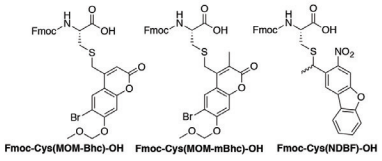
Photoremovable protecting groups are useful for a wide range of applications in peptide chemistry. In recent years, we have explored the Bhc protecting group that can be used to mask the thiol group of cysteine. While irradiation of Bhc-protected thiols can lead to C-S bond cleavage and the liberation of free thiols, it also frequently leads to the production of isomeric compounds where the thiol has migrated onto the aryl ring that are not useful. To address this problem, we have introduced two new thiol protecting groups that solve this problem and generate free thiols in high yield. In addition to being cleavable with UV light, they can also be removed with visible light via two-photon excitation. Forms of Fmoc-protected cysteine have been prepared and used to synthesize a number of peptides and peptidomimetics whose biological properties can be triggered with light. The synthesis of these materials, cleavage characteristics and biological activities will be presented. This includes their use as light-activated anti-cancer drugs.



L25 Using Theory and Computation to Design Polypeptide Materials

Jeffery Saven
University of Pennsylvania

Peptides and proteins provide can potentially form well-structured, highly functional biomaterials, but protein and macromolecular design often require the engineering of noncovalent as well as covalent interactions. Theoretical and computational methods can identify the properties of amino acid sequences consistent with desired structures and functions. Such methods leverage concepts from statistical mechanics and address the structural complexity of proteins and their many possible amino acid sequences. Computationally designed peptide-based systems can be used to realize prespecified, self-assembling biomolecular materials.

L26 Tertiary Alphabet for the Observable Structural Universe

C. O. Mackenzie[a], F. Zheng[b], J. Zhou[c], G. Grigoryan[a,b,c]
[a]QBS program; [b]Department of Computer Science; [c]Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

Understanding how amino-acid sequence encodes protein structure is a grand challenge of modern biophysics. Chief among the difficulties of describing this sequence-structure mapping is that the space of structural possibilities is immense and complex. We propose that this space should nevertheless be describable as a combination of discrete local structural patterns. We introduce the concept of a TERM (tertiary motif), which encapsulates the full structural environment around a given residue, and show that the protein structural universe is highly degenerate at the level of TERMs. In fact, only 650 TERMs describe over 50% of the structural database at sub-Angstrom resolution. We go on to show that such degeneracy enables the direct quantification of sequence-structure relationships. Local sequence models can be extracted for each TERM contained in a protein structure, based on the many occurrences of the TERM in unrelated proteins, with the overall protein structure described as a combination of these models. We have begun to demonstrate the broad applicability of such a framework: 1) protein design: we have fully redesigned (and validated) protein surfaces using TERM data alone; 2) structure prediction: we found that TERM-based sequence statistics identify accurate models; 3) we have shown that mutational stability changes are predicted quantitatively from TERM data alone. Earlier findings of degeneracies in the protein structure (e.g., for secondary and super-secondary motifs), have greatly advanced computational structural biology. TERM-based mining of structural data is the next logical step that should provide further quantitative insights into sequence-structural relationships.

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L27 Accurate Structural Modeling and High-affinity Design of Peptide-protein Interactions using Rosetta FlexPepDock

Ora Schueler-Furman, Nawsad Alam, Dima Kozakov, Michael Mares
Hebrew University of Jerusalem

Peptide-mediated protein interactions (i.e. interactions mediated by short, linear motifs isolated or embedded within proteins - often in unstructured regions) play major roles in cellular regulation and it is important to develop and apply accurate tools for their modeling and manipulation. I will our recent development of Rosetta PIPER-FlexPepDock: a novel global peptide-protein docking protocol of unprecedented accuracy that can be used to characterize novel peptide-protein interactions, and shortly describe the design of a high affinity (sub-nanomolar) protease inhibitor using an adapted protocol for the extension of peptides to nearby binding pockets, FlexPepDesign. Using these protocols, we can significantly extend our insight on the biophysical basis of peptide-mediated interactions, as well as provide new handles and information on novel biological targets.

(Collaborations with Kozakov lab at SUNY Stony Brook; Mares lab at the University of Prague, Czech Republic).

YI-L28 Development of a Peptide Macrocycle FXII Inhibitor for Safe Anticoagulation Therapy

Jonas Wilbs[†], Simon J. Middendorp[†], Raja Prince[‡], Anne Angelillo-Scherrer[‡] and Christian Heinis[†]
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Inhibiting coagulation factor XII (FXII) has been shown to reduce thrombosis without increasing the bleeding risk, a major side-effect of currently used anticoagulants. Until recently, several protein-based FXII inhibitors were developed but no high affinity small molecule inhibitor has been reported. In our laboratory we have generated a potent and highly selective FXII inhibitor based on a macrocyclic peptide format (MW <2000 kDa). Recently, we had improved the potency and stability of the inhibitor using unnatural amino acid incorporation. The final peptide shows inhibitory affinity in the picomolar range and selectivity with a high stability in plasma. The inhibitor prolonged intrinsic coagulation in human, mouse and rabbit plasma (EC_{2X} human = 1 µM). Pharmacokinetic studies in mouse and rabbit showed that the peptide was active *in vivo* and no signs of toxicity or abnormal bleeding were observed. In a FeCl3-induced thrombosis model in mice the peptide could reduce thrombosis substantially. Our results suggest that FXII inhibition by a peptide macrocycle can potentially offer a safe anticoagulation therapy.

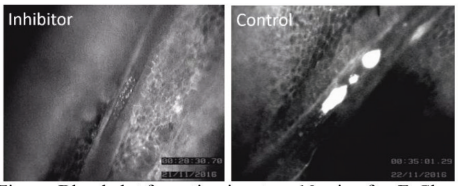

Figure: Blood clot formation in artery 10 min after $FeCl_3$ application

L29 Minimalist Protein ME47 Targets the Myc/Max:E-box DNA Network and Decreases Tumor Xenograft Growth

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Development of an anti-Myc drug has been the focus of many researchers toward creation of cancer therapeutics. Myc is a transcription factor that can become an oncoprotein; dysregulated Myc expression is involved in >50% of all cancers. Max is a transcription factor that heterodimerizes with Myc. The Myc/Max heterodimer binds to the E-box DNA target (CACGTG) and can upregulate a network of genes in uncontrolled manner. Thus, the Myc/Max:E-box network can be considered an oncogenic switch.

Using our minimalist protein design platform, we have engineered small proteins that specifically target the E-box DNA site and inhibit binding by Myc/Max. ME47 is a 66 aminoacid protein that suppresses tumor growth in cell culture and in mouse xenograft models. We will discuss ME47's anti-Myc activity in vivo, enhancement of its potency and specificity, and its effective delivery into cells using nanotechnology

L30 Impact of Backbone Modifications on Informational Properties of Peptides

Samuel H. Gellman
Department of Chemistry, University of Wisconsin - Madison

There has been growing interest in the possibility that functions of natural peptides and proteins might be recapitulated and perhaps even improved with oligomers based on unnatural backbones that manifest discrete folding preferences ("foldamers"). We have found that foldamers containing both alpha- and beta-amino acid residues ("alpha/betapeptides") can inhibit specific protein-protein interactions or augment signaling through polypeptide-activated receptors. The resulting signal can differ subtly from that of a prototype alpha-peptide. We will focus on the effects exerted by backbone modification via beta residue incorporation on the signaling profiles of agonists of two B-family GPCRs, the parathyroid hormone receptor-1 (PTHR1) and the glucagon-like peptide-1 receptor (GLP-1R). For both receptors, backbone modification of an established peptide agonist (PTH(1-34) or GLP-1) can generate agonists that display signal bias.

L31 Interfacing Oligourea Foldamers with α-peptides: An Approach to α-helix Mimicry and to the Creation of Composite Protein-like Architectures

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Significant progress has been made towards the synthesis of non-natural oligomers with high propensity to fold into regular secondary structures (i.e. foldamers[1,2]). The ability to precisely and chemically control monomer sequences in these synthetic systems opens up unique opportunities for mimicking proteins and for creating new functions. However, the design of foldamers that bind to specific protein-surfaces for inhibiting protein-protein interactions and the elaboration of more sophisticated folded architectures such as tertiary and quaternary structures, resembling proteins in terms of size and shape (and ideally function) still remain challenging endeavours Combining α-peptide and non-natural foldamer

backbones in a single chain is a promising approach to create complex architectures and to redesign peptides and proteins by replicating or modulating their structures and functions. We have recently started to explore this concept of foldamer/α-peptide chimeras with aliphatic oligoureas, a class of foldamers that adopt well-defined helical secondary structures akin to α-helices. In this presentation, we will show how key beneficial features of both species — such as natural epitope recognition of α-peptides and the innate helical stability of oligoureas — can be exploited in single chimeric constructs.

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L32 Peptide N-Amination: A Versatile Backbone Modification for Proteomimetic Constraint

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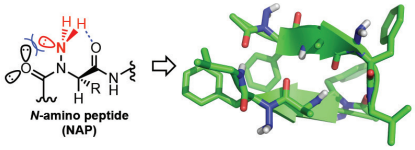
Backbone amide substitution has a profound impact on the conformation, bioactivity, and proteolytic stability of parent peptides. Although *N*-alkylation has been extensively employed in peptide SAR campaigns, heteroatomic amide substituents have received far less attention. Here, we describe a novel series of *N*-amino peptide (NAP) derivatives that mimic β-sheet-like secondary structure and exhibit enhanced resistance to aggregation and proteolysis. Tetrahydropyridazinedione (tpd)-constrained peptides feature a cyclic N_i-$C\alpha_{i+1}$ constraint leading to stabilization of extended backbone conformation by NMR and X-ray diffraction. 'Stitched' β-strand foldamers based on oligomeric tpd constraints thus represent a new class of β-strand mimics readily accessible by conventional SPPS. We have subsequently investigated the impact of simple NAP modifications on β-sheet stability and β-strand recognition by a variety of biophysical methods. Our results demonstrate, for the first time, that peptide *N*-amination supports β-sheet conformation despite the presence of a tertiary amide. This non-covalent stabilization is attributed to increased torsional strain, cis amide lone pair repulsion, and intraresidue C_6 H-bonding. Development of an efficient electrophilic amination approach toward chiral α-hydrazino acids corresponding to each primary proteinaceous amino acids further enables rapid 'NAP scanning' of lead sequences.



L33 Targeting Protein-Protein Interactions with Peptide-based Inhibitors

Kristian Strømgaard
Center for Biopharmaceuticals, Department of Drug Design and Pharmacology, University of Copenhagen

Protein-protein interactions (PPIs) are essential to vital cellular processes, and serve as potential targets for therapeutic intervention. We are particularly interested in the PPIs between integral membrane proteins and their intracellular protein partners. We have developed peptide-based inhibitors of the PSD-95/glutamate receptor interaction, by exploiting that PSD-95 contains a tandem PDZ1-2 domain. So we designed and synthesized dimeric peptides with low nanomolar affinities,[1] and have demonstrated that these ligands are potential treatment for ischemic stroke.[2] For the same PPI, we examined the importance of backbone hydrogen bond by employing amide-to-ester mutations in peptide ligands[3] and proteins.[4] Finally, we have exploited the principle of dimeric peptide-based ligands to perturb the PPI between the scaffolding protein gephyrin and glycine/$GABA_A$ receptors.[5,6] Most recently we have developed high affinity, cell-permeable peptides and demonstrated how these can modulate receptors and used to label synapses.[7]

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3. Eildal et al. *J. Am. Chem. Soc.* **2013**, *135*, 12998–13007.
4. Pedersen et al. *Nature Commun.* **2014**, *5*, 3215.
5. Maric et al. *Nature Commun.* **2014**, *5*, 5767.
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L34 Venoms to Drugs

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Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, University of Queensland, Brisbane 4072 Australia; Email: p.alewood@imb.uq.edu.au

Many organisms including snakes, spiders, scorpions, cone snails, anemones and some mammalian species have evolved venom as either a defence mechanism or a weapon for prey capture[1]. These venoms typically contain a complex cocktail of bioactive disulfide-bond rich polypeptides called toxins that target a wide range of receptors including enzymes, ion channels, GPCRs and transporters. Of interest to drug designers is their high potency and selectivity combined with their resistance to many proteases. Their high potency and exquisite selectivity for ion channels and receptors has led to several drug candidates undergoing preclinical and clinical trials.

Of particular interest are venoms from the *Conidae*[2,3], with smaller polypeptide chains of 10-40 amino acids that are highly constrained by one to five disulfide bridges and are structurally well defined. Their high potency and *exquisite selectivity* for ion channels and receptors has led to two drug candidates[4,5] from our laboratories.

In this presentation I will outline our program of discovery, describe the amazing diversity of molecular structures being discovered and regioselective chemistry that facilitates the replacement of disulfide bonds by diselenide[6], thioether and selenoether[7] bonds. This has led to mimetics that have similar or improved potency to the native molecule plus exceptional stability when exposed to reducing environments and in plasma. Together, these results underpin the development of more stable and potent peptide mimetics suitable for new drug therapies, and highlight the application of this technology more broadly to disulfide-bonded peptides and proteins.

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L35 From Twice-daily Injection to Once or Twice a Yearly Dosing of GLP-1 Agonist for Potential Treatment for Type 2 Diabetes

Ved Srivastava, Ph.D.
Vice President of Chemistry, Intarcia Therapeutics

Exenendin-4, a GLP-1 agonist is initially marketed as a twice-daily injection for the treatment of Type 2 Diabetes. Exendin-4 was subsequently formulated in PLGA microspheres as Bydureon™ for once weekly injection therapy for type 2 diabetes. This presentation will describe an exciting alternative approach for delivery of peptide drugs, with the flexibility of near immediate reversibility, for once or twice year dosing of GLP-1 agonist for potential treatment of T2D using Intarcia's Disruptive Technology - Medici Drug Delivery System™.

L36 Development of a Potent D-protein Inhibitor of VEGF-A with Reduced Immunogenicity and a Longer Half-life

Dana Ault-Riché, Ph.D.
CEO, Reflexion Pharmaceuticals, Inc.

Small proteins have historically been under utilized as human therapeutics because they are rapidly metabolized, have short half-lives and can be neutralized by anti-drug immune responses. However, proteins composed entirely of D-amino acids and achiral glycine (D-proteins) are resistant to proteases, which enables them to have longer half-lives and significantly reduced immunogenicity. Consistent with earlier studies by others, we have observed that unconjugated D-proteins fail to elicit an immune response even when repeatedly injected in the presence of a strong adjuvant. This observation has held across several different protein scaffolds injected as monomers or polymers suggesting that the lack of immunogenicity is a general property of D-proteins. This has important broad implications for new design opportunities for therapeutic proteins. Because D-proteins are chemically manufactured new designs can be created which are not economical or even possible using biologic manufacturing. Mirror image phage display technology has been used to create D-proteins that are potent inhibitors of VEGF-A, an important therapeutic target for treating eye diseases and cancers. In this process the therapeutic target protein is first chemically synthesized using D-amino acids to create a mirror image of the target protein. Phage display and structure-based design are then used to create L-proteins that bind to the mirror image of the target. Synthesis of the resulting binders using D-amino acids creates D-proteins that can modulate the activity of the natural target protein. This presentation will focus on the development of VEGF-A inhibitors as an illustration of the advantages of using D-proteins as therapeutics.

L37 Conformational, Topographical, Receptor and Dynamic Consideration in the Design of Biostable, Receptor Selective Ligands for the Melanocortin Receptors

Victor J. Hruby
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The melanocortin receptor system consists of five primordial receptors (MC1R, MC2R, MC3R, MC4R, and MC5R) that are involved in most human functions necessary for survival. Though numerous efforts to put melanocortin receptor ligands into clinical medicine only one ligand currently is in the clinic, a peptide we developed over 25 years ago. Many of these failures appear to be the result of ligands that lack appropriate receptor selectivity and/or had undesirable side effects. To overcome these deficiencies, we have been developing novel peptide and peptidomimetic ligands that are receptor selective agonists and antagonists that are bioavailable and do not have undesirable side effects. With these goals we have developed new 3D models of the 5 melanocortin receptors using domain swapping and site specific mutagenesis. These models have been used for ligand design in conjunction with conformational analysis of constrained ligands that are receptor selective agonists and antagonists using state of the art computational methods. We have designed novel peptide and peptidomimetic agonist and antagonist ligands that are receptor selective, biostable and bioavailable. This approach will be illustrated with novel structures that are highly selective for melanocortin receptors.

Supported by grants from the U.S. Public Health Service, NIH

L38 Breast Cancer Targeting Peptides and Peptide-Drug Conjugates for Increased Therapeutic Efficacy of Current Chemotherapeutic Drugs against Triple Negative Breast Cancer

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[a]Chapman University School of Pharmacy, Harry and Diane Rinker Health Science Campus, Chapman University, Irvine, CA, 92618, USA; [b]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2E1, Canada

Cancer treatment using chemotherapy 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 cancer site. In recent years, a number of peptides have been identified for delivering drugs and diagnostic elements specifically to cancer site. Using peptide array-whole cell binding assay, we have identified several breast cancer targeting peptides which selectively bind triple negative breast cancer (TNBC) cells. These peptides target specific receptors overexpressed on TNBC cell surface. Peptide-drug conjugates, synthesized by conjugation of targeting peptides to doxorubicin via different linkers, are evaluated for specific uptake by the TNBC cells, as well as their toxicity toward TNBC cells is determined. Targeting TNBC is important because it is more aggressive than other breast cancer subtypes, and

chemotherapy is the mainstay of treatment for TNBC. Here we will present our results for the design and evaluation of breast cancer targeting peptides and peptide-drug conjugates using *in vitro* and *in vivo* systems.

L39 Chemical Forces that Stabilize Proteins

R. T. Raines
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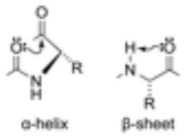
To manifest its biological activity, the amino-acid chain of a protein must fold into a particular three-dimensional structure. In the α-helices and β-sheets that dominate protein structure, a lone pair of electrons on the oxygen atom in peptide bonds accepts a hydrogen bond. Using peptidic model systems, we discovered that the other electron pair on that oxygen atom also participates in meaningful interactions in both of these architectural elements. In an α-helix, this interaction is an O···C=O n→π* interaction with the next carbonyl group in the main chain.[1] In a β-sheet, this interaction is an O···H–N hydrogen bond within the residue.[2] Both of these interactions entail the formation of 5-membered rings and involve significant overlap of non-bonding and anti-bonding orbitals. Whereas the canonical hydrogen bonds engage the *s* orbital of the oxygen, the n→π* interaction and "C5" hydrogen bond engage a *p* orbital that is orthogonal to the C=O bond. These latter interactions, which are enhanced by the orbital demixing that accompanies canonical hydrogen-bond formation, have measurable effects on protein structure and implications for biology.



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L40 HIV Glycosylation

Liwei Cao[1], Jolene K. Diedrich[1], Dan W. Kulp[2], Matthias Pauthner[2], Lin He[1], Sung Robin Park[1], Devin Sok[2], Ching Yao Su[2], Claire Delahunty[1], Raiees Andrabi[1], Dennis R. Burton[2], William R. Schief[2], John R. Yates III[1], *James C. Paulson[1,2]
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The HIV spike protein is a trimer of a highly glycosylated gp160 with 25-30 N-linked glycans that create a glycan shield against attack by the immune system, and confounds development of an effective vaccine. Yet, some chronically HIV-infected humans develop broadly neutralizing antibodies that have glycan-dependent epitopes. A consequence of the high density of glycans on gp160 is that glycan processing is variable, resulting in high-mannose glycans at some sites and complex glycans at others. To address the role of glycans in directing antibody responses to gp160, we have developed a mass spectrometry-based method to rapidly assess the degree to which each glycosylation site has no glycan, high-mannose type glycans or complex type glycans. The method utilizes the power of proteomics software to provide semi-quantitative site-specific analysis of the three glycosylation states, and could in principle be applied to any glycoprotein (Supported by NIH grant AI113867).

L41 Microbial Natural Products Discovery and Diversification

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Nature remains a productive source for molecules that display unique chemical and functional diversity by virtue of both the inherent core architectural scaffold and a remarkable array of core tailoring modifications. We have capitalized on the native substrate promiscuity of natural product tailoring enzymes (enhanced, in some cases, via structure-guided engineering and/or directed evolution), in combination with upstream synthetic chemistry and downstream chemoselective methods, to achieve the diversification of complex natural products and drugs. Specific areas that will be discussed include the discovery of novel microbial natural products from unique environments within the Commonwealth (with a particular focus on subterranean microbes from underground coal mines and deep well drilling operations) and the development of corresponding robust platforms for regio-/stereospecific differential glycosylation (glycorandomization) and/or differential alkylation (alkylrandomization). Hits identified relevant to novel targets in cancer, regeneration and/or drug abuse, via assays developed collaboratively between University of Kentucky investigators and the Center for Pharmaceutical Research and Innovation, may also be discussed.

L42 Elucidating the Features of Effective Vaccines

Laura L. Kiessling
University of Wisconsin

The carbohydrate coat of a cell can be a unique identifier. A long-term objective of our resear h is to understand how to generate synthetic carbohydrate-based vaccines. Immunity to carbohydrate antigens, especially glycoproteins, typically depends upon communication between antigen presenting B cells and T cells. B cells that recognize the carbohydrate antigen through the B cell receptor (BCR) take up the antigen and route it for processing. The resulting peptide epitopes are loaded into major histocompatibility complex II (MHC II) and presented to T cells. An understanding of the influence of antigen properties on B – T cell communication would facilitate vaccine design. Similarly, dendritic cells (DC) can also present carbohydrate antigens, and targeting carbohydrate antigens to achieve effective immune responses through DC – T cell interactions is another aspect of our program. We are optimizing the presentation of peptides and carbohydrates on a scaffold to promote immune responses. Our progress toward these goals will be described.

YI-L43 Engineering Dengue EDIII Immunogens to Elicit Broadly Neutralizing Antibody Responses

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Dengue is a mosquito-transmitted *Flavivirus*, of which there are four co-circulating serotypes, causing 100 million annual infections with no treatments available. Initial infection causes a febrile illness and lifelong immunity against the infecting serotype. Secondary infection with another serotype can result in rapid and potentially fatal progression to Dengue Hemorrhagic Fever or Dengue Shock Syndrome due to antibody dependent enhancement of infection, in which uptake of virus

by Fcγ receptor-positive cells is promoted by weakly neutralizing antibodies from the primary infection. Thus, development of a vaccine must elicit broadly neutralizing antibodies (bNAbs) that neutralize all serotypes. Analyses of the human and murine antibody response have shown that many potent bNAbs bind to domain III (EDIII) of the envelope glycoprotein E, and EDIII stimulates robust B-cell responses. Use of EDIII as an immunogen failed due to an immunodominant epitope, the AB-loop. Antibodies binding the AB-loop are cross-reactive but non-neutralizing. Contrastingly, the AG-strand epitope was shown to be broadly neutralizing, and is the epitope for bNAb 4E11. To optimize EDIII as an immunogen, we used phage display to "resurface" the AB-loop and surface-exposed residues via mutation to serine or alanine. *We hypothesize that resurfaced EDIII variants will provide improved and focused antibody responses when used as immunogens.* We prepared and screened limited diversity EDIII-based phage libraries allowing variation at the AB-loop and select surface positions, then selected this library for variants that maintained their capacity for recognition by bNAb 4E11. Current efforts include evaluation of these EDIIIs *in vitro* and i*n vivo*.

L44 Novel Peptide-like Inhibitors of the Proteasome Ubiquitin Receptor Rpn13 Proteasome Inhibitors and Their Mechanism of Action: A Promising New Approach to Chemotherapy

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Proteasome inhibitors such as Bortezomib have emerged as important drugs for the treatment of multiple myeloma (MM) and certain other hematological cancers, which produce prodigious amounts of protein and thus place an unusual burden on pathways to deal with mis-folded proteins, including proteasome-mediated degradation. However, they have failed in trials against solid tumors, apparently due to an insufficient therapeutic window (all cells require proteasome activity). Recently, the ubiquitin receptor Rpn13, a component of the 19S regulatory particle (RP) of the proteasome, has been shown to be non-essential in normal cells, but is highly overexpressed in a number of cancers, suggesting that Rpn13 is a "turbocharger" of the proteasome, a function that is critical in cancer cells, but not healthy cells. We developed a peptoid inhibitor of Rpn13 and found that it is toxic to a number of cancer cell lines, but displays no toxicity in normal cells.[1] We show that the peptoid abrogates Rpn13-mediated activation of the deubiquitylase (Dub) Uch37, strongly suggesting that unusually high Dub activity is critical for high-level proteasome function in cancer cells. The development of improved Rpn13 inhibitors will be also be described.

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L45 Automated Flow Peptide Synthesis: Toward Amide Bonds at Nature's Pace

Bradley Pentelute
Massachusetts Institute of Technology

Here we describe a rapid flow solid phase peptide synthesis methodology that enables incorporation of an amino acid residue in 40 seconds with amide-bond formation taking only 7 seconds. To demonstrate the broad applicability of this method, it was employed to synthesize hundreds of peptides and proteins.

L46 Organotrifluoroborates as Radioprosthetics that Enable Facile, User-friendly 18F-labeling for Turning Peptides into PET Imaging Agents

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Molecular imaging by PET (positron emission tomography) is being increasingly used to validate targets in vivo with high spatio-temporal resolution. Of several PET-useful isotopes, ^{18}F-fluoride has a track-record of FDA clearance and which can be produced at Curie-levels, on-demand. Nevertheless, the challenges for 18F-labeling peptides molecules are considerable; including a short half-life and the need for anhydrous conditions and/or at very high temperature, which lead to lower yields and cumbersome multistep routes. In seeking a wet reaction, we identified several organotrifluorborates for peptide conjugation to provide precursors that are 18F-labeled in a single aqueous step. To streamline labeling, we use isotope exchange whereby precursor = product. This allows for a time-efficient radiolabeling in ~20 min at 3 Ci/μmol in radiochemical yields of 20-40% using 1Ci of NCA 18F-fluoride. This method appears to be generalizable to many peptides: we have labeled RGD, bombesin, octreotate, bradykinin, as well as LLP2A (new), and diamino-acid ureas (new) for targeting prostate cancer. In pursuit of dual-mode fluorescent PET tracers, we have conceived of a "radiosynthon" that lets researchers graft any peptide and a fluorophore of choice to provide tracers that combine the sensitivity of PET with the brilliance of fluorescence for imaging RGD. Synthetic and

L47 Peptide Therapeutics Method Development: Increasing Efficiency and Purity of SPPS Using Parallel Synthesis Optimization with Induction Heating

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Peptide therapeutics have seen renewed interest with many being approved and many more in clinical studies in recent years. Cyclic peptides have become increasingly popular as therapeutic agents given their positive pharmacological properties, such as their improved stability, membrane penetration, and target specificity[1]. Methods for solid phase peptide synthesis (SPPS) of linear peptides have been improved by the use of heat (>50°C), resulting in shorter coupling cycles with higher crude purity. While cyclization chemistry has traditionally been performed in solution, different factors, such as ring size and sequence, can compromise efficiency. Using higher temperatures may provide advantages

for on-resin synthesis of cyclic peptides. Because a peptide's sequence dictates its synthetic difficulty, parallel condition optimization will facilitate a more robust synthesis method. Here we show the complete parallel synthesis, from swelling to cleavage, of different biologically relevant peptides under different conditions (50-90°C) on an automated peptide synthesizer with higher purity and yields in a reduced amount of time. Parallel heating allows simultaneous analog synthesis used for SAR studies of synthetically challenging sequences. An example of difficult linear peptides the JR-10mer peptide sequence, a known difficult sequence that serves as a great scaffold for optimizations, was synthesized[2]. A cyclic melanocortin receptor agonist, Melanotan II (MT-II), was also evaluated for the effects of higher temperatures during cyclization[3].

JR-10mer: WFTTLISTIM-NH2
MT-II peptide sequence: Ac-Nle-cyclo[Asp-His-D-Phe-Arg-Trp-Lys]-NH2

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2. T. Redemann and G. Jung, *Proc. 24th Eur. Pep. Symp.,* **1996**, *749*.
3. N. Tsomaia, "Peptide therapeutics: Targeting the undruggable space", **2015**.

L48 Cyclic Urethane Chemistry for Synthesis and Sequencing of Cyclic Peptides

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Cyclic peptides are attractive for drug discovery due to high binding affinity and specificity for the target. The therapeutic ability of cyclic peptides prompted the synthesis of cyclic peptide libraries for High Throughput Screening (HTS) against various biological targets. A major obstacle associated with this approach is the sequencing difficulty of hit cyclic peptides obtained by HTS using traditional methods. We have developed a one bead one compound (OBOC) approach for sequencing of hit cyclic peptides by selective modification of a peptide backbone chain at serine residue to a cyclic urethane moiety within the cyclic peptide.[1-3] Formation of the cyclic urethane moiety increases the susceptibility of the amide bond at the N-terminus of serine towards hydrolysis and leads to the opening of a cyclic peptide to its linear counterpart which was then sequenced by tandem mass spectrometry. The peptide macrocycles containing a cyclic urethane moiety promotes the formation of conformationally rigid turn structures in the macrocycles. The resulting cyclic urethane moiety containing cyclic peptides display high stability towards proteolytic degradation and can also lead to high passive membrane permeability, important characteristics required for the development of peptide-based therapeutics.

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L49 A New Level of Efficiency for SPPS

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CEM Corporation

The use of high efficiency solid phase peptide synthesis (HE-SPPS) provides benefits for the synthesis of peptides due to its high purity, rapid speed, and low chemical usage characteristics[1]. This has been advanced further through the development of a one pot coupling and deprotection process, a vacuum assisted post deprotection base removal at elevated temperature, and an improved carbodiimide coupling that enhances both O-acylisourea formation and the subsequent acylation[2]. Together these features maximized achievable synthesis purity, reduced the entire cycle time to < 3 minutes, and required only a single washing step per cycle. Therefore, using this process a difficult 10 mer peptide (0.1mmol scale) can be made in < 30 minutes with < 100mL total waste.

Realization of these benefits provides two practical benefits for peptide synthesis. First, enhanced synthesis purity compared to standard HE-SPPS conditions was achieved due to improvements in both the deprotection and coupling steps. Second, an automated sequential version of this process creates a powerful high throughput synthesis approach. For example, a batch of 24 different peptides (with lengths up to 75 amino acids) was synthesized automatically using this process within only a day while featuring an unprecedented reduction in chemical waste.

The application of this technology was then expanded through the use of the hyper-acid sensitive Cl-TCP linker [3]. This linker provides a unique combination of stability and protection against C-terminal side reactions at elevated temperatures compared to the well-known 2-Cl-Trt trityl and Trityl (TCP) linkers.

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2. US20160176918
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L50 Combinatorial Lanthipeptide Biosynthesis

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Ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a very large class of peptide natural products.[1] These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Lanthionine-containing peptides (lanthipeptides) are examples of this growing class and many members are highly effective peptide-derived antimicrobial agents that display nanomolar minimal inhibitory concentrations (MICs) against pathogenic bacteria (termed lantibiotics). These peptides are post-translationally modified to install multiple thioether crosslinks. During their biosynthesis, a single enzyme typically breaks 8-16 chemical bonds and forms 6-10 new bonds with high control over regio- and chemoselectivity.[2] This presentation will discuss investigations of the mechanisms of these remarkable catalysts as well as their use for the generation of non-natural cyclic peptides.

1. M.A. Ortega, W.A. van der Donk, *Cell Chemical Biology,* **2016**, *23*, 31-44.
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L51 Discovery of Macrocyclic Peptidomimetics Targeting Protein-Protein Interactions

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The majority of drugs on the market today target proteins with well-defined small-molecule binding sites, including enzymes. However, some of the most devastating diseases are associated with proteins that do not possess these natural binding sites, such as those involved in protein-protein interactions associated with many cancers. Molecules capable of inhibiting disease-related protein-protein interactions are thus valuable research tools to investigate molecular functions of target proteins and further could be developed as novel therapeutic candidates. However, discovering such inhibitors is a daunting task largely because protein interfaces involved in protein-protein interactions are relatively large and flat. Typical small drug-like molecules may not be suitable to effectively cover such extended protein contact areas. As such, macrocyclic peptides/peptidomimetics are attracting great interest as a promising class of molecules. Due to their relatively large size, they are more suitable to target protein interfaces than traditional small molecules. Compared to their linear counterparts, they should have conformational rigidity and preorganized structure, enabling them to bind to their target protein more tightly. Furthermore, they often have improved cell permeability and proteolytic stability compared to linear ones. Therefore, macrocyclic peptides/peptidomimetics could serve as an excellent source of modulators of many protein-protein interactions. Here, I will present our recent works on the discovery of macrocyclic peptides/peptidomimetics-based inhibitors of Skp2- and STAT6-mediated protein-protein interactions.

L52 Discovery of Soluble, Biophysically Stable Glucagon Analogs with Minimal Change to Native Sequence

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Glucagon serves an essential endogenous role in the maintenance of glucose control through stimulation of hepatic gluconeogenesis and glycogenolysis. It is also a well-established medicine used in the reversal of life-threatening hypoglycemia induced by excessive insulin action. Glucagon is being evaluated in several exploratory clinical settings including micro-dosing and bi-hormonal pump protocols. The conventional and exploratory clinical uses are impaired by its poor aqueous solubility, its chemical instability in acidic and alkaline formulations, and a propensity to fibrillate. These issues are not unique to glucagon and are also a persistent challenge in the optimization of peptide-based drug candidates. Our initial efforts employed isoelectric point adjustment coupled with chemical modification to fortify the secondary structure.[1] We now report two novel chemical strategies to address this problem that achieve a similar degree of stabilization with minimal modification to the native sequence, and should be broadly applicable to other biophysically challenging peptides. The first approach employs hydrophilic aromatic sidechains in the N-terminal region of the molecule through substitution with His and 3-pyridyl-Ala.[2] The second approach incorporates an isoacyl dipeptide in the sequence to achieve highly soluble, aggregation resistant prodrugs.[3] While chemically stable in acidic, aqueous formulations these analogs rapidly revert to the native hormone under physiological conditions. Select peptides derived from each series potently raised blood glucose in animal models in a comparable fashion to native glucagon, and possessed superior biophysical properties suitable for drug development.

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L53 Systematic Discovery and Development of Peptidic Molecular Guidance Systems for the Diagnosis and Treatment of Cancer

Kathlynn C Brown and Michael J McGuire
SRI International

Tumor targeting ligands are emerging components in cancer therapies. Widespread use of targeted therapies and molecular imaging is dependent on increasing the number of high affinity, tumor-specific ligands. Towards this goal, we biopanned five phage-displayed peptide libraries on a series of well-defined human non-small cell lung cancer (NSCLC) cell lines, isolating a panel of 15 novel peptides. The peptides show distinct binding profiles across 40 NSCLC cell lines and do not bind normal bronchial epithelial cell lines. Binding of specific peptides correlates with onco-genotypes and activation of particular pathways, such as EGFR signaling, suggesting the peptides may serve as surrogate markers. Multimerization of the peptides results in cell binding affinities between 0.0071-40 nM. The peptides home to tumors in vivo and bind to patient tumor samples. We have utilized these peptides as delivery agents for a wide variety of cargo, ranging from small molecular agents to nanoparticles. Progress in developing these peptides into clinically useful molecular imaging agents, therapeutics and immunotherapies for NSCLC will be presented.

L54 Sensitivity of the Opioid Activity Profile of CJ-15,208 Analogs to Ring Substitution

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Unlike linear peptides, the macrocyclic tetrapeptide CJ-15,208 (cyclo[Phe-D-Pro-Phe-Trp]) is stable to proteases and exhibits antinociception and kappa opioid receptor (KOR) antagonism after oral administration,[1] making it a promising lead compound for potential development of treatments for pain and drug abuse. As part of our exploration of the structure-activity relationships of this peptide, we are incorporating substitutions on the aromatic residues and assessing the opioid activity profile, both in vitro and in vivo, of the resulting analogs. The substitutions were generally well tolerated by KOR, as determined in radioligand binding assays *in vitro*. In the antinociception assay *in vivo* (the mouse 55 oC warm water tail withdrawal assay) the opioid activity profile, particularly the KOR antagonism, of the analogs evaluated was very

sensitive to the identity of the substitution. Thus incorporation of a fluorine on either of the phenylalanine residues resulted in the loss of KOR antagonism. Unexpectedly, one analog exhibited both antinociception and potent antagonism of all three opioid receptors in vivo following intracerebroventricular administration. Detailed pharmacological evaluation of the analogs, including activity in a model of relapse to drug seeking behavior, will be presented. This research supported by NIDA grant R01 DA023924.

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L55 The RaPID Discovery of Pseudo-natural Peptides

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This lecture will describe the most recent development in the genetic code reprogramming technology that enables us to express pseudo-natural peptides. The technology involves (1) efficient macrocyclization of peptides, (2) incorporation of non-standard amino acids, such as N-methyl amino acids, and (3) reliable synthesis of libraries with the complexity of more than a trillion members. When the technology is coupled with an in vitro display system, referred to as RaPID (Random non-standard Peptide Integrated Discovery) system, the non-standard macrocyclic peptide libraries with a variety ring sizes and building blocks can be screened (selected) against various drug targets inexpensively, less laboriously, and very rapidly. In this lecture, I shall describe recent successes in selecting highly active macrocycles against various drug targets related to cancer therapeutics.

Y. Iwane; A. Hitomi; H. Murakami; T. Katoh; Y. Goto; H. Suga*, "Expanding the amino acid repertoire of ribosomal polypeptide synthesis via the artificial division of codon boxes", *Nature Chemistry, 8*, 317–325 (**2016**)
K. Ito; K. Sakai; Y. Suzuki; N. Ozawa; T. Hatta; T. Natsume; K. Matsumoto; H. Suga "Artificial human Met agonists based on macrocycle scaffolds" *Nature Communications, 6*, 6373 (**2015**)

L56 From Natural Antibodies to Synthetic Proteins

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Affinity reagents that target and modulate proteins are of crucial importance for both basic research and therapeutic development. To date, antibodies derived by animal immunization have been the dominant source of affinity reagents, but in recent years, research in protein engineering has given rise to a new wave of technologies that promise to transform the field. In particular, our knowledge of protein structure and function has advanced to the point where it is now possible to develop small protein scaffolds that exhibit the high stabilities, specificities and affinities typical of large antibodies. By combining these small scaffolds with combinatorial phage display technologies, we have established high-throughput platforms that enable the rapid and cost-effective generation of affinity reagents targeting hundreds of proteins in a parallel manner. In many cases, the protein scaffolds are small and simple enough to enable complete chemical synthesis, thus linking the fields of high-throughput protein engineering and peptide synthetic chemistry.

In my group, we have designed libraries of ubiquitin variants that can be used to inhibit or activate virtually any of the hundreds of ligases and deubiquitinating enzymes in the ubiquitin system. These ubiquitin variants are adapted for intracelluar function, and thus, they can be introduced into cells to probe function in a living cellular context. In addition, we have developed small, optimized scaffolds that function like antibodies but are amenable to full chemical synthesis, thus enabling the incorporation of non-natural amino acids. The power of the technology has been demonstrated by the development of potent protein inhibitors composed entirely of D-amino acids. In sum, these advances in the design of synthetic binding proteins extend the applications for affinity reagents well beyond the range of natural antibodies and this should have a transformative effect on many areas of biological research.

YI-L57 Peptide Macrocyclization Inspired by Non-Ribosomal Imines

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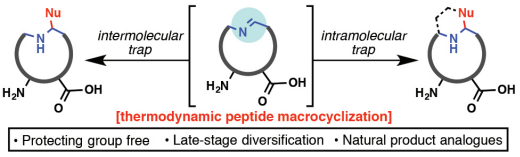
Peptides have enormous therapeutic potential but traditionally suffer from poor bioavailability. Macrocyclization is a key strategy for enhancing the drug-like properties of peptides, and new cyclization methods[1] are in exceedingly high demand in the pharmaceutical industry. Herein, a versatile strategy for macrocyclization inspired by the biosynthesis of non-ribosomal imine natural products is presented. Such macrocyclic natural products possess an intriguing head-to-tail imino linkage which forms upon spontaneous cyclization of the linear peptide amino aldehyde.[2] In this study,[3] imine macrocyclization is explored as a general, reversible mode of peptide cyclization, and the reactivity of the imine linkage toward inter- and intramolecular nucleophiles is exploited for late-stage peptide diversification. The cyclization proceeds in aqueous solution and in the absence of protecting groups. Application to the synthesis of four peptide natural products and the structural interrogation of relevant linear and macrocyclic peptides will also be discussed.



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L58 An Engineered Tumor Targeting Knottin Peptide is a Versatile Agent for Imaging, Drug Delivery, and Immunotherapy

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Cystine-knot miniproteins, also known as knottins, constitute a large family of structurally related peptides with diverse amino acid sequences and biological functions. Knottins have emerged as attractive candidates for drug development as they potentially fill a niche between small molecules and protein biologics, offering drug-like properties and the ability to bind to clinical targets with high affinity and selectivity. Due to their extremely high stability and unique structural features, knottins also demonstrate promise in addressing challenging drug development goals. Knottins from diverse sources, such as venoms and plants, naturally possess functions as protease inhibitors, toxins, and antimicrobials. To expand beyond nature's repertoire, we used rational and combinatorial protein engineering methods to generate a knottin peptide that binds tumor-associated cell adhesion receptors with high affinity. This engineered knottin peptide, when conjugated to molecular imaging probes, could be used as a non-invasive tumor imaging agent in a variety of murine cancer models. In addition, we showed that this peptide could localize to and illuminate brain tumors following intravenous injection. I will discuss recent work where we developed the engineered knottin peptide as a vehicle for targeted drug delivery and immunotherapy, expanding its application to tumor-directed therapy.

L59 Minimal Coiled Coils and their Potential as Protein-Protein Interaction Inhibitors

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New York University

Coiled coils are well-characterized protein motifs that orchestrate multimerization of various complexes important for biological processes. However, approaches that afford short peptides with defined coiled coil conformation remain elusive. We recently described a general strategy to constrain coiled coils by replacing an interhelical salt bridge with a covalent linkage, affording a stable, monomeric scaffold of Cross-linked Helix Dimers (CHDs). This presentation will discuss the genesis of this approach and the potential of CHDs to inhibit challenging protein-protein interactions.

L60 Targeting Kinase Regulation with Constrained Peptides

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Misregulation of kinases is implicated in a myriad of diseases and significant efforts have been put forth towards the development of targeted kinase inhibitors.However, many issues remain with current therapeutic strategies including specificity and inhibitor resistance.As an alternative approach for targeted inhibition, we have developed constrained peptide inhibitors targeting protein-protein interfaces (PPIs) that bind evolutionarily conserved structural features for a kinase of interest. We have used this approach to perturb spatiotemporal kinase regulation in the context of live cells.

A-Kinase Anchoring Proteins (AKAPs) scaffold PKA along with other proteins to form small signaling complexes in cells. While AKAPs play an important role in the spatial and temporal regulation of protein kinase A (PKA), many questions remain regarding their precise regulatory roles in cell signaling in normal and disease states. As a strategy to perturb AKAP signaling, we have developed inhibitors targeting protein-protein interfaces (PPIs) formed at AKAP complexes. These peptide-based inhibitors are chemically constrained to present a large binding surface area while locking the peptide scaffold into a pre-binding state. Since a single √-helix derived from AKAPs is used to anchor PKA-R, we developed a novel class of isoform-selective peptides targeting the AKAP binding site on PKA-R. These inhibitor peptides bind their intended target with low nM affinities and serve as cell-permeable tools to selectively disrupt AKAP signaling complexes. Additionally, novel peptide-based inhibitors are currently being developed to target novel PPIs at AKAP complexes that have previously not been targeted in order to achieve selective inhibition of a particular AKAP within the context of cells. Overall, the constrained peptides developed in this work demonstrate a unique strategy to perturb spatiotemporal regulation and broaden the possibility of targeting additional protein-protein interfaces that may not be accessible using a small molecule approach.

YI L61 Oxadiazole Grafts in Peptide Macrocycles

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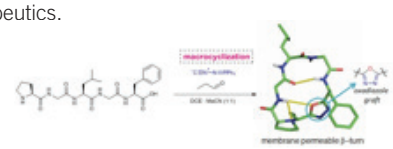
Peptide macrocycles are a class of structurally complex molecules with extended surfaces that are well suited to interrogate challenging protein-protein interactions. In contrast to linear peptides, the cyclized form can display improved binding affinity, increased proteolytic stability, and enhanced membrane permeability.[1] A new paradigm is emerging in which the conformation of peptide macrocycles is recognized as the primary determinant of their bioactivity. Incorporation of rigidifying structural elements into peptide macrocycles has emerged in our lab as a means to modulate the biological properties of these ligands, through conformational control and minimization of their polar surface area.[2] Our lab has recently developed a new strategy to rapidly generate cyclic peptides bearing a 1,3,4-oxadiazole incorporated into the backbone, via reaction between a linear peptide, aldehyde, and (*N*-isocyanimino)triphenylphosphorane.[3] Both the peptide sequence and aldehyde component can be varied to access macrocycles of different size and composition. The resulting oxadiazole-containing cyclic peptides can display a variety of rigid secondary structural motifs that are enforced by conserved, intramolecular hydrogen bonding patterns. In addition, oxadiazole-containing peptide macrocycles exhibit passive membrane permeability, which is an important property for the development of orally bioavailable peptide-based therapeutics.



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L62 How Do Cell-Penetrating Peptides Work?

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The cell membrane represents a major obstacle in drug discovery, especially for peptide-, protein-, and nucleic acid-based therapeutics. Over the past two to three decades, nearly 2000 different cell-penetrating peptides (CPPs) have been discovered and some of the CPPs have been widely used to deliver various cargos into mammalian cells. However, the first-generation CPPs have very low cytosolic delivery efficiencies (generally <5%) and their mechanism of action remains poorly defined. We have recently discovered a new family of small amphipathic cyclic peptides as exceptionally active CPPs, with cytosolic delivery efficiencies of up to 120%.[1] The cyclic CPPs are able to efficiently deliver small molecules, peptides, proteins, and nucleic acids into the cytosol of mammalian cells *in vitro* and *in vivo*. The availability of CPPs with high as well as varying efficiencies has, for the first time, allowed us to elucidate their mechanism of action.

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L63 Exploring Macrocyclic Peptide Cell Permeability: Stapled p53 Peptide Model System

Tomi K. Sawyer[1], Anthony Partridge[2], Chris Brown[3], Dawn Thean[3], Tareen Ho[2], Cynthia Chang[2], Kirsten Searle[2], Chandra Verma[4], Srinivasaraghavan Kannan[4], Pietro Aronica[4], Tan Yaw Sing[4], Charles Johannes[5], Tsz Ying Yuen[5], Fernando Ferrer[5], Bing Lim[2], Brian Henry[2], Shuhui Lim[2], Nicole Boo[2], Ahmad Sadruddin[2], Kristal Kaan[2], Angela Juang[2], Thomas Tucker[6], Jerome Hochman[6], Shiying Chen[7], Hui Wan[7], Xu Wang[8], Weixun Wang[8], Laura Surdi[1], Brad Sherborne[7], Sookhee Ha[7], Seth Clark[6], Danielle Molinari[1], and David Lane[3]
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Macrocyclic peptides can modulate protein-protein interactions with drug-like properties, but achieving cell permeability is challenging and is hindering their therapeutic potential. Accordingly, we have focused a multidisciplinary effort on systematically exploring macrocyclic peptides, including stapled peptides, to understand their structure-cell permeability relationships. As a benchmark and model system for such cell permeability studies, we have evaluated a series of analogs of ATSP-7041, a stapled peptide dual antagonist of MDM2 and MDMX. These include both Ala- and D-amino acid scanning to create an empirical dataset to compare biological properties (e.g., target binding and p53-dependent cellular activity) with biophysical properties (e.g., membrane permeability and partitioning) and structural/metabolic stability properties (e.g., helicity, amphipathicity, and intracellular stability). Ultimately, this work is focused on generating design rules predictive of macrocyclic peptide cell permeability.

L64 *In Vitro* Reconstitution of Thiopeptide Biosynthesis

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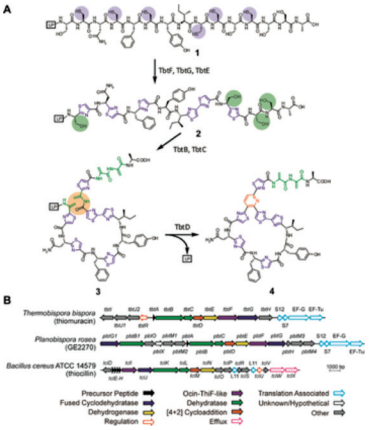
University of Illinois at Urbana-Champaign

Thiopeptides are architecturally complex natural products that potently inhibit bacterial protein synthesis by binding either to the 50S ribosomal subunit or to EF-Tu. Thiopeptides are biosynthesized by a remarkable series of post-translational modifications that transform a ribosomal precursor peptide into a polycyclic structure. This talk will cover recent work that describes the in vitro biosynthesis of thiomuracin with a focus on the timing of enzymatic events and substrate specificities. We show that cyclodehydration and oxidation of six cysteines to thiazoles precedes the dehydration of four serines to alkenes, which is catalyzed by two proteins in a $tRNA^{Glu}$-dependent manner. Subsequently, the macrocycle-forming enzyme, that also generates the 6-membered, nitrogenous heterocycle ubiquitous to thiopeptides, ejects the leader peptide as a C-terminal carboxamide. Mutagenesis studies of this enzyme identified residues important for the formal [4+2] aza-cycloaddition process. The core structure of thiomuracin exhibits similar antimicrobial activity to other known congeners, illustrating that in vitro biosynthesis is a viable route to potent antibiotics that can be explored for the rapid and renewable generation of analogs.



L65 Biosynthesis of RiPPs on the Coral Reef

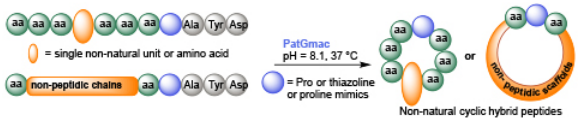
Eric Schmidt
University of Utah

In coral reef animals, diverse RiPP natural products are generated presumably as a defense against predation by animals. Perhaps for that reason, many marine RiPPs target animal biochemistry. I will describe the RiPP chemical space in marine animals, including novel biochemical transformations. Many of the marine RiPP enzymes appear to be designed for the biological purpose of generating chemically diverse products, and mechanisms of diversity-generating biosynthesis will be outlined. These mechanisms can be exploited in the designed synthesis of new materials, such as potential pharmaceuticals. Finally, new methods to directly access bioactive RiPPs for drug discovery, and the resulting hit compounds, will be described.

YI-L66 Enzymatic Macrocyclization of Non-natural and Hybrid Polyketide Peptides

E. Oueis[a], B. Nardone[a], N. J. Westwood[α], J. H. Naismith[a,b]
[a]School of chemistry, University of St Andrews, BSRC, North Haugh, St Andrews, KY16 9ST, UK; [b]State Key Laboratory of Biotherapy, Sichuan University, China

A large number of natural macrocycles are known to have potent biological and medicinal activities. Even though peptidic macrocycles have better chemical stability and higher structural rigidity, most are too polar to possess good physiochemical properties. Here we investigate the promiscuity of the macrocyclase enzyme PatGmac form the patellamide (cyanobactin) pathway to generate a series of macrocycles containing non-natural amino acids,[1] 1,4-substituted 1,2,3-triazoles as amide bond mimetics,[2] polyketides and non-natural scaffolds,[3] and proline mimetics. The amino acid content of some of the resulting hybrid macrocycles is reduced to three or just one. PatGmac has proven itself very useful for the macrocyclization of not only slightly modified peptides, but also highly hybrid backbones. PatGmac could be used as a biosynthetic tool for the generation of diverse hybrid macrocycles designed with control over properties.



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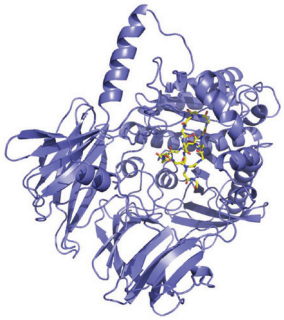
L67 Making and Breaking Lasso Peptides

A. James Link,[1] Mikhail Maksimov,[1] Joseph Koos,[1] Chuhan Zong,[1] Jon Chekan,[2] Satish Nair[2]
[1]Princeton University; [2]University of Illinois

Lasso peptides are a class of RiPPs with a unique threaded rotaxane structure. This talk will focus on the enzymes involved in the biosynthesis and catabolism of these peptides. Genome mining for lasso peptides has uncovered many highly conserved lasso peptide gene cluster in alpha-proteobacteria. As part of this work, our laboratory discovered lasso peptide isopeptidase, a prolyl oligopeptidase homolog that specifically hydrolyzes the isopeptide bond of lasso peptides, rendering the peptides linear. A combination of biochemical, mutagenesis, and structural studies has shed light on substrate recognition in this enzyme. The connection of lasso peptide isopeptidase to the proposed biological function will also be discussed.


Crystal structure of lasso peptide astexin-3 (yellow backbone) bound to an inactive form of the cognate isopeptidase AtxE2.

L68 Chemoenzymatic Platforms for the Discovery of New Peptide Therapeutics

Albert A. Bowers, Ph.D., Assistant Professor
Division of Chemical Biology and Medicinal Chemistry, University of North Carolina at Chapel Hill, Eshelman School of Pharmacy, Chapel Hill, North Carolina, USA

RiPPs are a rapidly growing class of peptide derived natural products. RiPP enzymes carry out remarkable chemistry en route to transforming short peptides into bioactive molecules. We have recently developed new technology for generating large and diverse libraries of RiPP natural products based on the promiscuity of key biosynthetic enzymes from RiPP pathways. Two examples will be presented. First, by employing members of the new pyridine synthase family of macrocyclases, discovered in our lab, we create non-natural analogues of the potent anti-MRSA antibiotics known as thiopeptides to explore their structure activity relationships. Second, we exploit the sactionine synthase AlbA from *Bacillus subtilis* to design stapled alpha-helical mimics for the inhibition of key therapeutic targets.

L69 The Design Evolution of Anticancer Peptides from Self-Assembled Hydrogels and How Cancer Cells Gain Resistance to their Action

Joel P. Schneider
Chemical Biology Laboratory, National Cancer Institute, Frederick, MD 21702

We previously designed a class of antibacterial hydrogels from self-assembling beta-hairpin peptides that kill a broad spectrum of drug–resistant bacteria on contact. Based on an understanding of the gels' lytic mechanism of action and the fact that bacteria and cancer cells share similar traits with respect to their cell surfaces, we have gone on to design a new class of non-gelling, soluble peptides that show cytotoxic activity against a broad range of cancer cells. These anticancer peptides (ACPs) adopt an ensemble of random coil, bio-inactive conformations in solution. However, when they encounter cancer cells, they bind to, and fold at, the cell's surface, adopting a highly lytic conformation capable of killing the cells. In general, most ACPs disrupt tumor cell membranes through rapid and non-stereospecific mechanisms, encouraging the perception that cellular resistance towards their action is unlikely to occur. Interestingly, although resistance towards small molecular chemotherapeutics has been well studied, the potential of tumor cells to avoid destruction by membrane-lytic compounds remains largely unexplored. We demonstrate that eukaryotic cells can, indeed, develop resistance to oncolytic peptides. Utilizing fission yeast as a model organism, we show that ACP resistance is largely controlled through the loss of cell-surface anionic saccharides. We then discovered a similar mechanism in mammalian cancer cells where removal of negatively-charged sialic acid residues directly transforms a peptide sensitive cell line into a resistant phenotype. These results demonstrate that changes in cell-surface glycosylation play a major role in tumor cell resistance towards oncolytic peptides.

L70 Intracellular Delivery of Therapeutic Peptides Using Enzymatically Cleavable Peptide Amphiphiles

Handan Acar[a,b], Mathew R. Schnorenberg[a,b,c], Matthew V. Tirrell[a,d], James L. LaBelle[b]
[a]Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA; [b]Department of Pediatrics, Section of Hematology/Oncology, University of Chicago, Chicago, IL 60637, USA; [c]Medical Scientist Training Program, University of Chicago, Chicago, IL 60637, USA; [d]Institute for Molecular Engineering, Argonne National Laboratory, Argonne, IL 60439, USA

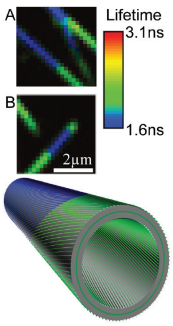
Peptides are promising tools for manipulating disease processes within cells, but their delivery to and into cells remains a major obstacle. Peptides amphiphiles (PAs), a peptide conjugated to a hydrophobic tail, are one tool to enable the intracellular delivery of therapeutic peptides. The hydrophobic tail drives self-assembly into nanoparticles (NPs). PA NPs are optimally sized (10-20 nm) to promote accumulation in tumor tissues via the enhanced permeability and retention effect, and their structure prevents hydrolysis and proteolysis of peptides in circulation. The internalization mechanisms have not been fully elucidated, but endocytosis plays an important role.

Despite these delivery benefits, internalized PAs remain trapped in endosomes, which prevents them from reaching their cytoplasmic targets and reduces therapeutic efficacy. Moreover, the hydrophobic tail promotes rapid excretion and sterically hinders binding of the therapeutic peptide with its target protein.

To overcome these obstacles after internalization, we recently introduced a cathepsin B (endosomal enzyme) cleavable linker between a PA's hydrophobic tail and peptide. We have used this system for intracellular delivery of two model peptides: $p53_{(14\text{-}29)}$ to reactivate tumor suppressor protein p53, and the BH3 death domain of BIM, a pro-apoptotic member of the BCL-2 family of proteins. In both systems, the PA is internalized while the peptide alone is not, and the cleavable PA promotes intracellular peptide accumulation, and in the case of the BIM peptide, induces apoptosis. The cleavable PA platform is a promising tool for intracellular delivery of peptides that could not otherwise reach their targets within cells.

L71 Protein Misfolding and Chemical Evolution

Allisandra Rha[a], Chenrui Chen[a], Junjun Tan[a], Tolulope O. Omosun[a], Ming-Chien Hsieh[b], Anil K. Mehta[a], Sha Li[a], Dibyendu Das[a], W. Seth Childers[a],Jay T. Goodwin[a], Rong Ni[a], Neil R. Anthony[a], Martha Grover[b], Keith M. Berland[a], & David G. Lynn[a]
[a]Departments of Biology, Chemistry, and Physics, Emory University, and [b]Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30322



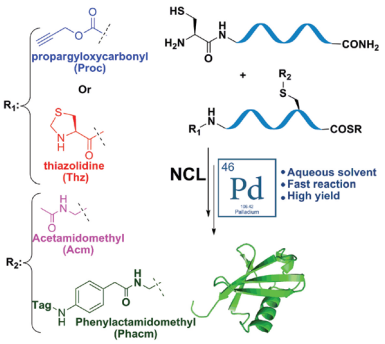
Protein misfolding is now implicated in at least fifty disease states that constitute personal and societal tragedies of large and growing proportions. In an effort to convert these infectious proteins into beneficial informational elements, we have tied covalent macromolecular synthesis to supramolecular assembly and used the tensions inherent in the combined dynamic chemical/physical networks to access new functions. The range and degree of order accessible to the coassemblies demonstrate how functional information can be propagated and selected in disease or exploited for needed structural and chemical functions.

Chen, C.; Junjun Tan, Ming-Chien Hsieh, Ting Pan, Jay T Goodwin, Anil K. Mehta, Martha A. Grover, and David G. Lynn **2017** Design of Multiphase Dynamic Chemical Networks, *Nat Chem*, doi:10.1038/nchem.2737. Omosun, T.O.; Hsieh, M-C.; Childers, W.S.; Das, D.; Anil K. Mehta, AK; Anthony, NR; Pan, T.; Grover, M.A.; Berland, K.M.; and Lynn, D.G. **2017**, Catalytic Diversity in Self-propagating Peptide Assemblies, *Nat Chem.* doi:10.1038/nchem.2738

YI-L72 Palladium in Chemical Protein Synthesis and Manipulation

Muhammad Jbara, Suman Kumar Maity, Shay Laps, Guy Mann, Guy Kamnesky, and Ashraf Brik
Schulich Faculty of Chemistry, Technion-Israel Institute of Technology Haifa, 3200008 (Israel)

Despite great advances in chemical and semi-synthesis of protein there are several challenges remain to be solved. One of these is the need for practical protecting groups for the synthesis of uniquely modified proteins. Thiazolidine (Thz) and Acetamidomethyl (Acm) protecting groups are widely utilized in peptide and protein synthesis, however, the reaction time and the harsh conditions of their removal limit their utilities. Developing optimal removal conditions for theses protecting groups remains a challenge. We have recently reported that water-soluble palladium (II) complexes



are excellent reagents for rapid (within 15 min) unmasking of Thz under NCL conditions.[1] Moreover, palladium (II) complexes are also able to rapidly remove propargyloxycarbonyl (Proc) protecting group from the N-terminal Cys in a similar efficiency. The utility of these conditions was exemplified in the efficient total chemical synthesis of Lys34-ubiquitinated H2B (H2BK34Ub) and neddlyated peptides.[1] Based on these conditions we also developed a new approach to control polypeptide structure by introducing a cleavable backbone thiazolidine linkage in peptides and proteins.[2] For the first time we showed also rapid and efficient Acm removal using palladium (II) complexes in fully aqueous medium, and demonstrated its use in the synthesis of ubiquitin like protein (UBL-5).3 This approach was also extended to the rapid cleavage of phenylactamidomethyl (Phacm) linker functionalized with a solubilizing tag for the synthesis of hydrophobic peptides and demonstrated its utility in the total chemical synthesis of Histone 4 protein (H4).[4]

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L73 Peptide-CRM197 Conjugate Vaccines – Considerations for Process Development

Anouk Dirksen
Pfizer Inc.

Cross Reactive Material 197 (CRM197), an inactive form of Diphtheria Toxin with a single point mutation in position 52 (G52E), is widely used as a carrier protein for conjugate vaccines. Modification of CRM197 leads to changes in its structure and properties. These changes have been found to impact stability and potentially immunogenicity and, as a result, need to be controlled. Key observations made during process development of peptide-CRM197 conjugate vaccines and a systematic approach to gain scientific understanding around these observations will be presented.

L74 Unlocking the Mysteries of Amyloid Diseases with Macrocyclic β-Sheet Peptides

Dr. James S. Nowick
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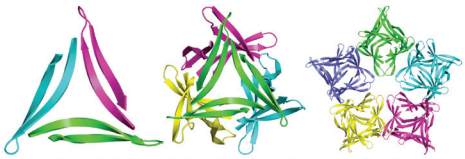
Amyloid oligomers have emerged as the key toxic species in amyloid diseases. Our laboratory is determining the structures and mechanism of action of oligomers of peptides and proteins associated with Alzheimer's disease, Parkinson's disease, frontotemporal dementias, type II diabetes, and other diseases involving protein aggregation. We are able to obtain high-resolution structures by constraining fragments of the peptides and proteins to β-hairpins and determining the structures of the oligomers that form by X-ray crystallography. Through these studies, in conjunction with biophysical and cell biology experiments, we are gaining new insights into the molecular basis of amyloid diseases.

Alzheimer's disease has been a major focus of our efforts. The 40-42 amino acid peptide Aβ aggregates to form fibrils and toxic oligomers. While the fibrils and the resulting plaques are the visible hallmark of the disease, the soluble oligomers are now thought to be the damaging species responsible for neurodegeneration. By constraining peptides derived from Aβ to a β-hairpin conformation and preventing fibril formation by *N*-methylation, we have discovered that triangular trimers constitute a fundamental building block of amyloid oligomers. Through X-ray crystallography, we have elucidated high-resolution structures of the trimers, as well as the hexamers, dodecamers, and annular pores that the trimers form. We are now beginning to correlate the biophysical and biological properties these oligomers with those formed by full length Aβ. This talk will describe our ongoing studies.

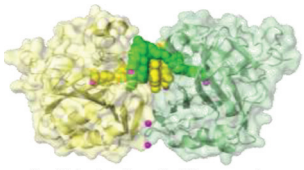


*X-ray crystallographic structures of a trimer, dodecamer, and annular pore formed by an $A\beta_{17-36}$ β-hairpin. (JACS **2016**, 138, 4864. PDB 5HOX)*

L75 Aromatic Foldamer-based Protein Mimicry and Recognition

I. Huc
European Institute of Chemistry and Biology, CRNS-Univ. Bordeaux, 2 rue Robert Escarpit, 33600 Pessac, France

Aromatic amide oligomers constitute a distinct and promising class of synthetic foldamers — oligomers that adopt stable folded conformations. Single helical structures are predictable, show unprecedented conformational stability, and represent convenient building blocks to elaborate synthetic, very large (protein-sized) folded architectures. They possess a high propensity to assemble into double, triple and quadruple helices, or to fold into sheet-like structures. Cavities can be designed within such synthetic molecules that enable them to act as artificial receptors and molecular motors. Water soluble analogues of these foldamers show promise in nucleic acid and protein recognition. This lecture will give an overview of the design principles of these functional molecular architectures.



Crystal structure of a protein-foldamer complex.

L76 Thioamide Probes for Studying Peptide Stability *In Vitro* and *In Vivo*

E. James Petersson
University of Pennsylvania

Our laboratory has demonstrated that thioamide substitutions of the peptide bond can be used as fluorescence quenchers to study protease activity, where thioamide placement allows one to observe cleavage at particular sites. During our investigations of protease sensor design, we have found that thioamide substitutions at the scissile bond can dramatically reduce cleavage of peptides by proteolysis. We have exploited this discovery to make versions of several important signaling hormones, including GLP-1, GIP, and NPY, that are stabilized by up to 1000-fold relative to native peptides, while retaining biological activity. Such stabilized peptides can be used as injectable therapeutics or imaging agents, and thioamides offer a facile way of making stable versions of newly discovered peptides for *in vivo* investigations.

L77 Targeting RNA with Protein Evolution Leads to Cyclic Peptide Drug Leads

Brian R. McNaughton
Colorado State University, Department of Chemistry, Department of Biochemistry & Molecular Biology, Fort Collins, Colorado, 80521, USA

Innovative approaches are needed to create new therapeutics that target HIV. Existing drugs can prolong patient lifespan by targeting multiple facets of the viral life cycle, but next-generation therapies are needed that act on new targets — especially those that resist mutation. HIV-1 TAR RNA is a validated drug target that is highly resistant to mutation. TAR binds to Tat, among other proteins, and this interaction is essential for proviral transcription and HIV-1 propagation. So far, TAR has evaded discovery of compounds with sufficient affinity and selectivity to warrant pharmaceutical development. To address this challenge, we undertook a 'semi-design and

protein evolution' approach that yielded many novel, high-affinity (K_D ~ 1.3 to 0.5 nM) TAR Binding Proteins (TBPs). We have determined the 1.80 Å resolution co-crystal structure of one variant, TBP6.7, in complex with TAR, revealing that the major binding interface consists of evolved loop β2-β3, which reads out the TAR RNA loop and upper stem. Most recently, we: (1) validated the observed TBP6.7-TAR interface; (2) synthesized cyclic peptide drug leads—derived from our crystal structure—that bind TAR and inhibit its interaction with Tat, and; (3) assessed the ability of these cyclic peptides to inhibit HIV-1 propagation in mammalian cells. Taken together, this talk will describe research that dovetails protein evolution of a new protein-RNA interaction, evaluation of the protein-RNA complex, and structure-guided development of cyclic peptide inhibitors of HIV-1 propagation.

References:

Blakeley, B.D.; Shattuck, J.; Coates, M.B.; Tran, E.; Laird-Offringa, I.A.; McNaughton, B.R. "Analysis of Protein - RNA Complexes Involving an RNA Recognition Motif Engineered to Bind RNA Hairpins with 7- and 8-Nucleotide Loops" *Biochemistry* **2013**, *52*, 4745.

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Crawford, D.W.; Blakeley, B.D.; Chen, P.-H.; Sherpa, C.; Le Grice, S.F.J.; Laird-Offringa, I.A.; McNaughton, B.R. "An Evolved RNA Recognition Motif That Suppresses Tat/TAR-Dependent Transcription" *ACS Chemical Biology* **2016**, *11*, 2206.

L78 Inventing Synthetic Peptide & Protein Chemistries to Reveal How Enzymes Work

Stephen Kent
University of Chicago

From its origins, the goal of peptide chemistry was to understand the molecular basis of enzyme catalysis. In modern terms, using chemistry you could *in principle* control every aspect of the properties of an enzyme-substrate complex, and thus probe in detail the physical organic chemistry of enzyme-catalyzed reactions.

Effective total synthesis of enzyme protein molecules is needed in order to experimentally implement this concept. Today I will describe the evolution of solid phase peptide and native chemical ligation synthetic methods, driven by total chemical syntheses of the HIV-1 protease (HIV-1 PR). Case studies of HIV-1 PR will include 'backbone engineering' to quantitatively evaluate catalytic dipoles, and 'Ramachandran space engineering' to probe the role of the flexible HIV-1 PR flaps in catalysis. Site-specific ^{13}C labeling was used to reveal the ionization states of the catalytic Asp side chains.

The synthetic tools developed in order to study HIV-1 PR have enabled us to apply chemistry to a wide range of other protein molecules. Proteins with enhanced therapeutic properties have been developed. Protein enantiomers and diastereomers have been used to probe protein folding and stability. Novel topological protein analogues not found in nature have been designed and built. (Quasi)racemic crystallography has been used to elucidate the structures and molecular basis of function of recalcitrant proteins.

In the near future, advanced physical techniques enabled by total chemical synthesis of proteins will reveal a detailed understanding of the physical organic chemistry of enzyme catalysis. Then, it will be possible to *'de novo'* design, and use chemistry to build, protein-inspired molecular devices with predetermined catalytic properties.

Rainbow Park Beach

Peak2Peak

POSTER ABSTRACTS

YI-P001 AntiMicrobial Peptide Database, a Collaborative Bioinformatic Toolkit

Abraham Herrera, Tripti Garg, Sunandha Acharya, Joan Peckham, Lenore Martin
URI

The Internet has become a significant instrument in our day-to-day activities — from an entertainment source to a research tool linking people and information through computers and other digital devices. In this age of "Big Data", we are constantly exposed to stimuli that can make us forget that the primary objective of the World Wide Web is to foster collaboration: the support of group discussions, the exchange of electronic correspondence, and access to distant databases. Having useful information available and accessible from a user-centered database is as important as being able to process this information and use it in research projects.

The Antimicrobial Peptide Editable Database (AMPed) database, developed by Professor Martin's peptide research group at the University of Rhode Island is a high quality annotated collection of normalized data describing antimicrobial peptides, their sources, their 3D structures, and their target organisms. AMPed is designed to enable users to perform quick queries as well as enabling the entire peptide community to contribute to the repository. The AMPed database and website have all the user and administrative features gleaned from diverse existing databases in order to become the number-one repository of antimicrobial peptide information worldwide.

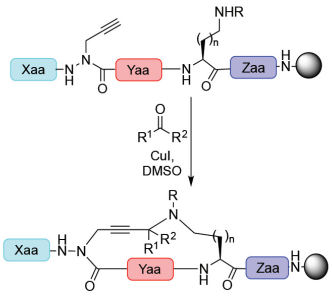
The cross-disciplinary team that designed and implemented this project went through multiple design iterations in search of a general method for organizing information about peptides in a format that is open-source and endlessly able to expand to accommodate user needs. A suite of software tools that will assist the user in analyzing the data is underway.

YI-P002 Cyclic Azapeptide Synthesis by A^3-Macrocyclization

Ahsanullah, Jinqiang Zhang, Ramesh Chingle, Ragnhild Ohm and William D. Lubell
Department of Chemistry, Université de Montréal, Montréal, QC, Canada

A series of potent cyclic azapeptide analogues of Growth Hormone eleasing Peptide-6 (GHRP-6) have been recently synthesized by a diversity-oriented A^3-macrocyclization approach eaturing copper-catalyzed addition of Mannich reagents onto propargylglycine residues.[1] Our presentation will focus on current developments in the study of the scope and limitations of this novel synthetic method for making cyclic peptides.

1. Zhang, J.; Mulumba, M.; Ong, H.; Lubell, W. D. "Diversity-Oriented Synthesis of Cyclic Azapeptides by A^3-Macrocyclization Provides High-Affinity CD36-Modulating Peptidomimetics." *Angew Chem Int Ed Engl.* **2017**, doi:10.1002/anie.201611685.



YI-P003 Redesigning Hemolytic Peptides for cCytosolic Antibody Delivery

Misao Akishiba[1], Toshihide Takeuchi[1], Yoshimasa Kawaguchi[1], Kentarou Sakamoto[1], Ikuhiko Nakase[2] and Shiroh Futaki[1]
[1]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan; [2]Nanoscience and Nanotechnology Research Center, Research Organization for the Twenty First Century, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan

Antibodies have high abilities in molecular recognition and targeting. Intracellular antibody delivery could thus achieve controlling cellular events, such as protein-protein interaction and post-translational modification. This suggests the potential applicability of antibodies to attack intracellular therapeutic targets. Many approaches for intracellular delivery of biomacromolecules have been reported up to the present time. However, few of them are efficient enough to deliver high-molecular-weight proteins such as antibodies into cytosol to effectively modulate cell functions.

In order to deliver various membrane-impermeable molecules into the cytosol effectively and efficiently, we developed a novel endosome-destabilizing peptide by engineering the structure of a hemolytic peptide derived from a spider toxin. This peptide showed significant stimulation of cytosolic release of endocytosed molecules, including polydextran (10kDa), Cre recombinase and antibodies (IgG). Here, we demonstrate successful recognition of intracellular targets by the intracellularly delivered antibodies which was confirmed by confocal microscopic analysis and the effect on signal transduction.

YI-P004 pH Switchable β-sheets

Jordan M. Anderson[a] and Niels H. Andersen[a]
[a]University of Washington, Department of Chemistry Seattle, WA 98105, USA

Conformational switching has emerged as an exciting method to control when a protein is in an active state. Usually done by coupling a motion to a binding event, pH change or light irradiation, most designs require large protein specific structural changes or the incorporation of non-natural entities. Herein we describe a pH switchable β-turn (-HPATGK-), which, when mutated into a fold-nucleating hairpin of a larger structure, can create a β-sheet which is fully folded at physiological pH and unfolded at low pH. Using a standard three-stranded

sheet model (the WW domain), it was found (using NMR and circular dichroism CD) that upon acidification (from pH 8 to pH 2.5) the molar fraction folded (χ_F) changed from 0.97 to < 0.26 ($\Delta\Delta G_F$ = > 11 kJ/mol). It was also found that this effect was still seen when the turn was in the center of a long loop (-G4HPATGKG4-), with a reduction in T_m from 37 °C to < 5 °C, pH 8 and 2.5 respectively. With this advance, conformational control of larger beta proteins should be possible.

P005 A Novel One-Pot Synthesis Strategy for Bicyclic Peptide Assembly

Y. Angell, Y. Wang
Department of Peptide Chemistry, ChemPartner SF, 280 Utah Avenue, Suite 100, SSF, CA 94080

Bicyclic peptides are polypeptides forming two circular units. The cyclic structures often exhibit improved stability, higher potency and bioavailability. Therefore, they are considered as a novel therapeutic class, which lies between small molecules and monoclonal antibodies Bicyclic peptides can be prepared by both solution phase and solid phase synthesis; however, their synthesis remains a challenge. The multiple steps of synthesis, cyclization, and purification often result in low overall yield. Therefore, the synthesis strategy plays a critical role.

We recently synthesized a 13-mer bicyclic peptide, containing one disulfide bridge and one triazole bridge. Multiple synthetic routes were tested, including both on-resin cyclization and cyclization in solution. The original protocol using step-wise cyclization in solution required multiple steps of cyclization and purification, which gave an overall yield of 18%. Using a novel, one-pot synthesis strategy, we were able to carry out two, sequential cyclizations in one reaction solution and purify the final product with only one final purification step to give an increased yield of 30%. For the production of 100mg of final, bicyclic peptide product, the entire synthesis time was shortened by one week using the one-pot reaction protocol. We believe scale up of the reaction process to produce gram quantities of final pure, bicyclic compound is feasible, and no further process development will be needed. This novel strategy is applicable to facilitate the synthesis of a broad range of bicyclic peptides when the preparation of a disulfide bridge and triazole bridge within a single sequence is required.

YI-P006 Application of Leucocin A Peptide and Gold Nanoclusters for Selective Detection of *Listeria monocytogenes*

H. Hossein-Nejad-Ariani[a], T. Kim[a], K. Kaur[a]
[a]Chapman University School of Pharmacy (CUSP), Harry and Diane Rinker Health Science Campus, Chapman University, Irvine, CA, 92618-1908, USA

Listeria monocytogenes is a gram-positive, food-borne pathogen that is frequently the cause of listeriosis. The mortality rate from such infections exceeds 20 percent, which designate the microbe as a high threat to humans.[1] Highly sensitive and selective detection of pathogenic bacterial strains from various samples ranging from human specimens to food samples are one of the many challenges in industry and medical fields.[2] In this study, we specifically target the receptors present on *Listeria monocytogenes* surface using a peptide, to develop a peptide-based assay for selective and quick detection of *Listeria monocytogenes*. We utilized Leucocin A peptide from class IIa bacteriocins which selectively binds to *Listeria monocytogenes* specific receptor, namely, mannose phosphotransferase system permease. Additionally a growing interest on fluorescent gold nanoparticles and more recently gold nanoclusters (AuNC) emerged in the last few decades. These appeared to be practical not only in bioimaging but also in the detection of infectious diseases. AuNCs prepared with $HAuC_{l4}$ and 3-mercaptopropionic acid as a capping agent display high fluorescence with excitation and emission wavelengths of 300 nm and 600 nm, respectively. Leucocin A immobilized on glass surface is used to bind *Listeria monocytogenes* specifically from spiked samples, which are then labelled with AuNCs allowing for quick detection of bacteria with a limit of detection (LOD) of 10^4 bacteria in each 10 microliter sample.

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P007 Doxorubicin Conjugate for Targeting Breast Cancer Cells

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A significant challenge with chemotherapeutic drugs is their toxicity to non-cancerous (normal) cells and tissues.[1] As a result, the clinical application of these drugs is limited by side effects such as nausea, myelosuppression and cardiotoxicity.2 In order to target chemotherapeutic agents specifically to cancerous cells, various methods have been designed. One of the most effective methods is the use of targeting ligands such as peptides that bind appropriate receptors on specific types of tumor cells.[1] Despite promising results with peptides as targeting ligands, incorporating peptides in targeted drug delivery for clinical applications is hindered due to their fast proteolytic degradation *in vitro* and *in vivo*.[2] Here we have designed and synthesized a novel peptide-drug conjugate using a 11-mer peptide 18-4 (NH_2-ABWxEAAYQrFL-$CONH_2$) with high proteolytic stability and specificity for breast cancer cells, an established crosslinker, SMCC, which was used in a FDA approved drug conjugate, and a chemotherapeutic agent doxorubicin (Dox).[1-3] First, we conjugated peptide 18-4 to SMCC crosslinker.[3] And then, using Traut's reagent, Dox was thiolated to facilitate the conjugation of peptide-SMCC to Dox. We hope that the conjugation of peptide 18-4 to Dox through SMCC linker will improve the stability (half-life) and in vivo properties such as therapeutic efficacy of potentially many chemotherapeutic drugs including Dox.[3]

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P008 Synthesis of Synthesis *O*-Sialylglycopeptide Using TFA-labile Protecting Groups

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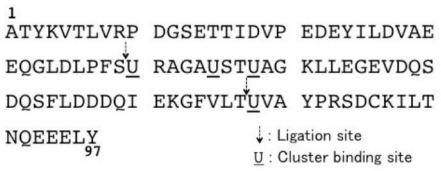
Recently, our group realized the efficient synthesis of glycopeptide thioesters using the TFA-labile labile protecting groups for alcohols of the carbohydrate.[1] In the synthesis, the glycosylated amino acid carrying the TFA-labile protecting

groups on carbohydrates was synthesized and used for the Fmoc solid-phase peptide synthesis. The advantage of this method is that the deprotection of the carbohydrate portion can be simultaneously carried out during the peptide deprotection by TFA cocktail. In this presentation, the strategy was applied to the synthesis of sialylated *O*-glycopeptide. The synthesis of sialyl-glycopeptide is challenging, since α-sialyl linkage is acid sensitive and gradually cleaved even under slightly acidic conditions.

The sialylgylcoamino acid protected with TFA-labile groups, 4-methoxyphenylmethyl (MPM) group for sialic acid and 4-methylbenzyl (MBn) group for *N*-acetylgalactosamine moiety, was prepared via α-selective sialylation followed by glycosylation with Ser derivative. It was then used for the solid-phase synthesis of peptide thioester composed of the repeating unit of MUC-1 by the *N*-alkylcysteine-assisted thioesterification method.[2] The obtained thioester was used for the ligation by the thioester method[3] and successfully afforded the MUC-1 tandem repeat peptide carrying sialyl-Tn antigens.



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P009 *N*-2-Hydroxybenzyl-Cysteine Peptides as Efficient Thioester Surrogates for Native Chemical Ligation

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Recent advances in Fmoc-based solid phase synthesis of peptide α-thioesters significantly contributed to push further the boundaries of the convergent synthesis of proteins via native chemical ligation (NCL). Complementary to other methodologies, β-mercapto amide-based thioesterification devices exploiting an *N*→S acyl shift rearrangement have emerged. If most systems require an acid catalysis, a few can rearrange *in situ* under neutral NCL conditions: peptides bearing such devices are called crypto-thioesters. To improve ligation kinetics of existing systems, which are markedly slowed down as compared to preformed thioesters, we recently reported an *N*-(2-hydroxy-4-nitrobenzyl)cysteine-based device (*N*-Hnb-Cys)[1] which allows routine automated synthesis of crypto-thioesters from inexpensive building blocks. Conveniently, no post-SPPS steps are required, and these thioester surrogates are perfectly stable to handling, storage and purification. We demonstrated the potential of this methodology for the synthesis of long,[1a,d] cyclic[1b] and N-terminal Cys-containing[1c] disulfide-rich peptides. To assess the scope and limitations of the method, we undertook systematic kinetics studies that showed that NCL reactions using *N*-Hnb-Cys-based crypto-thioesters are only 5-to-6 fold slower than those using a benchmark preformed alkyl thioester under optimal NCL conditions. Such fast kinetics likely arise from our bio-inspired design, aimed at mimicking intein-like intramolecular catalysis with a well-positioned phenol group.[1a] To rationally design further optimized devices, we aimed at understanding the molecular bases of the fast rearrangement. These efforts gratifyingly led to a very promising 2nd generation device displaying NCL kinetics close to preformed alkyl thioesters.

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YI-P010 Bioactive and Stabilized Cationic Peptide Capped Metallic Nanohybrid Materials

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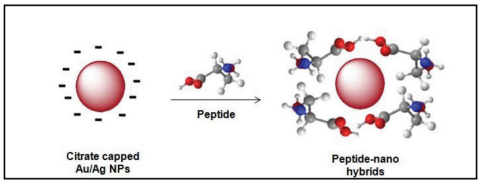
Peptide based biomolecules have been known to be an important component of the innate immune systems of a wide variety of animals, plants and bacteria.[1] Recently, short cationic peptide based mimics have proven to be consisting of antimicrobial properties individually.[2,3] Also, different size and morphology based gold/silver nanoparticles (AuNPs/AgNPs) has been reported using natural amino acids as reducing/capping agent.[4] However, the combined effect of metallic nanoparticles and short peptides has not been explored yet for biomedical applications. The present work discusses the synthesis of short cationic peptide labeled AuNPs/AgNPs and their characterization using various spectroscopic and microscopic techniques (Scheme 1). The synthesized peptide-nano conjugates were observed to possess potent efficacy against tested microbial strains. Notably, it is the first extensive study that discusses the conjugation of dipeptides with metallic nanoparticles at molecular level as most of the earlier works in this direction have been performed with proteins and larger peptides; thereby further highlighting the cost effectiveness of the present approach.



Scheme 1. AuNPs/AgNPs based novel peptide nanohybrids

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YI-P011 Structural Studies on the Mechanism of Action of Lacticin 3147, a Two Peptide Lantibiotic

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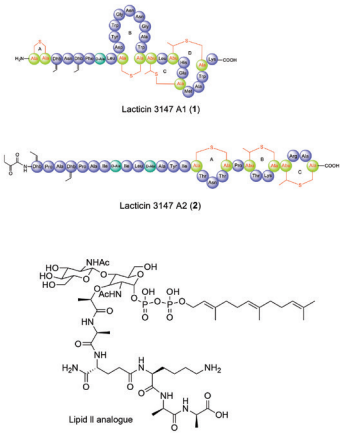

Lacticin 3147 is a two peptide lantibiotic produced by *Lactococcus lactis* subspecies *lactis* DPC3147. Its constituent A1 and A2 peptides synergistically exhibit strong antimicrobial activity in nM concentration against a broad range of Gram-positive bacteria (including antibiotic resistant organisms).[1] It is known that some antimicrobial peptides exert their activity through inhibition of bacterial formation of peptidoglycan and/or through creation of pores in bacterial membranes. Some peptides also interact with the precursor of peptidoglycan, namely lipid II.[2] We have examined the mechanism of action of lacticin 3147 A1 and A2 peptides, and their interaction with lipid II and its analogs, using a variety of techniques, including isothermal titration calorimetry (ITC), fluorimetry and nuclear magnetic resonance (NMR) studies. The results provide a three dimensional picture of peptide-lipid II interaction and of the function of the A1 and A2 peptides.

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P012 Engineering Hybrid Peptidomimetics for Improved Pain Treatments

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To address the different types of pain, different classes of medications, mainly non-steroidal anti-inflammatory drugs and narcotics (opioids), are used. The alleviation or treatment of moderate to severe pain states, in particular, commonly invokes the use of opioids. Unfortunately, their chronic administration induces various undesirable side effects. One strategy to overcome these major side effects and to prolong the antinociceptive efficiency of the applied drugs involves the creation of multifunctional compounds which contain hybridized structures.

Combination of opioid agonist and antagonist pharmacophores in a single chemical entity has been considered and extensively investigated, but opioids have also been combined with non-opioid bioactive neurotransmitters and peptide hormones that are involved in pain perception (e.g. substance P, neurotensin, etc.).[1] Such novel chimeras (also called designed multiple ligands or DMLs), may interact independently with their respective receptors and potentially result in more effective antinociceptive properties. The designed multiple ligands presented in this work include peptide-based opioid-non-opioid dimer analogs, such as for example opioid-neurokinin 1 receptor,[2,3] opioid-nociceptin[4] and opioid-neuropeptide FF DMLs.[5] Some of the prepared ligands demonstrated to be dually effective in both acute and neuropathic pain models. Additionally, compounds with reduced (cross-)tolerance (with morphine) and respiratory depression were unraveled.

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YI-P013 Probing the Protein-protein Interaction between Mint-2 and Amyloid Precursor Protein as Putative Treatment for Alzheimer's Disease

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Protein-protein interactions (PPIs) are vital for cellular and biochemical processes and hence are promising drug targets. However, targeting PPIs is often challenging as the binding sites are typically shallow and devoid of obvious binding pockets.

The Munc18-interacting (Mint) protein family, Mint1-3, are multidomain scaffolding proteins comprising a phosphotyrosine binding domain (PTB) and two PSD-95/discs large/zonula occludens 1 (PDZ) domains. The two members, Mint-1 and Mint-2, are primarily expressed in neurons and being assigned to key functions in synaptic vesicle exocytosis, protein transport and synapse formation. Furthermore, Mint-1 and Mint-2 are important for amyloid precursor protein (APP) processing through a direct interaction via the endoplasmatic sorting motif of APP with the PTB domain in Mint. Proteases process APP into the plaque forming Aβ peptide, which is the main component of the toxic amyloid plaques found in excess in brains of patients suffering from Alzheimer's disease.

By mapping the interaction between APP and Mint-2, we envisioned to design novel PPI inhibitors for the Mint-2/APP-interaction. After determining the minimal binding peptide sequence of APP, this peptide was subjected to extensive mutational scans allowing us to highlight structural properties in the APP sequence crucial for the interaction with Mint-2. Based on this data, constrained peptides characterized by significantly improved affinity and metabolic stability have been developed. Lead compounds have further been tested in neurons.

P014 Inhibition of Ebola Virus Infection using Hydrocarbon-stapled Alpha-helical CHR Peptide Mimics

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Ebolavirus (EboV) is a fatal disease that causes severe hemorrhagic fever. Viral infection of a host cell is mediated by a transmembrane envelope glycoprotein (GP) via formation of a six-helix bundle, consisting of trimeric N-helix and C-helix regions (NHR and CHR). Once the virus is taken up by the host cell's endosomal system, viral entry is mediated by fusion with the endosomal membrane. Following endosomal maturation, collapse of an extended "pre-hairpin" intermediate into the six-helix bundle completes the membrane fusion and allows for viral entry. We hypothesize that hydrocarbon stapled peptide mimics of CHR could inhibit viral entry by blocking this 'pre-hairpin' collapse.

We synthesized a family of small inhibitor peptides designed to mimic the structure and function of CHR in the critical six-helix bundle. Here we demonstrate by circular dichroism (CD) spectroscopy that hydrocarbon stapling enforces the peptide helical secondary structure which is maintained when analyzed under acidic and thermal denaturing conditions. We also measured biophysical properties such as binding affinity and stability enhancement of designed a 5-helix EboV construct in complex with our stapled peptides to probe our mechanism of action.

Fluorescently-tagged stapled peptides were synthesized to visualize cellular uptake of our peptides by fluorescent microscopy. Additionally, cellular analysis of the stapled peptides confirm Ebola virus infectivity is significantly reduced (5-fold) relative to wild type CHR in Huh7 cells after 48 hours. We also measured favorable cytotoxic levels with half of the peptides analyzed. These results confirm that CHR stapled peptide mimics are effective therapeutics for inhibiting Ebola virus infection.

YI-P015 New Strategies for the Development of Selective and Optimized Delta Opioid Receptor Agonists Inspired from Leu-Enkephalin

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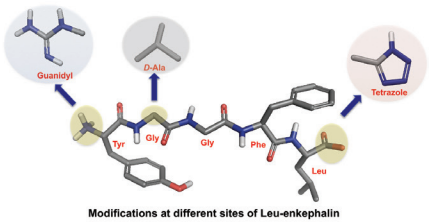
Clinical treatment of pain relies mainly on Mu opioid receptor (MOP) agonists such as Morphine, associated with many adverse side effects (constipation, addiction, dysphoria, somnolence…). In the case of patients with chronic pain, allievement can barely reach 50 % efficacy, making long-term treatment harmful. Studies show that selective activation of the Delta Opioid Receptor (DOP), could provide good analgesia without the adverse effects linked to traditional opioids. Due to their relative selectivity for DOP over MOP, the simplicity of their synthesis, and their low toxicity, endogenous peptides such as enkephalins (Tyr-Gly-Gly-Phe-Leu/Met) are key targets. However, they suffer from poor blood brain permeability and plasma stability, due to quick enzymatic degradation.



Modifications at different sites of Leu-enkephalin

This issue in mind, several analogues of Leu enkephalin were designed and synthesized to improve their pharmacokinetics profile. To do this, modifications were carried on the free ammonium group of Tyr, the carboxylic acid of Leu, and the side chain of Gly2. Furthermore, several modifications on the Phe residue were also tested, to study the influence of phenyl ring substitution on these parameters. Finally, such strategies were applied to macrocycles analogues, since they are also known for improving permeability and stability. Their biologic profile toward DOP and MOP were then assessed. Preliminary results show sub nanomolar DOP affinity and potency for some candidates, while dramatically improving plasma stability. Thus, such strategy could be of interest by improving blood brain permeability and plasma stability, while keeping good activity at DOP.

YI-P016 Synthesis, Antimicrobial Activity and Structural Analysis of Pediocin PA-1 and its Analogs

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Bacteriocins are an attractive alternative to antibiotics in the food, medical and veterinary sectors[1]. Pediocin PA-1, a small bacteriocin of the class IIa family, is an antibacterial peptide of 44 amino acids containing two disulfide bonds isolated from *Pedioccocus acidilactici*[2]. Even if members of this class have been widely studied, only few structures have been determined to date. In the case of pediocin PA-1, the presence of a readily oxidizable Met residue makes the synthesis, purification and structural analysis significantly challenging. Using a combination of different synthetic strategies, pediocin PA-1 and various pediocin-derived peptides were prepared to perform structure-activity studies and structural analysis by NMR. The total synthesis and characterization of pediocin PA-1 and various analogs, their antimicrobial activity and the determined 3D structure of an antilisterial pediocin PA-1 analog will be presented.

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P017 Genetically-encoded Fragment-based Discovery of Glycopeptide Lisgands for DC-SIGN

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[a]*University of Alberta;* [b]*Imperial College London*

In this project we have employed genetically encoded glycopeptide libraries to identify ligands for dendritic cell-specific ICAM3 grabbing non-integrin (DC-SIGN). Dendritic cells play a major role in both innate and adaptive immune responses. These important cells survey, capture and process antigens for presentation to the immune system and thus, are the first-in-line natural targets for antigen delivery.

Presentation of antigens by dendritic cells increases by 100-fold when specific uptake receptors are engaged. In dendritic cells, the majority of these receptors recognize carbohydrate-based ligands. DC-SIGN is of particular interest, as it recognizes both high-mannose and Lewis^X containing glycans. Although compounds that bind DC-SIGN have been the subject of many publications, further development of synthetic ligands with higher affinity and specificity for DC-SIGN, compared to other receptors, remains an important and unsolved problem.

To this end, we have applied the recently developed technology of genetically-encoded fragment-based discovery (GE-FBD) to identify novel glycopeptides with specific affinity to DC-SIGN. In GE-FBD, a canonical, readily available phage-displayed library of peptides is equipped with a monosaccharide fragment. The monosaccharide fragment serves as an anchor domain for targeting of the glycan-binding site. This anchor domain assists in the search for a synergistic peptide sequence that has affinity for an uncharacterized secondary binding site that is proximal to the glycan-binding site of DC-SIGN.

Using GE-FBD methodology on multimeric DC-SIGN extracellular domain (ECD), we have identified several glycopeptides with higher potency than the parental monosaccharide ligand anchor domain. These compounds represent the first examples of a novel class of inhibitors for DC-SIGN.

P018 Catalytically Active Peptide Foldamers

Berlicki,L. Szefczyk, E. Weglarz-Tomczak, M. Drewniak, K. Ozga, A. Krzyszton, E. Rudzinska-Szostak, J. Krzciuk-Gula
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De novo construction of protein-like three dimensional structures using peptide scaffolds remains a great challenge. The incorporation of constrained, cycloalkane-based β-amino acid in the peptide chain is one of the possibilities of rational approaches to this problem. It has already been shown that α/β-peptide foldamers can fold to different secondary structures but studies on more complex systems are rare.

Here, we would like to present the results of exploration of structures and functions of α/β-peptide foldamers containing *cis*-2-aminocyclopentanecarboxylic acid residue. Initially, short peptides with various sequence patterns (αβ, ααββ, ααβ, αβαβ and αααβ) were studied and it was proven that both geometry and handedness of helices could be effectively controlled[1,2]. Subsequently, α/β-peptides containing two helical fragments joined by oligo-glycine linker were designed, synthesized and structurally evaluated. Spectroscopic studies indicated that conformational stability was dependent on the possibility of interactions between helices. Finally, the elaborated foldameric helix-loop-helix scaffolds were used for construction of catalytically active molecules. A set amino acid residues, distributed in geometrical relationship analogous to that observed in native enzymes, was placed on the chosen scaffold. Aldolase and hydrolase mimetics were obtained. In both cases, significant enhancements of reaction rate were achieved and the kinetics of the catalyzed reactions confirmed an enzyme-like mechanism. In summary, we have proven that α/β-peptide foldamers are excellent platforms for construction of enzyme mimetics.

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P019 Blocking Bacterial Transcription Factor Function with Sigma-54 Stapled Peptides

Bernal, F.,[a] Payne, S.R.,[a] Pau, D.I.,[b] Whiting, A.L.,[b] Boddy, C.N.,[a]
National Cancer Institute; University of Ottawa

The emergence of antibiotic-resistant bacteria has outpaced the development of new antibiotics. Thus, research involving novel antimicrobial compounds is highly pertinent to global health. One promising class of molecules is hydrocarbon-stapled alpha helical peptides. Through an all-hydrocarbon restraint incorporated in the amino acid sequence, stapled peptides can exhibit the stabilized alpha-helical structure and target-binding affinity of a biologic, as well as the cell permeability and proteolytic resistance of a small molecule. We have identified sigma factor 54 (σ^{54}). Sigma 54 enables microbial cell survival under nitrogen-limiting conditions, but has also been implicated in the virulence and pathogenicity of several species of bacteria. Sigma 54 interacts with its promoter via several domains, one of which is a highly conserved alpha helix known as the RpoN-box.

We have designed and synthesized a panel of stapled peptides modeled after the RpoN-box. Stapled σ^{54} peptides (Sσ^{54}) incorporate hydrocarbon staples at different positions across the RpoN box designed to enforce alpha-helicity, which we confirmed by circular dichroism spectroscopy. Fluorescence microscopy in tandem with flow cytometry indicate that Sσ^{54} penetrate gram-negative bacterial cells. In addition, in vitro data show that Sσ^{54} bind to the σ^{54} promoter sequence, as well as disrupt production of a σ^{54}-dependent protein, glutamine synthetase. Through effective targeting of σ^{54} promoters, stapled peptides have potential therapeutic value for several types of bacterial infections and related diseases and could prove useful in the quest for novel antibiotics.

YI-P020 Developing Quorum Sensing Modulators in Gram-Positive Symbiotes & Pathogens

Erin Tiwold[a], Jonathan Le[a], Yftah Tal-Gan[b], Michael A. Bertucci[a]
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Regulation of group behaviors in gram-positive bacteria is mediated by short peptide pheromones in a process known as quorum sensing (QS). Our research group is focused on establishing structure-activity relationships for these peptides in human symbiotes of the genus *Lactobacillus* and the human

pathogen *Streptococcus pneumoniae*. A protocol has been established for the synthesis of the cyclic pheromone LamD of *L.plantarum*. A subsequent alanine scan to divulge the influence of side chain interactions in binding and activation is currently underway. In collaboration with the Tal-Gan laboratory, the impact of the hydrophobic face of the peptide CSP-1 in stimulating competence in *S. pneumoniae* is being assessed. Initial results indicate tolerance of single amino acid substitution with nanomolar EC_{50} values comparable to the natural ligand. Obtained dose-response and structural data is being integrated to develop potent agonists and antagonists of the QS pathway.

YI-P021 Generation of Homogeneous Multi-glycan Glycopeptides for HIV Reverse Vaccinology

Michael J. Bird and Philip E. Dawson
The Scripps Research Institute, Department of Chemistry, La Jolla, California

The search for an effective HIV vaccine has been ongoing for many years with limited success in the form of a single trial showing low levels of protection. The only immunogenic surface epitope of the HIV virion is the highly glycosylated envelope protein trimer, Env. Efforts to use soluble Env protein trimers to stimulate development of broadly neutralizing antibodies (bNAbs) have shown little success, and it has been found that the germline precursors of known bNAbs do not bind existing solubilized trimers. As such there is interest in producing smaller epitopes that can direct germline sequences toward the production of bNAbs. One such epitope known to induce potent broadly neutralizing antibodies is the V1/V2 epitope at the trimer apex. This structure forms a greek key motif of four beta-strands, with bNAb binding dependent on appropriate glycosylation at multiple N-X-S/T sites in the monomer sequence, and in some cases on appropriate quaternary structure of the epitopes in the timer. We explore strategies to produce glycopeptides mimicking the native V1/V2 epitope with independent insertion of specific glycans at the various glycosylation sites within the epitope. The glycopeptides resulting from these efforts can be used in binding studies with known bNAbs and their putative germline precursors as early work in the design of a vaccination strategy.

YI-P022 Targeting Strategies for Enhanced Activity of a Novel Class of Antibacterial Peptides, Cationic Amphiphilic Polyproline Helices (CAPHs), for the Treatment of Intracellular Bacteria

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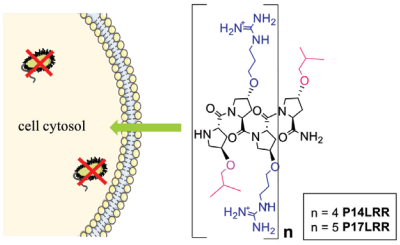

Cell penetrating peptides have shown promise as an effective means of intracellular delivery of therapeutic cargo. Furthermore, serious health threats posed by intracellular bacterial infection emphasize the need to establish new antimicrobial agents. In an effort to address the necessity for new antibacterial therapies, features of cell penetrating peptides were incorporated into the development of a dual action antimicrobial peptide, cationic amphiphilic polyproline helices (CAPHs). CAPHs have proven to be an effective antimicrobial agent to combat an array of both Gram negative and Gram positive bacteria, including the intracellular bacteria *Brucella*, *Listeria monocytogenes*, and *Salmonella enterica*[1,2]. To further enhance the antimicrobial efficiency of CAPHs against intracellular pathogens, targeting strategies, including cell type specificity and subcellular localization, have been investigated for the targeted delivery of CAPHs. Results describing the addition of such targeting capabilities into CAPHs to increase efficacy in the clearance of intracellular bacteria will be presented.

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P023 Design of a Library to Explore the Phallotoxin Chemical Space

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*Phallotoxin family is an excellent template for use in the design of privileged chemical libraries on solid phase in order to study the mechanisms involved in allosteric inhibition of protein targets. Phallotoxins inhibit F-actin depolymerization and are characterized by a defined rigid bicyclic structure consisting of a head-to-tail cyclized heptapeptide with a transannular linkage known as a tryptathionine bridge. The latter is prepared via the Savige-Fontana tryptathionylation of the oxidized tryptophan derivative 3α-hydroxypyrrolo[2,3-*b*] indoline in neat TFA. Therefore, to achieve the synthesis of phallotoxin-based library on solid phase, the linker must be stable both in TFA and during the peptide synthesis. Such a linker was elaborated via a tartrate-based linker. As proof of this concept, following completion of the linker synthesis, a linear phallotoxin precursors was prepared by standard Fmoc-SPPS. The tryptathionine bridge was achieved by the Savige-Fontana reaction and the second cyclization was performed by a head-to-tail macrolactamization, all on resin. The bicyclic peptide was cleaved off from the linker with the mild sodium periodate oxidant, purified by RP-HPLC and characterized by mass spectra and UV spectra. In addition, a modified phalloidin was anchored on solid phase and used as positive control to design a fluorescent F-actin polymerization experiment on phalloidin linked to beads.

P024 Efficient Oxidation of *N*-protected Tryptophan and Tryptophanyl-dipeptides by *in situ* Generated Dimethyldioxirane Provides Hexahydropyrroloindoline-Containing Synthons Suitable for Peptide Synthesis and Subsequent Tryptathionylation

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A series of hydroxypyrroloindoline (Hpi) containing dipeptides along with the corresponding monomeric Hpi-α-amino acid (Hpi-2-carboxylate), were prepared by reacting a series of Nα-protected-tryptophans in aqueous or biphasic [water/cyclopentyl methyl ether (CPME)] solutions containing Oxone® (potassium peroxymonosulfate) and acetone. This procedure

avoids the tedious distillation of unstable dimethyldioxirane (DMDO), which is commonly used to oxidize indoles. Monomers N^{α}-Boc-Hpi-OH and N^{α}-Fmoc-Hpi-OH were readily incorporated by solid-phase peptide synthesis (SPPS) into a peptide containing a cysteine; in trifluoroacetic acid (TFA), the Hpi underwent intramolecular dehydrative condensation with the cysteine thiol to afford the anticipated tryptathionine crosslink. This eco- and user-friendly oxidative methodology greatly simplifies the synthesis of Hpi derivatives while enabling the synthesis of tryptathionine crosslinks characteristic of phalloidin and amanitin, two potent peptide toxins of present interest.

P025 Synthesis on Solid Phase of a Bioactive Tryptathionine Octreotate Analog

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Development of somatostatin analogues (SSAs) plays a central role in the improvement of diagnostic and therapeutic tools in the treatment of neuroendocrine neoplasms (NENs). These analogues target the somatostatin receptors (SSTRs) 1-5 which are often overexpressed on the outer membrane surface of many tumor cells. Poor selective modulation of a single SSTR subtype by SSAs leads to a wide range of side effects, limiting their clinical impact. The high affinity of somatostatin (SST) and its analogues that bind to SSTRs results from a short subsequence (5-9 amino acids), usually within a rigid beta-turn motif. Tryptathionine bridges (Ttn) represent a privilege scaffold for the beta turn pharmacophore and contribute to high affinity, selectivity and significant metabolic stability of peptidic toxins. We have prepared a trimmed somatostatin analogue, whereby the disulfide bond found in octreotate was replaced by a tryptathionine bridge ((Ttn)-TATE). The resulting soluble (Ttn)-TATE displayed a high affinity in vitro for membrane solubilized SSTR2 and for SSTR2 expressed on whole Ar42J cells. Similar results were obtained from (Ttn)-TATE anchored on TentaGel microbeads. This work lays down the foundation for a one-bead-one-compound (OBOC) combinatorial tryptathionine peptidic library to isolate selective and avid binders of a desired SSTR subtype and cellular phenotype outcome.

YI-P026 Synthesis and Spectroscopic Conformational Studies of Ac-Nit-NHMe Peptide Model

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3-Nitrotyrosine (Nit) occurs naturally in organism as a result of oxidative stress and was observed in some neurodegenerative and cardiovascular diseases.[1] To better understand the effect of nitration on structure of peptides, we proposed a synthesis of prototypical peptide model for peptides containing 3-nitrotyrosine residues, Ac-Nit-NHMe (Fig. 1).[1,2] We prepared both enantiomers of Ac-Nit-NHMe and Ac-Tyr-NHMe.

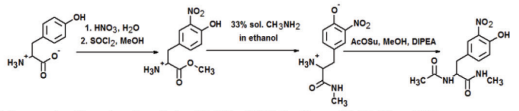

Figure 1: Synthesis of Ac-D-Nit-NHMe from H-D-Tyr-OH

We compared their conformational spaces using MD and DFT simulation in combination with various spectroscopic methods (NMR, IR, Raman, CD, VCD, and ROA spectroscopy) carried out in several solvents.

This work was supported by Czech Science Foundation (reg. no. 17-00121S).

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P027 Ehancing Specific Disruption of Intracellular Protein Complexes by Hydrocarbon Stapled Peptides Using Lipid Based Delivery

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Linear peptides can mimic and disrupt protein-protein interactions involved in critical cell signaling pathways. Such peptides however are usually protease sensitive and unable to engage with intracellular targets due to lack of membrane permeability. Peptide stapling has been proposed to circumvent these limitations but recent data has suggested that this method does not universally solve the problem of cell entry and can lead to molecules with off target cell lytic properties. To address these issues a library of stapled peptides was synthesized and screened to identify compounds that bound Mdm2 and activated cellular p53. A lead peptide was identified that activated intracellular p53 with negligible non-specific cytotoxicity, however it still bound serum avidly and only showed a marginal improvement in cellular potency. These hurdles were overcome by successfully identifying a pyridinium based cationic lipid formulation, which significantly improved the activity of the stapled peptide in a p53 reporter cell line, principally through increased vesicular escape. We then demonstrated that the formulated peptide was efficacious in p53 wild-type SJSA-1 derived xenografts in immuno-compromised mice, where it caused a dramatic reduction in tumor growth. These studies underscore that stapled peptides, which are cell permeable and target specific, can be identified with rigorous experimental design and that these properties can be improved through use with lipid based formulations. This work should facilitate the clinical translation of stapled peptides

P028 Studies on the Interactions of Selective N-Methylated Cyclic SHU9119 Melanotropins with Melanocortin Receptors: Conformational and Docking Studies

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Systematic *N*-methylated derivatives of the SHU9119, with all possible backbone *N*-methylation combinations have led to multiple binding and functional selectivity towards melanocortin receptor subtypes 1, 3, 4 and 5. However, the N-methylation induced conformation changes of backbone and side chains which contribute the melanocortin receptor selectivity is still unknown. In this study we did comprehensive conformational studies in solution of two selective antagonists of the hMC3R from N-methylated SHU9119, namely, Ac-Nle-c[Asp-His[6]-NMe-

D-Nal(2')[7]-NMe-Arg[8]-Trp[9]-Lys]-NH_2 **(15)** and Ac-Nle-c[Asp-His[6]-D-Nal(2')[7]-NMe-Arg[8]-NMe-Trp[9]-NMe-Lys]-NH2 **(17)**. It is shown that the peptides have an anti-parallel β-sheet structure and the pharmacophore (His[6]-DNal[7]-Arg[8]-Trp[9]) occupies a βII-turn like region with the turn centered about DNal[7]-Arg[8]. The analogues with different selectivity showed distinct differences in the spatial arrangement of individual amino acid side chains. We also did molecular interaction studies of these two peptides with homology model of the hMC3R. Earlier chimeric human melanocortin 3 receptor studies revealed insights regarding the binding and functional sites of the hMC3R selectivity. The docking study of peptides **15** and **17** to these sites of the hMC3R revealed that the Arg[8] and Trp[9] side chains are involve in majority of the interactions with the hMC3R binding pocket. While Arg[8] forms polar contacts with D154 and D158 of the hMC3R, the Trp[9] is oriented for π-π interactions with F295 and F298 on the hMC3R transmembrane domain. It is observed that the Trp[9] is critical important for the agonist activity for hMC3R. The lesser the contacts of the Trp[9] with hMC3R the better is the antagonistic activity. The absence of any interactions of the *N*-methyl groups with hMC3R suggests that they are acting as pure conformation control motifs on the ligands

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P029 Fast Synthesis of 84-mer Human Parathyroid Hormone for the Study of Osteoporosis and Hypoparathyroidism

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Human parathyroid hormone (1-84) (PTH) is produced by the parathyroid glands and regulates calcium and phosphate metabolism. PTH acts on PTHR1 receptors to stimulate bone formation and is used as a treatment for osteoporosis and hypoparathyroidism, a rare deficiency of parathyroid hormone[1,2]. There are limited published studies on full length PTH due the difficulty of obtaining the full sequence in high purity[2]. Others have used Boc-chemistry and combinations of Fmoc- based solid phase peptide synthesis (SPPS) with Native Chemical Ligation[3]. Here we explored PTH's complete synthesis using fast protocols on an automated peptide synthesizer, to obtain high purity PTH peptide and it's analogs in a reduced amount of time which can be used to further understand PTH's role in SAR studies or enhancing bioavailability and stability of PTH based therapeutics.

H-SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAP RDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ-NH_2
Figure 1. PTH structure.

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YI-P030 Design of a New N-Terminal Linker for the Capture-and-Release Purification of Biologically-Relevant Disulfide-Rich Peptides

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Disulfide-rich peptides (DRPs) are bioactive natural products binding a wide number of therapeutically-relevant targets. They are considered as promising drugs candidates and pharmacological tools. However, the chemical synthesis of long DRPs (> 40 amino acids) is severely limited by complex and time-consuming HPLC purifications leading to low yields and poor purities. We recently started re-investigating the use of N-terminal linkers as "chemical tags" for the non-chromatographic catch-and-release purification of peptides.[1,2] Such linkers can be selectively introduced at the N-terminus of a target peptide, leaving unreacted truncated acetylated peptides, the main co-products of SPPS. After cleavage from the SPPS resin, the target peptide is immobilized on a second solid support through a chemoselective ligation reaction. A simple filtration step then removes truncated peptides. Cleavage of the linker finally releases the purified peptide into solution. Previously developed linkers involve ligation or cleavage reactions difficultly compatible with unprotected cysteines. We report herein a novel linker fully compatible with cysteine-rich peptides, making use of the native chemical ligation reaction (NCL) for the immobilization step, and its application to the production of several biologically-relevant long DRPs. This work also paves the way to an application to the synthesis of longer proteins, through multiple successive solid supported NCLs.

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YI-P031 DPEG, DNPEG and PyPEG as Novel Polymeric Supports for Membrane-Enhanced Peptide Synthesis

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Membrane Enhanced Peptide Synthesis (MEPS),1 which combines Liquid Phase Peptide Synthesis (LPPS) with organic solvent nanofiltration (OSN), has emerged as a new methodology to tackle the most serious challenges faced by Solid Phase Peptide Synthesis (SPPS). The last methodology (SPPS) is actually the strategy of choice for the preparation

of peptides.[2] The new technology platform (MEPS) offers advantages over SPPS by combining solution chemistry with a simple purification procedure. Herein, screening of three novel soluble polymeric supports for MEPS was carried out. Its application through the preparation of a model peptide was also demonstrated.

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YI-P032 Effect of a Fusion Peptide by Covalent conjugation of a Mitochondrial Cell-penetrating Peptide and a Glutathione Analog Peptide

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Previously, we designed and synthesized a library of mitochondrial antioxidative cellpenetrating peptides (mtCPPs) superior to the parent peptide, SS31, to protect mitochondria from oxidative damage. A library of antioxidative glutathione analogs called glutathione peptides (UPFs), exceptional in hydroxyl radical elimination compared with glutathione, were also designed and synthesized.

Here, a follow-up study is described, investigating the effects of the most promising members from both libraries on reactive oxidative species scavenging ability. None of the peptides influenced cell viability at the concentrations used. Fluorescence microscopy studies showed that the fluorescein-mtCPP1-UPF25 (mtgCPP) internalized into cells, and spectrofluorometric analysis determined the presence and extent of peptide into different cell compartments. mtgCPP has superior antioxidative activity compared with mtCPP1 and UPF25 against H2O2 insult, preventing ROS formation by 2- and 3-fold, respectively. Moreover, we neither observed effects on mitochondrial membrane potential nor production of ATP.

These data indicate that mtgCPP is targeting mitochondria, protecting them from oxidative damage, while also being present in the cytosol. Our hypothesis is based on a synergistic effect resulting from the fused peptide. The mitochondrial peptide segment is targeting mitochondria, whereas the glutathione analog peptide segment is active in the cytosol, resulting in increased scavenging ability.

P033 Synthesis of a Novel Highly Hydrophilic Dimeric RGD Peptide

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In recent years, noteworthy and considerable achievements have been seen in the development of radiolabeled cyclic RGD peptides targeting integrin $\alpha_v\beta_3$ for their use as imaging and therapeutic agents. Many different monomeric and multimeric RGD peptides, in combination with a variety of pharmacokinetic modifiers, prosthetic groups or bifunctional chelators and radionuclides, have been utilized and reported as diagnostic probes in animal studies and human clinical investigations in the areas of oncology and cardiology. Among the multimeric compounds, dimeric RGDs are considered to be the most valuable subclass. We designed the structure and synthesized a novel highly hydrophilic member of this group of peptides. It is an analog of Galacto-RGD_2[1] in which SAA was replaced with another pharmacokinetic modifier (D-Glucamine) to increase the hydrophilic character of the vector peptide and improve the biodistribution of a probe.

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P034 The Role of Protein Kinase C Epsilon in Simulated Hypoxia-induced Cell Damage

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Protein kinase C (PKC) is a signal molecule normally mediating various processes, such as immune responses and cell growth. However, under ischemia and/or hypoxia, PKC activation causes cell damage. PKC includes 12 isoforms, and PKC epsilon, a novel PKC, has shown to regulate mitochondrial ATP-dependent potassium channels, which can collapse the mitochondrial membrane potential if opened too long. Our lab has shown that a selective PKC epsilon inhibitor peptide (N-Myr-EAVSLKPT, MW = 1,054), not a selective PKC epsilon activator peptide (N-Myr-HDAPIGYD, MW = 1,097), significantly reduced infarction when given at reperfusion. To elucidate underlying mechanisms of PKC epsilon, we first evaluated the effects of PKC epsilon inhibitor and activator peptides in cobalt chloride induced cell injury. We used tetrazolium to differentiate metabolically active and inactive cells/tissues and evaluate cell damage by measuring absorbance at 450 nm. After 24 hrs or 48 hrs of simulated hypoxia using cobalt chloride (800 µM, n=6), H9C2 rat myoblast cells, showed cell death of 54 ± 5% or 60 ± 3%, respectively. By contrast, when PKC epsilon inhibitor peptide (n=3), not PKC epsilon activator peptide (n=4), was given after 24 hr incubation of cobalt chloride (800 µM), dose-dependently (5-20 µM) improved cell survival up to 30±11% relative to cells subjected to cobalt chloride incubation alone. However, both PKC epsilon inhibitor and activator peptides (both 5-80 µM, n=5) given before incubation of cobalt chloride (800 µM) did not show an increase in cell viability. In summary, the preliminary results confirm that inhibition of PKC epsilon under hypoxia may salvage cells.

This study was supported by Division of Research and Department of Bio-Medical Sciences at Philadelphia College of Osteopathic Medicine.

P035 Novel Linear and Cyclic Peptides Derived from FGF2 for Anti-cancer Therapy

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Fibroblast growth factor receptors (FGFRs) are a subfamily of receptor tyrosine kinases and have five distinct members including FGFR1, FGFR2, FGFR3, FGFR4 and a closely-related receptor FGFRL1. FGFRs play key roles in tumor progression and are very important therapeutic targets for cancer therapy. Currently, several small molecule inhibitors and antibodies of FGFR are in various stages of clinical trials. In our effort to develop FGFR2-targeting peptides, computer modeling studies using FGFR2 and its ligand FGF2 as a model were performed, resulting in six short peptide hits derived from the FGF2 sequence. After re-synthesis, the six peptides were evaluated for their anti-cancer activity. Among them, the P5 peptide (LQLQAEER) inhibited the proliferation of Du145 prostate cancer cells *in vitro* and tumor growth in vivo in Du145 xenograft mice with a dose-dependent manner. P5 did not show obvious toxicity as revealed from the blood chemistry, liver and renal panel tests. Results from HE staining of mice organs did not show visible lesions after treatment with P5. An ITC assay determined that P5 has variable affinities to FGFR2. FGFR3, VEGFR2 and IGFR. The binding of P5 to FGFR2 on the cell surface was verified by an indirect fluorescence assay. Phosphorylation of ERK was decreased with treatment of P5. After cyclization with a disulfide bond, cyclic P5 retained inhibition of tumor growth in mice but with a little longer half-life *in vivo*. Both linear and cyclic P5 have the potential to be developed into new peptide drugs for cancer therapy.

P036 Specificity and Mechanism of Action of Alpha-helical Membrane-active Peptides Interacting with Model and Biological Membranes by Single-molecule Force Apectroscopy

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Antimicrobial peptides (AMPs) are important and effective components in innate host defenses against infectious pathogens.[1] A large number of antimicrobial peptides, which exhibit broad-spectrum activity against microorganisms, including Gram-positive and Gram-negative bacteria, fungi, protozoa, viruses, and even tumors, have been identified from a wide variety of animals, including humans.[2, 3] In this study, we systematically investigated the specificity of membrane-active peptides (MAPs) on different types of cell membranes and evaluated the effects of MAPs on different large unilamellar vesicles mimicking prokaryotic, normal eukaryotic, and cancer cell membranes using AFM.[4] The charge and hydrophobicity of peptides markedly affect the interaction probability and unbinding force between peptides and liposome membranes. *Acholeplasma laidlawii*, 3T3-L1 and HeLa cells were used to represent prokaryotic cells, normal eukaryotic cells, and cancer cells in AFM experiments, respectively, we found that the interaction probabilities significantly correlate with peptide hydrophobicity on the interactions of different types of cell membranes. Antimicrobial and anticancer activities of MAPs exhibited strong correlations with the interaction probability determined by AFM, which illustrates strong correlations of peptide biological activities and peptide hydrophobicity and charge. Peptide specificity significantly depends on the lipid compositions of different cell membranes, which validates the *de novo* design of peptide therapeutics against bacteria and cancers.

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YI-P037 Design, Synthesis, Pericyclic Chemistry and Biomedical Applications of Azopeptidess

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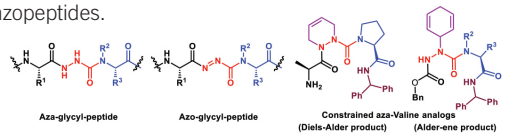
Azopeptides feature an imino urea component that serves as amino amide surrogate. Oxidation of aza-glycine residues has proven effective for making azopeptides which have been employed in pericyclic chemistry and examined by X-ray crystallography.[1-3] Diels–Alder cyclization and Alder–ene reactions on azopeptides enabled respectively access to constrained aza-pipecolyl and azaallylglycinyl residues. Employing the products from azopeptide chemistry as constrained aza-valine analogs, mimics of the Ala-Val-Pro-Ile sequence from the second mitochondria derived activator of caspases (Smac) protein were synthesized and demonstrated ability to induce apoptosis in breast cancer cells.[2,3] Our presentation will describe recent research in the synthesis, pericyclic chemistry and biomedical applications of azopeptides.



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YI-P038 Mechanistic and Structural Studies of Faerocin MK, A Type IIa Bacteriocin

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With antibiotic resistance on the rise, alternative avenues are actively being explored. One such avenue has led to the study of bacteriocins, which are antimicrobial peptides produced by bacteria. Previous work from our group has uncovered a nucleotide sequence in the genome of an Enterococcus species that encodes for a polypeptide with high homology to type IIa bacteriocins, a subclass of these peptides known for their antilisterial properties[1]. The 43-residue peptide, faerocin MK, was chemically synthesized and activity assays confirmed its potent activity against Listeria monocytogenes and other gram-positive bacteria. Genetic and heterologous expression studies have demonstrated that faerocin MK production is dependent on two gene products: the bacteriocin and an immunity protein. This immunity protein, which protects the producing organism from its own bacteriocin, is of interest as it can shed light on the interactions taking place at the cell membrane. The mode of action of these bacteriocins is thought to involve a mannose transport protein complex on the cell surface of gram-positive bacteria; however, this area is not well understood[2]. Our current work is focused on the NMR solution of faerocin MK's three-dimensional structure, with the hope of investigating its mechanism of action.

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P039 Application of Small Synthetic Antimicrobial Peptide (ssAMP) to Control Streptomycin Resistant Pathogens of Citrus Canker Disease.

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Citrus canker caused by X*anthomonas citri* subsp. *citri (Xcc)* decreases the fruit quality and yield significantly. Emerging of streptomycin-resistant (SR) strains threatens the citrus industry seriously because of a lack of proper control agents. It has been suggested ssAMPs could be a promising alternative. Fourteen potential hexapeptides were designed and synthesized based on the positional scanning of synthetic peptide combinatorial libraries (PS-SPCL). Majority of them showed antimicrobial activities against variety of microbes including *Bacillus*, *Pseudomonas*, *Xanthomonas* and *Candida* species. Three hexapeptides, BHC06 and 11, and KCM21 were selected and tested to control citrus canker using 5 years old Sathuma mandarin leaves (*Citrus unshiu*). Each hexapeptide drastically reduce the canker symptom development caused by wild type as well as SR strains. The results showed great potential of ssAMPs to fight against emerging antibiotics-resistant pathogens in agriculture.

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P040 Phage Display Derived Fragment-based Discovery of Antigens for Antibodies Associated with Mycobaterial Infections

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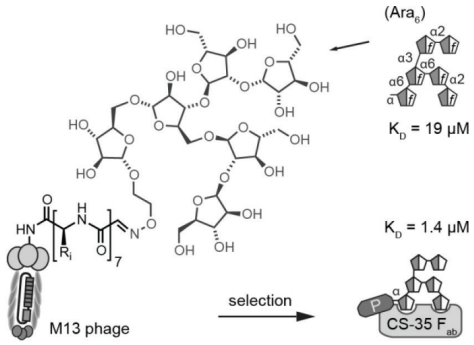
Accurate identification of tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is important for optimal global disease management. Often, the cost and the lack of access to reliable TB antigens limit the effectiveness of TB screening, especially in resource-limited settings. Point-of-care serological tests may improve TB diagnosis; however, commercially available serodiagnostics provide wide ranges of specificities or sensitivities. We described a genetically encoded fragment-based approach that uses selection of phage-displayed glycopeptides to facilitate the search for specific glycopeptide ligands for antibodies associated with mycobacterial infections. Starting from a library of 10^8 glycopeptides with an arabinofuranosyl-containing hexasaccharide moiety (Ara_6), we identified 80 putative hits against CS-35, a well-characterized carbohydrate-binding antibody against the mycobacterial cell wall component LAM. The synthetic glycopeptide hit Ara_6-ANSSFAP exhibited 14 fold enhancement in affinity over the parent carbohydrate Ara_6. Microarray suggested selectivity: Ara_6-ANSSFAP demonstrated pronounced selectivity towards CS-35 over a closely-related carbohydrate-binding antibody 906.4321, whereas the carbohydrate Ara_6 exhibits minimal selectivity. The improved affinity and selectivity from discovered ligands using phage display derived fragment-based approach provides new insight to searching more reliable and effective ligands for antibodies associated with mycobacterial infections.



YI-P041 Scandium(III) Triflate as a Lewis Acid Catalyst of Oxime Ligation

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Imine-forming reactions are widely applicable in bioconjugation settings due to their high chemoselectivity. The ligation of a ketone or aldehyde with an aminooxy functional group to form a physiologically stable oxime bond is often employed to link complex and precious biomolecules. While the reaction proceeds modestly in acidic solution, a low population of protonated carbonyl at pH 7 limits its utility in many biological applications. The use of nucleophilic aryl amines, such as aniline or a phenylenediamine, allows for a high population of protonated Schiff base to form and transiminate to the oxime product. While this method affords up to a 400-fold rate enhancement at pH 4.5, a diminished improvement

of 40-fold is seen at pH 7. Here we employ Scandium(III) trifluromethanesulfonate, a uniquely water-stable Lewis acid, as a co-catalyst with *ortho*-phenylenediamine in the oxime ligation to yield up to an order of magnitude rate increase at near neutral pH.

P042 Taking the Sting out of Tunicamycin: A Novel Approach Significantly Reduces the Cytotoxicity of this Nucleoside Antibiotic

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Antibiotic resistance constitutes one of the most significant challenges to human health, with the continued emergence of multidrug resistant bacteria decreasing the efficacy of existing antibiotics. A worst-case scenario was realized last year when a patient in the USA died from an infection caused by a strain of *Enterobacteriaceae* that was resistant to all available antibiotics.[1] Thus, there is an urgent need for new antibiotics. Tunicamycin is a nucleoside antibiotic that inhibits peptidoglycan biosynthesis by binding to the UDP-MurNAc-pentapeptide phosphotransferase MraY.[2] However, tunicamycin also binds to dolichyl-phosphate-GlcNAc-phosphotransferase in eukaryotes, which is the first committed step in post-translational glycosylation.[3] This results in significant cytotoxicity and therefore removes the utility of tunicamycin as an antibiotic. Our lab has developed new tunicamycin analogues with potent activity against *Mycobacterium tuberculosis* that display very low cytoxicity.

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P043 Understanding the Structure Activity Relationship of the Nrf2-KEAP1 Protein-protein Interaction Site using an Nrf2-neh2 Peptide Library Design Approach

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Oxidative stress has been postulated as a hallmark of HD pathology. The transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2), regulates the expression of antioxidant and cell detoxifying enzymes in response to oxidative stress. Agents capable of activation Nrf2 hold promise for HD.

Cellular basal levels of Nrf2 are regulated by a mechanism involving the binding of Neh2 domain of Nrf2 by the oxidative stress sensor protein keap[1]. It has been proposed that one Neh2 molecule interacts with two identical Keap1 sites via two binding interactions, a stronger binding containing an amino acid motif ETGE and a weaker binding DLG motif. Crystallography studies have revealed that both DLG and ETGE epitopes are complemented by an arginine-rich and highly positively charged keap1 site. Given the fact that this protein-protein interaction is driven by many intermolecular charged interactions, designing a small neutral molecule keap1 binder with adequate binding potency, cellular potency, and most importantly, brain penetration poses a great drug design challenge.

Aided with structure-based tools such as NMR, X-ray crystallography and molecular modeling, we decided to approach this challenge with a strategy to utilize the ETGE neh2 peptide epitope for systematic understanding of the structure activity relationship (SAR) of peptides capable of interacting with the interphase. We specifically aimed at modification of the acidic residues so that this SAR understanding could be applied to designing neutral brain penetrating small molecule that could serve as proof-of-concept tools for HD studies.

YI-P044 Structure-Permeability Relationship of Macrocyclic Semi-Peptides

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Macrocycles have generated an increasing level of attention as drug candidates, for their ability to combine the advantages of small molecules in terms of PK properties, combined with their potential interacting surface areas that are larger than small molecules, which makes them more likely to tackle difficult targets such as protein-protein interactions. Cyclic peptides are known to possess some pharmacokinetic advantage as well over their linear counterparts: their rigid structure tends to reduce the rate of cleavage by proteases — especially at lower ring sizes — and head-to-tail cyclization effectively masks the two polar terminal functions, enhancing their lipophilicity. However, multiple amide bonds remain a source of polarity and it has been observed that N-methylated amides can show increased permeabilities. The similar idea of replacing amino acids by their N-alkylated glycine analogs ("peptoids") also showed improvements.

This project aims to study the structure-permeability relationship of a small library of macrocycles comprising both peptoids and various linking units. Those linkers differ only in the position of a methyl group, as we hypothesized that minute changes in the structure (i.e. its position and stereochemistry) could have a large influence on conformational landscape and thus on permeability. Both PAMPA and Caco-2 assays were performed and show broad range of results, with interesting trends.

YI-P045 Accurate *De Novo* Design of Heterochiral Constrained Peptides

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Short constrained peptides present attractive opportunities for drug design as they can be designed to have specificity like larger proteins, and the cell permeability, shelf life, and oral bioavailability seen in small-molecule drugs. We describe the development of computational methods for *de novo* design of heterochiral conformationally-restricted peptides. We used these computational methods to design peptides with diverse shapes and sizes. These computationally designed peptides were found to be exceptionally stable to thermal and chemical denaturation, and NMR solution structures of the peptides are nearly identical to the design models. These *de novo* design methods are highly accurate, and can be extended further for designing peptides with enhanced bioavailability and cell permeability. These novel computational design methods and the extremely stable scaffolds provide a basis for the generation of new peptide-based drugs.

YI-P046 Developing Covalent Core Constrained Peptide Scaffold to Inhibit Protein-Protein Interactions

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Protein protein interactions (PPIs) function in many aspects of biological processes,[1,2] and often underlie serious human diseases.[3] Here, we propose to develop a covalent core constrained tricyclic peptide as a new class of organic-protein hybrid scaffold with considerable potential to inhibit complex protein-protein interfaces. In this scaffold, we use macrocyclization and crosslinking to constrain the peptide to adopt an exquisitely well-defined tertiary structure which has not been seen before. Cyclization and crosslinking renders these scaffold based peptides much more resistant to protease digestion.[4] The exquisitely well-defined secondary and tertiary structures provide many different surfaces for potential binding with proteins to inhibit protein-protein interactions. The size of this scaffold peptide (~60 amino acids) is smaller than almost all natural proteins, thus it gives us the power to produce the molecule relatively easily either through chemical synthesis or protein expression, and we also have total control to modify the molecules beyond natural occurring amino acids. Through computational design,[5,6] phage display[7,8] and chemical protein synthesis,[9] we plan to develop this intrinsically stable and versatile peptide scaffold as a robust platform for the discovery of various PPIs inhibitors. To showcase the effectiveness and power of our designed peptide scaffold, we aim to develop an inhibitor for PD-1, and ultimately disrupt the interaction of PD-1 and PD-L1, a clinically significant PPI in cancer immunotherapy.[10]

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YI-P047 Supercharging Helical Peptides for Silencing Protein-Protein Interactions through Covalent Irreversible Bonding

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Unlike small organic molecules, helix-constrained peptides have emerged as promising effective modulators of protein-protein interactions (PPIs) involved in human disease, particularly those occurring inside the cell, opening up new opportunities to pharmacologically exploit a range of well-established, yet "undruggable" intracellular targets. However, many reported helical peptides display only moderate cell activity, requiring high doses for in vivo efficacy. Here, we aim to improve potency of helical peptides by applying the concept of covalent inhibition. Covalent targeting has long been exploited to increase therapeutic efficacy of small organic drugs, particularly for irreversible enzyme inhibitors. Over the last years, a steady number of covalent drugs are coming to market, validating the medicinal success of this strategy.

Here we expand the covalent drug scope to include helical peptide macromolecules that act on intracellular PPIs. We insert both a helix-inducing constraint and an appending electrophilic warhead into the peptide ligand BIM to target the oncogenic protein Bcl2A1. Upon binding, the peptide warhead is positioned in close proximity to a reactive cysteine at the protein binding interface allowing selective covalent bonding between the peptide inhibitor and its target Bcl2A1. The modified BIM peptide is capable of entering cells and binding irreversibly to cytosolic Bcl2A1. This innovative approach to increasing receptor residence time of helical peptides demonstrates the potential to selectively silence a PPI inside cells, allowing longer duration of action, non-competition with other endogenous ligands and avoiding peptide clearance once bound to target protein.

P048 Δ-Myrtoxin-Mp1a is a Helical Heterodimer from the Venom of the Jack Jumper Ant with Antimicrobial, Membrane Disrupting and Nociceptive Activities

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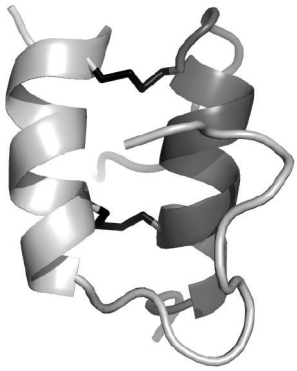

Δ-Myrtoxin-Mp1a (Mp1a), a 49-residue heterodimeric peptide from the venom of *Myrmecia pilosula* is comprised of a 26-mer A chain and a 23-mer B chain connected by two disulfide bonds in an antiparallel arrangement. Combination of the individual synthetic chains via aerial oxidation remarkably resulted in the self-assembly of Mp1a as a homogenous product without the need for directed disulfide bond formation. NMR analysis revealed a well-defined, unique structure containing a pair of antiparallel α-helices. DPI analysis showed strong interaction with supported lipid bilayers and insertion within the bilayers. Mp1a caused non-specific calcium influx in SH-SY5Y cells with an EC_{50} of 4.3 μM. Mp1a also displayed broad-spectrum antimicrobial activity, with the highest potency against Gram-negative *A. baumannii* (MIC = 0.025 μM). Intraplantar injection (10 μM) in mice elicited spontaneous pain and mechanical allodynia. Additionally, non-native analogues explored here showed diminished bioactivities, highlighting the antiparallel connectivity as a crucial feature of this toxin.

YI-P049 Sec-Scan: A New Approach for Reliable Disulfide Connection Assignment

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Cysteine-rich peptides are important leads in drug development. Biological activity of these compounds depends on tertiary structure and correct disulfide bridge connectivities. Known approaches for assignment of disulfide bond connectivities in cysteine-rich peptides imply laborious MS methods or NOE analysis by NMR and often yield ambiguous results. Here, a new approach of disulfide bond connectivity determination using selenocysteine (Sec) scanning (Sec-Scan) and NMR spectroscopy is demonstrated. This approach involved substitution of single cysteines by selenocysteines into a chemically synthesized peptide and comparing 13C Cβ and 1H Hβ chemical shift changes of these single Sec mutants from natural abundance 13C-1H HSQC spectra relative to reference spectra of the native peptide. Our data show that in model peptides and proteins incorporation of a single selenocysteine leads to the same structure as the corresponding native form. Substitution of S atoms by Se results in strong upfield shifts (~8 ppm) of the Cβ atom in Sec itself, but also induced a through-bond upfield shift (~1 ppm) of the cysteine Cβ atom that is present in the mixed Se-S bond. Based on assigned Cβ chemical shifts alone, this observation allows to determine which particular cysteine is covalently attached to the Sec residue that was introduced at a predefined position in the amino acid sequence. Sec-Scan was successfully validated on various-sized peptide and protein model systems with known structures having two to six cysteine residues linked in a complex disulfide network even containing multiple neighbouring CC-sequences (Arg-Vasopressin, Kalata B1, and μ-conotoxin KIIIA). Finally, disulfide connections were established in Evasin-3, a protein with hitherto unknown structure and disulfide connectivity.

P050 Deletion Sequences in Fmoc SPPS – Root Cause Analysis and Prevention Strategies

Frank Dettner
BACHEM

The formation of deletion sequences is one of the most serious problems encountered in solid phase peptide synthesis (SPPS). Deletion sequences only differ by the lack of one or a few amino acids from the desired target peptide and are typically challenging to separate in later purification steps. The longer the peptide sequence the more difficult the separation of these impurities usually becomes. This can be regarded as a major drawback of stepwise linear SPPS.

In this presentation, causes for and experiments regarding the formation of deletion sequences will be discussed. Different strategies to prevent the formation of deletion sequences are applied and will be presented accordingly.

YI-P051 Rational Design and Synthesis of a Highly Potent and Selective Peptide Inhibitor of PACE4 by Additive Stabilization of its β-Strand Conformation and Salt Bridge Interaction at Position P3

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PACE4 is a prostate and ovarian cancer validated target, which belongs to the proprotein convertase's (PCs) family of serine proteases. Our group has developed a potent and selective inhibitor with the following structure: Ac-LLLLRVKR-NH2. We noticed a difference in the S3 pockets of PACE4 and Furin (another member of the PC family). To increase the selectivity of this inhibitor, we exchanged its P3 Val residue with modified basic residues interacting with Asp160 of PACE4 rather than Glu256 of Furin. Thus, we examined different basic amino acids at P3 to optimize the side chain length. We found that the inhibitor with Apg (2-amino-3-guanidinopropionic acid) was the most potent in a series of eleven new analogues.

Proteases substrates form a β-sheet with the active site residues. β-branched amino acids like Val and Ile are known to stabilize β-strand conformations. We synthesized the β-branched analogue of Apg to take advantage of both its "conformation stabilization" and an additional "interaction with Asp160". By replacing the Val residue (at P3) of our original inhibitor with this residue, enhanced selectivity and potency were indeed observed. Comparing to the lead compound, it is two times more selective (40fold vs. 20fold) and eight times more potent for PACE4 (K_i: 2.7nm vs 22nm). Molecular dynamics simulations confirmed the stabilizing effect of branched residues on the β-sheet conformation of our inhibitors. It is worth noting that this discovery could be applied in the drug design of other proteases as well.

YI-P052 The Binding of Nisin to Lipid II: A Chemical and Structural Biology Study

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We are interested in the synthesis and structural analysis of lantibiotic peptides, particularly nisin and its close structural relative mutacin I. These lantibiotics have complex cyclic structures created by the thioether-bridged dipeptides lanthionine and methyllanthionine, which give this class of peptides its name, as well as containing the dehydrated amino acids dehydroalanine and dehydrobutyrine. In order to synthesise truncated analogues of nisin and mutacin I *via* SPPS, ways to introduce these unusual residues have to be found. To this end, 2 orthogonally protected (methyl) lanthionines have been synthesised, as well as a number of different precursors to the dehydrated residues. With these in hand, a number of novel truncated analogues of nisin and mutacin I have been synthesised. Once prepared, the solution phase NMR structures of the peptides were collected and analysed using XPLOR-NIH, as well as that of nisin WT 1-12 obtained from the digestion of a commercially available preparation.[1] From these studies we have observed key conformational differences between the individual rings A and B of the synthesised truncated analogues in comparison to nisin WT 1-12.

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P053 Radiolabeled Tetrameric NGR Peptide for Molecular Imaging of Neovascularization after Myocardial Infarction

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Angiogenesis plays an important role in restoration of blood perfusion after myocardial infarction (MI). CD13 is selectively expressed on angiogenic endothelium and binds the tripeptide motif Asn-Gly-Arg (NGR). Aim of this study was to design and synthesize cyclic Asn-Gly-Arg (NGR)-based imaging probes with improved stability and efficacy for non-invasive SPECT of angiogenesis.

Linear CNGRG-MpaL thioester peptide was synthesized by Boc-based solid-phase peptide synthesis and cyclized using native chemical ligation instead of disulfide bridging. This monomeric cyclic backbone coNGR peptide contained a sulfhydryl group that was conjugated to maleimide-DTPA. Additional to this monomer, a tetrameric coNGR peptide was synthesized by coupling coNGR to a tetrameric scaffold containing a thiaproline (Thz) at the core. The sulfhydryl group of Thz was decrypted and reacted with maleimide-DTPA. Resulting DTPA-$[SMCC\text{-}coNGR]_4$ and its monomeric counterpart DTPA-coNGR were radiolabeled with $^{111}InCl_3$.

Backbone-cyclized mono- and tetrameric coNGR demonstrated a markedly higher stability in blood compared to disulfide-cyclized cNGR. Uptake patterns of ^{111}In-labeled mono- and tetrameric coNGR peptides coincided with CD13 immunohistochemistry on excised hearts. In addition, tetrameric coNGR showed a significantly higher specific uptake in infarcted myocardium compared to monomeric coNGR imaging agent. Dual-isotope SPECT allowed simultaneous imaging angiogenic endothelium and perfusion in infarcted myocardium. Radiolabeled tetrameric coNGR is a promising sensitive imaging agent for detection of angiogenesis in infarcted myocardium.

YI-P054 Venomous Insulin Molecules: A Bioinspired Approach for the Treatment of Diabetes

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For people with diabetes, maintaining optimal blood glucose levels is effective in delaying or even preventing long-term complications. Tremendous efforts have been made in developing fast-acting and long-lasting analogues to provide better glycemic control compared to native insulin. Unfortunately, most people are unable to meet their target glycemic range, and aggressive efforts to reach this goal can cause frequent and potentially life-threatening episodes of hypoglycemia. A key challenge lies in the slow onset and long duration of fast-acting insulin action, which affects the feasibility of intensive insulin therapy. To address this challenge, we take a bioinspired approach to develop an ultrafast-acting insulin (UFI) to overcome the obstacles. Fish-hunting cone snails use a specialized venom insulin to rapidly induce hypoglycemic shock in fish. We and collaborators have shown that this venomous insulin is monomeric, binds to human insulin receptor, activates the insulin signaling pathway, and reduces blood sugar levels in mice. These strong preliminary results provide a rational basis to develop an UFI based on the snail venom insulin structure. In this project, we seek to study the biochemical and cellular properties of a series of venomous insulin molecules. We further utilize the knowledge learned from the cone snails to develop human monomeric insulin with full insulin potency.

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P055 Studies with Prenylated Peptides Demonstrate that Isoprenoid Analogues are Bioactive Surrogates for Natural Farnesyl Groups

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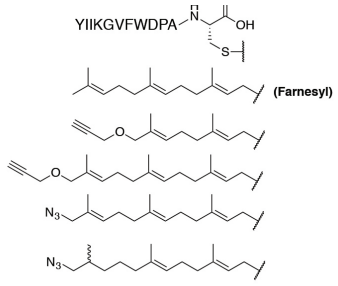
Protein prenylation involves the attachment of C_{15} and C_{20} isoprenoids to proteins. Prenylated proteins are key to many signal transduction pathways in eukaryotic cells. Recently, alkyne- and azide-modified isoprenoids have been used in metabolic labeling experiments to identify prenylated proteins and to evaluate how their levels change in various disease states. An important question in those experiments concerns how isoprenoid modification affects the bioactivity of prenylated proteins. In this study, we first demonstrate that a-factor, a farnesylated dodecapeptide, retains biological activity even when modified with alkyne- and azide-modified isoprenoids in a yeast mating assay. Next, we show that a-factor precursor

peptides containing modified isoprenoids are converted to their mature C-terminal methyl esters with efficiencies comparable to the wild type peptide. These experiments suggest that alkyne and azide modifications have minimal effects on the bioactivity of prenylated proteins and that metabolic labeling with these analogues does not significantly perturb their function.



YI-P056 Differential Regulation of Endothelial Derived Nitric Oxide Release by Selective Protein Kinase C Isoform Peptides

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Protein kinase C (PKC) beta II (βII) and PKC zeta (ζ) activation decreases endothelial nitric oxide synthase (eNOS) activity via phosphorylation on Thr-495 and Thr-497, respectively. By contrast, PKC epsilon (ε) activation increases eNOS activity via phosphorylation on Ser-1177. Myristoylation (myr) of peptides increases cell permeability to rapidly affect biochemical processes. Myr-PKC βII peptide inhibitor (10 μM) (N-myr-SLNPEWNET), myr-PKC ζ peptide inhibitor (10 μM) (N-myr-SIYRRGARRWRKL), and myr-PKC ε peptide inhibitor (10 μM) (N-myr-EAVSLKPT) have not yet been compared to their native peptides in regulating NO release. We hypothesized that myr-PKC βII and myr-PKC ζ inhibitors would enhance eNOS-derived NO release, while myr-PKC ε inhibitor would decrease this effect. Thoracic aortas from male Sprague-Dawley rats (275-325 g) were extracted, cut into equivalent segments (~10 mg/segment), and pinned with the endothelium facing up in a 24-well culture dish containing 37°C Kreb's buffer. Aortic NO release was determined using a calibrated NO sensor. Basal aortic NO release was 7.4±0.4 pmol/mg (n=7). Myr-PKC βII (n=16) and myr-PKC ζ (n=7) peptide inhibitors significantly increased basal NO release by 5.86±0.76 and 8.58±2.46 pmol/mg, respectively, compared to their native peptides which only increased NO release by 1.99±0.78 (n=17) and 2.00±0.66 (n=14), respectively ($p<0.05$). Furthermore, myr-PKC ε peptide inhibitor (n=29) significantly decreased basal NO release by 6.01±1.02 pmol/mg, compared to PKC ε native peptides (n=17) which only decreased NO release by 1.69±1.10 pmol/mg ($p<0.01$). These findings suggest that myr-conjugated peptides more effectively regulate PKC isoform function compared to their native peptides possibly due to enhanced cell membrane permeability.

This study was supported by the Pennsylvania Department of Health Grant (#4100057680), Center for Chronic Disorders of Aging, Division of Research, and the Department of Bio-Medical Sciences at Philadelphia College of Osteopathic Medicine.

YI-P057 Synthesis and evaluation of 1,3,4-benzotriazepin-2-one Turn Mimics as Allosteric Modulators of the Urotensin II Receptor

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Modulation of the urotensin II receptor (UT) offers promise for treating atherosclerosis and pulmonary arterial hypertension.[1] Considering the two endogenous cyclic peptide UT ligands, urotensin II (UII, H-Glu-Thr-Pro-Asp-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) and urotensin II-related peptide (URP, H-Ala-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH), and the potential for their common Trp-Lys-Tyr motif to adopt a turn conformation,[2] we designed pyrrolo[3,2-*e*][1,4]diazepin-2-ones that modulated selectively the potency (EC50) and efficacy (E_{Max}) of UII and URP in a rat aortic ring ex vivo bioassay.[3] With a new method for a diversity oriented synthesis of related 1,3,4-benzotriazepin-2-ones in hand,[4] we have now prepared a library of Trp-Lys-Tyr mimics and examined their ex vivo activity in a rat aortic ring bioassay. Certain analogs exhibited capacity to block aortic ring contraction selectively in the presence of UII and URP. Our presentation will describe the synthesis and bioactivity of these novel UT modulators.

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YI-P058 Synthesis of Acid-Stable Anti-Thrombotic Peptides

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Thrombin is a serine protease that plays a key downstream role in the blood coagulation cascade. The central role of thrombin in the formation of fibrin clots makes it an attractive target for the development of inhibitors to treat thromboembolic diseases. Several naturally occurring anti-thrombotic peptides such as hirudin have been isolated and shown to be potent direct thrombin inhibitors (DTIs). Previous work in our group has shown that tyrosine sulfation plays an important role in the activity of DTIs, with order of magnitude decreases in inhibition constants for thrombin.[1] Tyrosine sulfation, however, suffers from well-documented acidic lability.[2] Therefore, there is a need to design acid-stable analogues sulfotyrosine which retain biological activity against thrombin. Such analogues would improve the ease of synthesis and purification of thrombin inhibitors and should improve biological stability *in vivo*. Based on prior work by Taylor and co-workers,[3] we designed a synthetic route to five Fmoc-protected analogues of sulfotyrosine (sTyr) (Fig. 1.) *via* a key Negishi coupling step. Incorporation of these unnatural amino acids into two known potent DTIs, tsetse thrombin inhibitor (TTI) and variegin, using Fmoc solid-phase peptide synthesis provided the acid-stable analogues of doubly sulfated TTI and singly sulfated variegin. Biological evaluation of these peptide analogues is currently being undertaken.

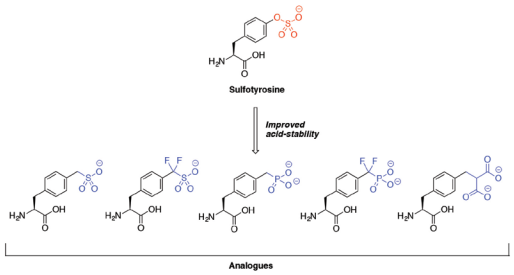

Fig. 1. Sulfotyrosine (above) and acid-stable target analogues (below) for incorporation into peptidic direct thrombin inhibitors.

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YI-P059 Oxazolidinone Mediated Cleavage of Peptide Bonds and its Applications

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Modification of serine's hydroxymethyl side chain to a 2-oxazolidinone moiety activates the peptide backbone chain and increases its susceptibility towards cleavage. Due to the versatility of this moiety, 2-oxazolidinone has been used to explore various applications such as protease mimics, formation of peptide thioesters, and modified C-terminal peptides. When used as a protease mimic, formation of 2-oxazolidinone allows for site-selective cleavage of extremely unreactive peptide bonds using neutral aqueous conditions. This method exhibits broad substrate scope and selectively cleaves various bioactive peptides with post-translational modifications (e.g. N-acetylation and N-methylation) and mutations (D- and β-amino acids), which are unsuitable substrates for enzymes. Further application of this method has been demonstrated by sequencing of cyclic peptides which is difficult to achieve by utilizing traditional methods such as Edman's degradation and MS/MS. Identifying the sequence of macrocyclic peptides is vital in exploring potential therapeutic candidates created through split and pool techniques. Building on the susceptibility of 2-oxazolidinone to cleavage, this moiety was also utilized for the formation of peptide thioesters, which is of significance in native chemical ligation for synthesis of large proteins. This approach allows the synthesis of peptide thioesters by using Fmoc SPPS, which is usually an incompatible method due to the nucleophilic secondary amine required for Fmoc removal. Moreover, 2-oxazolidinone was used for the synthesis of various C-terminally modified peptides such as acids, esters, N-alkyl amides, and alcohols, which are otherwise unattainable without the use of specialized resins, handles, or linkers.

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YI-P060 A Submonomer-based Approach towards the Total Synthesis of L-156,373

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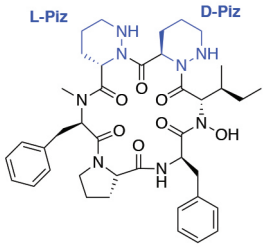
The piperazic acid (Piz) residue is found in a number of biologically active natural products and there exist numerous methods for its synthesis and incorporation into host peptides. Most approaches introduce pre-formed orthogonally protected Piz residues, synthesized in many steps, onto a growing peptide chain. L-156,373, a cyclic hexapeptide and oxytocin antagonist isolated from *Streptomyces silvensis*, features two consecutive enantiomeric forms of the Piz residue. Here, we present our efforts towards the first total synthesis of L-156,373 via a sub-monomer-based electrophilic amination approach. Our strategy relies on a tandem Mitsunobu cyclization to afford both Piz residues in one step and employs L- and D- glutamic acid residues as chiral synthons. This approach provides a means to investigate the impact of the Piz constraint on the efficiency of late-stage macrocyclization. These results, in addition to analogue synthesis, will also be presented.



L-156,373

YI-P061 Automated Ligator (Aligator) – A Computational Tool for Designing Efficient Chemical Protein Synthesis Schemes

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Chemical protein synthesis combines solid-phase peptide synthesis and native chemical ligation to produce proteins that cannot be prepared recombinantly, such as mirror-image proteins. Particularly for long proteins, there are many challenges in designing an efficient chemical protein synthesis route, including selection of optimal ligation sites, solubility challenges (in individual segments and assembly intermediates), and minimizing the number of ligations while maintaining segment synthesis feasibility. These complex considerations are currently evaluated by a tedious manual analysis. Here, we introduce Aligator (Automated Ligator), a Python suite of scripts that automates the total chemical synthesis design process. Using the input protein sequence, as well as the synthesis tools available to the user (e.g., pseudoprolines, list of preferred ligation junction residues, thiolated amino acids, ligation chemistries), Aligator analyzes the protein of interest to identify and rank potential protein assembly pathways. Aligator's modular design will enable updates as new chemical tools become available (e.g., our recently introduced "helping hand" semi-permanent solubility aid[1]). We demonstrate the utility of Aligator by systematically examining the set of proteins comprising a functional *E. coli* ribosome. We envision this tool to be most useful for

designing total chemical syntheses of large and/or multi-protein complexes (e.g., a D-ribosome).

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YI-P062 Effect of the Aromatic Interactions into the 3D Structure and Binding Affinity of New Somatostatin Analogs

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Somatostatin (SST), or somatotropin release-inhibiting factor (SRIF), is a 14-amino-acid peptide discovered in 1973.[1] It is a natural hormone whose biological activity is linked to five identified receptors: SSTR1-5.[2] The vast majority of the analogs commercialized until now, used as a gastric anti-secretory drugs, are shorter ones (6-8 residues) which mimic that pharmacophore; such as octreotide and lanreotide among others.

Our approach consists of modifying the sequence of the original structure but maintaining the 14-residue peptide structure. Previous work done in the group allowed us to obtain, for the first time, an accurate 3D view of some 14-residue somatostatin analogs where aromatic interactions between residues 6, 7 and 11 where key to the conformational stability of the peptide.[3] Herein, we will describe the effect of replacing Phe these positions by 2 non-natural electron-poor aromatic amino acids, L-3-(3'piridyl)alanine and L-3-(4'piridyl)alanine, so as to enhance the aromatic interactions, naturally present in the hormone between Phe6, 7 and 11. We will also describe the effect of the non-natural electron-rich aromatic amino acid L-3-(3',5'-dimethylphenyl)alanine in the same positions in comparison with other electron-donating amino acids. The aim of these modifications is to find a highly SSTR selective analog through which we can develop a new drug delivery system where the drug attached to the peptide can be released by light.

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P063 Structure-Based Design of Macrocyclic Tetrapeptides Intended to Modulate the Opioid Receptors

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The opioid receptors (μ, β, and κ) — best known for their involvement in analgesia — have recently shown promise as targets for the treatment of a variety of diseases including mood disorders. Peptide-based modulators of the opioid receptors often possess limited metabolic stability and oral bioavailability, hampering their therapeutic utility. CJ-15,208 and other macrocyclic tetrapeptides (MTPs) have shown stability to peptidases and oral bioavailability. Unfortunately, they often have markedly different *in vitro* and *in vivo* opioid activities, which makes optimization of the MTP scaffold challenging. We are currently using molecular modeling to gain insight into these differences and to design improved MTPs. Here, we describe modeling experiments intended (a) to examine possible active conformations of the MTPs, (b) to understand how the MTPs may bind to opioid receptors, (c) to explain the activity of known MTPs, and (d) to design novel MTPs with improved activity. This research was supported by NIDA grant R01 DA018832.

YI-P064 Lactams as Disulfide Bond Mimetics for Adrenomedullin

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Adrenomedullin (ADM) belongs to the CGRP family of peptide hormones, which also includes intermedin (IMD) and calcitonin gene related peptide (CGRP).[1] It exerts its vasodilatory and angiogenic effects primarily by activation of the ADM1 receptor (AM1R), which is a heterodimer of the calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 2 (RAMP2).[2] Adrenomedullin contains an intramolecular disulfide bond consisting of six amino acids that is known to be essential for receptor activation.[1] Since disulfide bonds in peptides undergo reductive cleavage under physiological conditions, their stabilization is a prerequisite for therapeutic applications. The successful replacement of disulfides by metabolically more stable groups, such as lactams or thioethers has been reported for various biologically active peptides.[3,4] Hence, it is a useful tool to increase the metabolic stability for medical purposes.

Here, we describe a strategy for the synthesis of different lactam-based disulfide bond mimetics of an N-terminally truncated adrenomedullin using Fmoc/tBu solid phase peptide synthesis.[4,5] Signal transduction assays were performed to characterize the activity of different analogues as well as the impact of the modification on selectivity within the ADM/CGRP receptor system. Wildtype-like receptor activation could be demonstrated for one of the variants. Furthermore, we found that the position and orientation of the lactam bond strongly influences the potency and efficacy. These structure-activity insights might be of use in the development of new and alternative therapeutics for cardiovascular diseases.

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YI-P065 Towards Selective and Mild Lactamization of Expressible Peptides and Proteins

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In solution peptides exist in an equilibrium of conformational states and backbone conformational constraints have long been utilized to control peptide topology. Amino-γ-lactam bridged dipeptides, commonly known as Freidinger Lactams, have been shown to stabilize Type II' β–turns, a common motif in peptide secondary structure. While lactams have been incorporated into peptides before, they must either be synthesized as bridged dipeptide units for use in chemical peptide synthesis or cyclized on resin through an alkylated methionine residue under harsh conditions and long reaction times. Here we employ a mild alkylation of Selenomethionine in aqueous solution at pH 6, followed by lactam cyclization of the alkylated peptide while adsorbed to Reverse Phase silica resin in DMSO. The utilization of Selenomethionine and mild conditions enable selective access to Freidinger lactams in expressible peptide and protein systems.

YI-P066 Di-iodotyrosinated Peptides as Targeted Radio-Contrast Agents

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Current radio-contrast agents in medical imaging are non-targeted. The poor sensitivity of X-ray based imaging techniques makes antibody-based probe design impractical. We outline here a rapid and effective strategy for the synthesis of targeted radiocontrast media via the introduction of multiple 3,5-diiodo-l-tyrosine ('**Y**') residues (which would absorb Xrays) into tissue-specific peptides during Fmoc-solid phase peptide synthesis. This approach was used to carry out Di-iodotyrosinated Peptide Imaging of Cartilage (DIPIC) by computed tomography (CT). DIPIC has potential use in the evaluation of osteoarthritis, where one of the earliest features is the deterioration of cartilage within the joint. The WYRGRL peptide binds to the highly abundant type-II collagen in cartilage. To determine the number and location of '**Y**' residues that may be tolerated by WYRGRL before type-II collagen binding was compromised, 23 unique peptides were synthesized and their cartilage binding determined using *ex vivo* micro-CT imaging of mouse tibiae. The calculated half-life ($t_{1/2}$), which ranged from 0-96 h, was used as an indicator of peptide binding strength. Intra-articular injection of a lead peptide, '**Y**'-*AEEA*-WYKGKL into mice (*ex vivo* t1/2 = 13.52 h, *AEEA* = [2-(2- aminoethoxy)ethoxy]acetic acid) enabled visualization of articular cartilage by *in vivo* micro-CT. The strength of di-iodotyrosinated peptide imaging lies in the ability to use Fmoc-SPPS to introduce '**Y**' into any tissue-specific peptide sequence to carry out targeted imaging of diseases using the clinically relevant technique of CT.

YI-P067 Screening Ubiquitin Specific Protease Activities Using Chemically Synthesized Ubiquitin and Ubiquitinated Peptides

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Ubiquitin, a 76 amino acid protein, is a key component that contributes to cellular protein homeostasis. The specificity of this modification is due to a series of enzymes: ligases, attaching the ubiquitin to a lysine, and deubiquitinases, which remove it. More than a hundred of such proteins are implicated in the regulation of protein turnover. Their specificities are only partially understood. We chemically synthesized ubiquitin, attached it to lysines belonging to the protein sequences known to be ubiquitinated. We chose the model protein "murine double minute 2" (mdm2), a ubiquitin ligase, itself ubiquitinated and deubiquitinated. We folded the ubiquitinated peptides and checked their tridimensional conformation. We assessed the use of these substrates with a series of fifteen deubiquitinases to show the potentiality of such an enzymological technique. By manipulating the sequence of the peptide on which ubiquitin is attached, we were able to detect differences in the enzyme/substrate recognition, and to determine that these differences are deubiquitinase-dependent. This approach could be used to understand the substrate/ protein relationship between the protagonists of this reaction. The methodology could be customized for a given substrate and used to advance our understanding of the key amino acids responsible for the deubiquitinase specificities.[1]

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YI-P068 Total Chemical Synthesis, Refolding, and Crystallographic Structure of Fully Active Immunophilin Calstabin 2

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Chemical biology is a growing field to which the chemical synthesis of proteins, particularly enzymes, makes a fundamental contribution. However, the chemical synthesis of catalytically active proteins (enzymes) remains poorly documented because it is difficult to obtain enough material for biochemical experiments. We chose calstabin,[1] a 107-amino-acid proline isomerase, as a model. The enzyme was synthesized using the native chemical ligation approach. Thanks to the SEA resin, several tens of milligrams of the peptide were obtained with fair yield and purity. The polypeptide was refolded properly, and we characterized its biophysical properties, measured its catalytic activity, and then

crystallized it in order to obtain its tridimensional structure after Xray diffraction. The refolded enzyme was compared to the recombinant, wild-type enzyme. In addition, as a first step of validating the whole process, we incorporated exotic amino acids into the N-terminus. Surprisingly, none of the changes altered the catalytic activities of the corresponding mutants. Using this body of techniques, avenues are now open to further obtain enzymes modified with exotic amino acids in a way that is only barely accessible by molecular biology, obtaining detailed information on the structure function relationship of enzymes reachable by complete chemical synthesis.[2]

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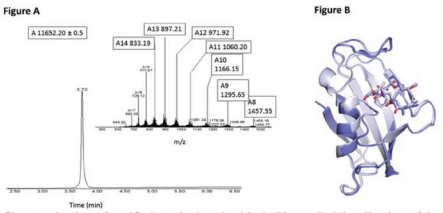

Figure A. Characterization of purified synthetic calstabin 2; **Figure B**. Visualization of the synthetic calstabin crystal

P069 Loosening of the Lipid Packing by Curvature Inducing Peptides Promotes Cell Entry of Oligoarginine

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Arginine-rich cell-penetrating peptides (CPPs), including HIV-1 TAT peptide and oligoarginines, represent a well-established class of CPPs. There are numerous reports of successful intracellular delivery using arginine-rich CPPs. However, the details of their entry pathways are still elusive.[1] We have recently reported that the direct membrane translocation of octaarginine (R8) could be enhanced in the presence of a positive membrane-curvature inducing peptide, derived from the N-terminus of epsin-1 (EpN18).[2] Some arginine-rich CPPs have been reported to have potential ability to induce membrane curvature.[3] Modulation of lipid membrane curvature should be accompanied by alterations in the lipid packing. We thus hypothesized that the lipid packing defects may effectively promote the energy-independent translocation of oligoarginine peptides through the plasma membranes.

Lipid packing was analyzed in terms of generalized polarization (GP) of plasma membranes using an environment-sensitive fluorescent probe, di-4-ANEPPDHQ. Among seven known curvature-modulating peptides studied, EpN18 and Sar1p(1-23) were found to fluidize plasma membranes and to significantly promote R8 translocation. Additionally, we have previously reported that the membrane translocation of arginine-rich CPPs was markedly promoted in the presence of externally added hydrophobic counter anions such as pyrenebutyrate (PyB).[4,5] We found that PyB also induces a positive membrane curvature and loosens lipid packing. These results imply a possible general mechanism of enhancement in R8 translocation, based on the distortion of the lipid packing, and suggest that similar enhancers can be identified through screening, *e.g.*, of the curvature modulating peptides.

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P070 Small but Potent: Snail Insulin Activates Human Insulin Receptor

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Con-Ins G1 is the smallest known insulin found in nature and is used by the fish-hunting cone snail *Conus geographus* to facilitate prey capture.[1] Con-Ins G1 is rich in posttranslational modifications (PTMs), a well known feature of cone snail toxins and unusual for insulins. Here we report the crystal structure of Con-Ins G1, its activity against the human insulin receptor (hIR) and a computational model of the Con-Ins G1- hIR interaction.[2] Although Con-Ins G1 lacks the C-terminal fragment of the B chain of hIns (residues Arg^{B22} through Thr^{B30}), the Phe^{B24}, a residue critical for human insulin activity, it has compensating structural elements that allow it to maintain activity on hIR. Additionally, we demonstrate that cone snail venom insulins form a diverse class of novel hIR ligands, which might be useful as pharmacological tools and potential drug leads for diabetes.

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YI-P071 Synthesis of Teixobactin Analogues: Structure-Activity Relationship Study of Small Cyclic Lipopeptidomimetics

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Antibiotics were introduced as drugs in the 1940s, and many of these are still widely used today. However, the development of bacterial resistance over time, combined with the lack of novel compounds, has resulted in a drastic need for new antibiotic drugs. The structure of teixobactin, a cyclic undecapeptide, was published in January 2015,[1] in a paper describing the use of an "iChip" to successfully grow a number of previously unculturable bacteria. Teixobactin, isolated from the newly identified E*leftheria terrae*, has been found to have μM activity against many Gram-positive bacteria, including *Clostridium difficile* and MRSA.

Teixobactin is a cyclic depsipeptide made up of 11 amino residues. Its unusual structure contains four D-amino acids, a methylated terminal residue, an ester linkage between a D-threonine hydroxyl and the C-terminal acid, and the unnatural residue L-*allo*-enduracididine.

In this research, a systematic structure-activity relationship study was designed to fully investigate the role of the linear hydrophobic tail, and the effect of mutating the unnatural L-*allo*-enduracididine residue at the 10 position. Seventeen analogues were synthesised with acetylation or prenylation in place of residues 1-7, and enduracididine was mutated to seven different residues varying in functionality, basicity and structure. Of the compounds tested, four were found to have antibiotic activity, with one compound sharing a similar minimum inhibitory concentration (MIC) value (0.5 µg/ mL) as native teixobactin itself.

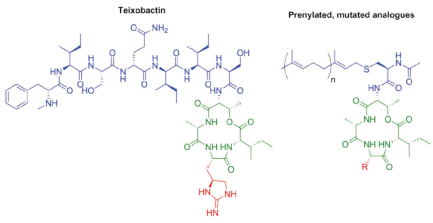

Figure 1 – Native teixobactin, and general structure of mutated synthethic analogues

This presentation discusses the design of these teixobactin analogues, the synthethic routes employed to create the final products, and the results and conclusions from the MIC assays.

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YI-P072 Effect of 25-hydroxycholesterol on the Interaction of the Fusion Inhibitor Peptide C34 with Model Membranes and Human Blood Cells

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The fusion between the viral and the target cell membrane is a crucial step in the life cycle of enveloped virus. The blocking of this process is a well-known therapeutic approach that led to the development of the fusion inhibitor peptide enfuvirtide, clinically used against HIV[1]. Despite this significant advance on HIV treatment, the appearing of resistance has limited its clinical use[2]. Such limitation has led to the development of other fusion inhibitor peptides as C34 that present the same structural domain as enfuvirtide (heptad repeat sequence), but have different functional domains, like pocket- and lipid-binding domains[3].

Recently, the antiviral properties of 25-hydroxycholesterol (25HC) were demonstrated, which boosted the interest on this sterol[4]. The combining of two distinct antiviral molecules, C34 and 25HC, may help to suppress the emergence of resistant viruses.

In this work, we characterize the interaction between C34-25HC conjugate with biomembrane model systems and human blood cells. Lipid vesicles and monolayers with defined lipid compositions were used as biomembrane model systems. C34-25HC interacts preferentially with membranes rich in sphingomyelin (a lipid enriched in lipid rafts), and presents a poor partition to membranes composed solely of phosphatidylcholine and cholesterol. We hypothesize that cholesterol causes a repulsive effect that it is overcome in the presence of sphingomyelin. Importantly, the peptide shows a preference for human peripheral blood mononuclear cells (PBMC) relative to erythrocytes, which shows its potential to target CD4 positive cells.

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P073 Analysis of Hydration Dynamics of Synthetic Antifreeze Glycopeptide (AFGP) Analogues

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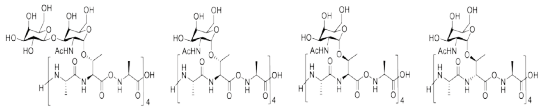
Biological antifreezes are an important class of proteins and peptides found in Arctic and Antarctic species of fish, as well as insects, plants and amphibians. They exhibit unique properties, which allow them to inhibit ice crystal growth and ice recrystallization, which enables the survival of a variety of organisms in extreme climate conditions, where sub-zero temperatures are encountered on a daily basis. Certain species of fish adopted this strategy in order to endure the harshness of polar and subpolar oceans and are known to produce antifreeze glycopeptides (AFGPs). In a model case scenario AFGPs are composed of 4-55 repetitions of a tripeptide unit (Ala-Ala-Thr), in which the secondary hydroxyl group of threonine is glycosylated with β-D-galactosyl-(1→3)-α-N-acetyl-D-galactosamine. Despite their discovery in the 1960s, the adsorption mechanism of AFGPs, which underlies their ability to hinder ice growth, is not fully defined. Nowadays, a growing demand for non-toxic and highly effective cryoprotectants for medical, pharmaceutical, agricultural and food industries has spurred the research in the field of antifreeze glycopeptides. Recently studies on AFGPs revolve around explaining the phenomena that occur in hydration dynamics[1,2] of the glycopeptides at temperatures close to the freezing point of water. It is believed that through analysis of this mechanism and the conformational behavior of the AFGPs that triggers it we may be able to obtain a clearer understanding of the

antifreeze activity. Here we present the analysis of hydration shell dynamics for synthetic AFGP analogues using molecular dynamics simulations with TIP4P/Ice water model.



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P074 Ribosomal Synthesis of Bicyclic Peptides using Artificial Amino Acids with Tunable Reactivities

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Some bioactive peptides consist of complex macrocyclic scaffolds. For instance, conotoxins, cyclotides and defensins have multicyclic frameworks, which have been thought to be important to their potent bioactivities. Thus, macrocyclic peptides have gained interest and have been utilized as scaffolds for drug discovery. Several methods involving the chemical macrocyclization of peptides to generate artificial macrocyclic frameworks which mimic the benefits seen in naturally occurring peptides have been reported thus far.

We have previously devised an in vitro translation system[1], in which the genetic code is reprogrammed to generate peptides bearing an N-terminal chloroacetyl (ClAc) group. After ribosomal synthesis of a linear precursor peptides, the N-terminal ClAc group spontaneously reacts with the sidechain sulfhydryl group of a downstream Cys to form a sulfide bond, resulting in thioether macrocyclic peptides as translation product[2]. This synthetic strategy has also made it possible to generate macrocyclic peptide libraries with diversities up to the trillions and discover novel macrocyclic peptides with various bioactivities[2-4].

We here report a series of new artificial amino acids that exhibit different and tunable reactivities with nucleophilic functional groups on peptides. We also demonstrated ribosomal synthesis of bicyclic peptides with controlled ring toporogies by means of the artificial amino acids.

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YI-P075 Effects of Cysteine Substitutions on the Structural Properties of Chlorotoxin Explored by Molecular Dynamics Simulations

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Chlorotoxin (CTX) is a 36 amino acid peptide isolated from the venom of the Deathstalker scorpion. It has 8 Cys residues with four disulfide bonds. CTX has high affinity for ClC-3 channel and MMP-2 enzyme making it a promising chemotherapeutic agent. Replacement of the Cys^{2}-Cys1^{9}, Cys^{5}-Cys^{28}, Cys^{16}-Cys^{33}, Cys^{20}-Cys^{35} residues with L-α-aminobutyric acid (Abu) has been performed and their binding properties determined. Here, we compared the conformational space of the following CTX analogs: wild type, Abu^{2}-Abu^{19}, Abu^{5}-Abu^{28}, Abu^{16}-Abu^{33}, Abu^{20}-Abu^{35}, and $Abu^{2,5,16,19,20,28,33,35}$ by performing 4 μs molecular dynamics simulations at 310 K using the CHARMM36 force field. The Rg of all analogs remains compact at ~0.9 nm with the exception of $Abu^{2,5,16,19,20,28,33,35}$. Likewise the RMSD of the analogs compared to the 1CHL NMR structure are low: WT 0.35 ± 0.01nm, Abu^{2}-Abu^{19} 0.35 ± 0.03, Abu^{5}-Abu^{28} 0.24 ± 0.04, Abu^{16}-Abu^{33} 0.25 ± 0.05, and Abu^{20}-Abu^{35} 0.28 ± 0.03, with the expected exception of $Abu^{2,5,16,19,20,28,33,35}$ 0.93 ± 0.18 nm. The α-helix at residues 13-20 was shortened in the wild type to residues 15-20. In Abu^{5}-Abu^{28} and Abu^{16}-Abu^{33} the helix was well conserved through residues 15-20, but was extended to residues 9-20 for over half of the simulation. The α-helix was poorly preserved in the other three analogs. The β-strands, found at residues 27-29 and 32-34 in the NMR structure, are well conserved in all analogs with the exception of $Abu^{2,5,16,19,20,28,33,35}$ in agreement with CD spectroscopy. Preservation of the C-terminal β-strands may inhibit proteolysis of CTX and its analogs but not affect biological activity.

YI-P076 Metal and Polyoxometalate Driven Assembly of a Tripeptide

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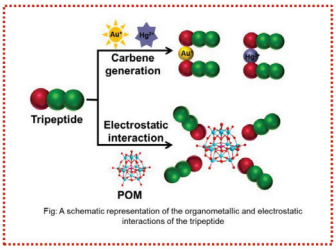

Fig: A schematic representation of the organometallic and electrostatic interactions of the tripeptide

We present the strategic design of a novel tripeptide that has the capacity to generate a carbene on its side chain. This tripeptide was used for the synthesis of an organometallic carbene complex by reaction with appropriate gold and mercury precursors. These complexes showed the characteristic features of carbene complexes i.e. disappearance of peak for the proton where carbene is generated in 1H NMR and deshielding of the carbenoid carbon peak in the 13C NMR spectra. Their structures were elucidated by Density Functional Theory (DFT) calculations.

The tripeptide alone showed fibrous morphology, but showed the formation of spherical nanostructures due to electrostatic interaction with polyanionic phosphotungstate salt. Their interaction was elucidated by various techniques like FTIR and XRD. Their applications have also been studied in the present work.

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YI-P077 Solid Phase Synthesis of Peptide Selenoesters

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Given the growing interest in peptides as medicines, the development of efficient methods for their synthesis has become exceedingly important. The selenocystine-selenoester ligation is a rapid and efficient method for the construction of native amide bonds between peptide fragments (Figure 1).[1] The reaction proceeds without the use of additives, is complete within minutes, and can be used in concert with one-pot deselenization chemistry. A bottleneck in the utility of the Se-mediated ligation is the synthesis of the peptide selenoester component. Currently, the selenoester is synthesized over four steps, including a solution-phase selenoesterification reaction. Considering the inefficiencies in the current methodology, a more robust and rapid approach was developed.

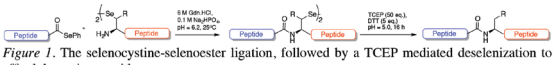

Figure 1. The selenocystine-selenoester ligation, followed by a TCEP mediated deselenization to afford the native peptide.

This work presents the on-resin synthesis of peptide selenoesters which proceeds with higher yield and in an overall shorter reaction time than the traditional synthesis. The strategy involves an initial side chain immobilization of an allyl ester protected Fmoc-amino acid to a suitable resin. Following peptide elongation through solid-phase peptide synthesis, the C-terminal carboxylic acid can be unmasked via a palladium catalyzed allyl ester deprotection. On-resin treatment with diphenyldiselenide and tri-n-butylphosphine, after cleavage and purification, provides peptide selenoesters in excellent yields without significant epimerization.

Figure 2. On-resin selenoester synthesis via a side chain anchoring strategy.

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YI-P078 Derivatization of a Macrocyclic Tetrapeptide Kappa Opioid Receptor Antagonist to Improve Solubility and Physiochemical Properties

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Within the past decade, kappa opioid receptor (KOR) antagonists have shown promise for the treatment of mood disorders and substance abuse. Our lab is exploring macrocyclic tetrapeptides displaying selective KOR antagonism that prevent stress-induced reinstatement of cocaine-seeking behavior in mice.[1] The lead antagonist demonstrates blood-brain barrier permeability following oral administration.[2] However, its highly hydrophobic nature limits aqueous solubility, which may adversely affect its absorption and administration *in vivo*. To improve solubility while retaining the pharmacological properties of the parent peptide we are synthesizing a series of macrocyclic tetrapeptide derivatives containing a handle that can be derivatized with polar functionalities while maintaining important aromatic side chains. The agonist and KOR antagonist activity of the derivatives *in vivo* in the mouse 55oC warm water tail withdrawal antinociception assay will be presented. This research is supported by NIDA grant R01 DA023924.

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P079 Facile Optimization of Peptide and Protein Purification via Flash Chromatography

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Advances in chromatographic technologies are now allowing for the purification of peptides and proteins via comparatively low-pressure chromatographic methods while maintaining high purity. Indeed, traditional affinity chromatography of expressed proteins can leave multiple impurities that are easily removed by reversed-phase flash chromatography. In addition, while peptides have traditionally been purified by high-pressure systems, high recoveries and purities can be achieved by employing flash techniques with the added benefit of increased speed and throughput. A variety of packing materials are available and method development can be expedited due to the high throughput platform of flash chromatography and the reduced cost of flash cartridges compared to traditional high-pressure columns.

Here, we present data on the facile optimization of purification methods for simple, linear peptides, peptide macrocycles, and small recombinant proteins by exploring traditional, and sometimes forgotten, packing materials in a low pressure format. A comparison of high-pressure chromatography and low-pressure flash is presented for peptide purification as well as the application of reversed-phase flash chromatography to polish recombinant proteins following affinity purification.

P080 Silyl-protected Alkynes for Multiple Labeling of Chemically Synthesized Protein

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Engineered proteins installed with one or more unnatural moieties have potential to expand protein function. To site-specifically introduce functional molecules (e.g. fluorescent dyes, crosslinkers, and PEGs) into proteins of interest, reactive handles such as azide and alkynes are often used for bioorthogonal conjugation. However, there seems to be no such molecular handles that stably exist during the whole process of chemical protein synthesis, which generally consists of solid-phase peptide synthesis (SPPS), native chemical ligation (NCL),[1] and desulfurization (Cys to Ala conversion).[2] Here, we demonstrate that silyl-protected alkynes can tolerate all of the reaction conditions of Fmoc-SPPS, peptide ligation, and desulfurization and are useful for labeling peptides or proteins. We synthesized peptides bearing three types of sily protecting groups with different stability (DMES, TES, and TBS) by Fmoc SPPS and tested their stability in desulfurization reaction by monitoring HPLC charts and identifying molecular weight. Then, appropriate deprotection conditions were explored to

remove these protecting-groups in orthogonal fashion. We found that concentration of potassium fluoride (KF) and/or silver nitrate ($AgNO_3$) is critical to control the deprotection order. We also revealed that deprotection of silyl-protecting groups and subsequent copper-catalyzed azide-alkyne cycloaddition (CuAAC) proceeded in one-pot. By using a peptide having two different silyl-protecting groups of DMES and TBS, two consecutive one-pot deprotection-CuAAC reactions were demonstrated. In addition, doubly-labeled nuclear transition protein 1 (TP1) with Cy3 and Cy5, was chemically synthesized through NCL, desulfurization and tandem dye-labeling reaction using DMES- and TBS-protected alkynes. We reasoned that silyl-protected alkyne would become a powerful tool when introducing functional molecules into chemically synthesized peptides or proteins.

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P081 Disulfide-bond Formation Reagents Based on Solid-phase 3-nitro-2-pyridinesulfenyl (Npys) Derivatives

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The first reported use of Npys-Cl was published in 1980 by Matsueda and Walter[1]. In the presence of a base, the Npys chloride reacts readily with thiols, amines or alcohols to produce asymmetrical disulfides sulfenamides or sulfenates, respectively. One of the most successful applications of the Npys group has been in the formation of mixed disulfides. Indeed, Npys protects sulfur atoms and acts as an activator for disulfide bond formation, thereby facilitating further oxidation of free thiols to the corresponding disulfides[2]. The selectivity and mild reaction conditions opened up a wide range of applications in chemical biology, in particular for thiols conjugation[3]. Recently, our group developed solid-phase supported reagents for the selective biotinylation[4] and oligoarginylation[5] of SH groups. The latest reported developments around solid phase reagents led to the synthesis of a universal disulfide bond formation reagent that proved to be particularly useful for disulfide formation in peptides[6]. This method was used to successfully synthesize oxytocin, a cyclic nonapeptide, after intramolecular amide bond formation and subsequent deprotection. Herein are reported the latest developments of such innovative solid-supported Npys derivatives for their application to peptide synthesis and chemical biology, through several examples for disulfide containing peptides, such as -conotoxin and human ANP.

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YI-P082 Tuning GLP-1 and GLP-1 Ligands into Biased Agonists of the GLP-1R through Structural Constraints and Amino Acid Substitutions

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Glucagon-like peptide (GLP-1) is an endogenous hormone that induces insulin secretion from pancreatic islets and modified forms are used to treat diabetes mellitus type 2. Understanding how GLP-1 interacts with its receptor (GLP-1R) can potentially lead to more effective drugs. Our approach was to first examine GLP-1 bound to our GLP-1R homology model and use molecular dynamics simulations to predict the bound conformation of the N-terminus of GLP-1. These conformations were used to design conformational constraints of individual regions of GLP-1 using simple sidechain-to-sidechain or sidechain-to-mainchain lactam constraints. The resulting effects of peptide structure were determined by measuring GLP-1R affinity, cAMP stimulation, β-arrestin 2 recruitment and insulin release. β-arrestin 2 has a key role in GPCR desensitization, internalization and down regulation. The structural components of GLP-1 important for β-arrestin signalling or insulin secretion have not been examined previously. An understanding of how the GLP-1 structure and sequence influence GLP-1R binding, intracellular signalling, and insulin release may lead to more effective and safer modulation of GLP-1R for the treatment for type 2 diabetes. Structural constraints of both the N-terminal region and the introduction of Lys-Asp (i, i+4) crosslinks in the middle and at the C-terminus increased alpha helicity and cAMP stimulation without much effect on binding affinity or beta-arrestin 2 recruitment. Using these approaches we have also developed compounds, which lose the ability to signal through β-arrestin 2 but maintain good cAMP stimulation. Here we will discuss the design and development of such biased compounds and some of the biological consequences of removal of β-arrestin 2 signalling ability in GLP-1 agonists.

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P083 *In Vitro* Selection for Macrocyclic Peptides that Bind to ALK1

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Bone Morphogenetic Proteins (BMPs) are members of the Transforming Growth Factor β (TGF-β) family, which are cytokines that play a role in the differentiation and proliferation of cells during development. A cell surface receptor for BMP, Activin-like Kinase (ALK)1 was discovered to partake not only in developmental but also in pathological angiogenesis. Knockdown of ALK1 in mice resulted in severe vascular deformities, suggesting that regulation of ALK1 overexpression in tumors could provide a means for mitigating tumor angiogenesis. Unfortunately, targeting ALK1 has been met with difficulty. A promising anti-ALK1 antibody has recently failed in a Phase 2 clinical trial, by showing low potency[1] and side effects such as thrombocytopenia. Instead of pursuing biologic ligands or small molecule therapeutics, our group has focused our efforts on the discovery of macrocyclic peptides that bind to ALK1 and inhibit its protein-protein interactions needed for signal propagation. Macrocyclic peptides are resistant to proteolytic degradation compared to their linear counterparts, and they have the potential to penetrate the cell membrane due to their small size and reduced hydrogen binding to water. Toward this goal, we have employed an in vitro selection technology called the Random non-standard Peptide Integrated Discovery (RaPID) system.[2] This technique combines the ribosomal incorporation of non-standard amino acids for the production of macrocyclic peptides with mRNA display. After 6 rounds of selection, we were able to identify a promising macrocyclic peptide that binds to ALK1.

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P084 Positively Charged Amphipathic α-Helical Peptides as Standards to Evaluate Column Performance in Reversed-Phase Chromatography

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Amphipathic cationic α-helical peptides represent a class of compounds which offer a potent test, especially in the pharmaceutical industry, for new packing materials for reversed-phase chromatography (RPC). Such materials can then be used effectively in the purification of synthetic peptides in order to meet FDA purity expectations. Amphipathic peptides are particularly suitable for RPC standards due to the preferred binding of the non-polar face to the hydrophobic stationary phase. The ability of different reversed matrices to separate mixtures of peptide standards with only subtle hydrophilicity/hydrophobicity variations in both the non-polar and polar face of the peptides can then be assessed. We have designed *de novo* a mixture of six 26-residue all D-conformation amphipathic cationic α-helical peptides with a single, positively charged lysine residue in the center of the non-polar face and an increasing number of lysine residues (4-9 residues) replacing neutral residues in the polar face, resulting in an overall net positive charge of +5 to +10. Thus, the non-polar, preferred RPC binding face remains constant, with only the polar face varying in hydrophilicity/hydrophobicity. Mixtures of these peptides were applied to columns of varying functional group properties (C8, C18 or phenyl; polar endcapped, polar embedded; porous, non-porous core with superficially porous surface) under mobile phase conditions varying in ion-pairing reagent trifluoroacetic acid (TFA) concentration or buffer pH. Our results clearly demonstrate the excellent potential of these novel peptide standards to enable the selection and development of effective RP materials for peptide separations and purification.

Acknowledegements
We thank the John Stewart Endowed Chair in Peptide Chemistry to Robert S. Hodges.

P085 "Specificity Determinants" Improve Therapeutic Indices of *De Novo* Designed Antimicrobial Peptides (AMPs), Encode Selectivity for Gram-negative Pathogens and are Active against *A. baumannii* Strains Resistant to Polymyxin B and Colistin

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We have designed *de novo* four 26-residue all D-conformation amphipathic α-helical cationic antimicrobial peptides (AMPs) with two "specificity determinants" (Lys residues K13 and K16) in the center of the non-polar face which provide specificity for prokaryotic cells over eukaryotic cells[1,2]. We tested the AMPs against 14 different *A. baumannii* clinical isolates, 7 of which were resistant to polymyxin B and colistin (antibiotics of last resort), 6 isolates of *P. aeruginosa*, and 17 strains of *S. aureus*, 8 of which were methicillin resistant (MRSA) strains. The four AMPs (D33, D34, D35 and D36) differ from one another by the arrangement of the six positively charged residues on the polar face. The four AMPs without specificity determinants, A13 and A16 instead of K13 and K16, have an average therapeutic index of 1.45 whereas the four AMPs with specificity determinants have an average therapeutic index of 210. The best AMP, D33, has a 371-fold improvement in the therapeutic index when comparing the identical AMPs with and without specificity determinants. The four *de novo* designed AMPs with specificity determinants have a large preference for the Gram-negative pathogen, *A. baumannii* compared to the Gram-positive pathogen, *S. aureus* (selectivity factor as low as 16 to as high as 88). Our results suggest we can control Gram-negative pathogen selectivity by changing the location of the positively charged residues on the polar face of the helix. Specificity determinants also maintain excellent antimicrobial activity in the presence of human serum by preventing high affinity binding to serum proteins. Our AMPs without specificity determinants were inactive against *A. baumannii* in the presence of human serum proteins due to high affinity binding. This study clearly shows the potential of these amphipathic α-helical AMPs as therapeutics to replace existing antibiotics.

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Acknowledgements
We thank the John Stewart Endowed Chair in Peptide Chemistry to Robert S. Hodges.

P086 Application of Orthogonal Thioester Presursors to the One-Pot Synthesis of Histone H4

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Previously, we developed a cysteinylprolyl ester (CPE) method[1] and *N*-alkylcysteine (NAC) method[2] for preparation of the peptide thioester by the Fmoc method. Two moieties introduced during the solid-phase method have different characteristics. The CPE moiety can be converted to peptide thioester under weakly basic conditions (pH ~8), whereas NAC moiety can be converted to peptide thioester under slightly acidic conditions (pH ~5). The difference in the reactivity between two thioester precursors for the thioesterification led us to develop a novel one-pot native chemical ligation reaction.

In this presentation, the novel method was demonstrated using histone H4 as a model. The entire sequence of H4 was divided into 3 segments at the N-terminus of two Ala residues and the native chemical ligation followed by desulfurization to perform conversion from Ala to Cys residue was performed. Thus, the N-terminal peptide with CPE and the middle segment with NAC, and C-terminal peptide were synthesized by the solid phase method. The first ligation of the N-terminal and the middle segment was performed at pH 7.8, keeping the NAC moiety intact. The C-terminal segment dissolved in acetate buffer (pH 5.0) was then added to perform the second ligation at 5.5. Two sequential ligation followed by desulfurization successfully gave the desired H4[3]. The application of the newly developed 2-(4-pyridyl)-2-propyl group for the protection of side chain carboxy group of Asp at the ligation site will also be reported.

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YI-P087 Trifluoromethylated Proline Surrogates as Part of "Pro-Pro" Turn-inducing Templates for the Design of β-hairpin Mimetics.

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Proline is often found as a turn inducer in peptide or protein domains. Exploitation of its restricted conformational freedom led to the development of a D-Pro-Pro segment as a 'templating' unit, frequently used in β-hairpin peptidomimetics.[1] Recent studies revealed the stabilizing effect of CF_3-proline surrogates on β-turn conformation, especially by blocking the cis-trans isomerization of the amide bond.2 In view of finding templates with a stronger capacity to fix the β-hairpin conformation, we investigated different fluorinated analogues of the D-Pro-Pro segment as β-turn promoter.

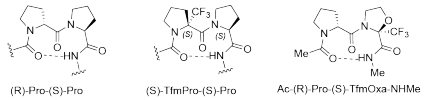

Figure 1 (*R*)-Pro-(*S*)-Pro, (*S*)-TfmPro-(*S*)-Pro and Ac-(*R*)-Pro-(*S*)TfmOxa-NHMe as a model for β-turn formation

A conformational study was performed on a set of 12 non-natural variants of the D-Pro-Pro hairpin sequence, incorporating for example TfmPro and TfmOxa. Out of these combinations only TfmPro-Pro, TfmPro-TfmPro, TfmOxa-Pro, D-Pro-TfmPro, D-Pro-TfmOxa exhibited a strongly stabilized β-turn conformation, relative to D-Pro-Pro. Considering synthetic feasibility, TfmPro-Pro and D-Pro-TfmOxa (Figure 1) were selected for the incorporation into cyclic peptides. The increased preference of two selected peptides for the β-hairpin conformation was confirmed by molecular dynamics. To support those calculations, pseudo-tetrapeptides like Ac-D-Pro-TfmOxa-NHMe were synthesized and studied as models for β-turn induction. In a final study, TfmPro surrogates were incorporated in a designed cyclic peptide sequence for NMR conformational study.

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P088 Role of Disulfide Linkages in the Folding and Activity of Scyllatoxin-based BH3 Domain Mimetics

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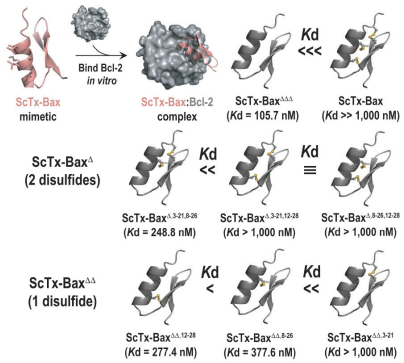

Anti-apoptotic Bcl-2 proteins are implicated in pathogenic cell survival and have attracted considerable attention as therapeutic targets. We recently developed a new class of synthetic protein based on scyllatoxin (ScTx) designed to mimic the helical BH3 interaction domain of the pro-apoptotic Bcl-2 protein Bax. Fully oxidized ScTx-Bax mimetics containing three disulfides did not bind Bcl-2 *in vitro*, while fully reduced forms bound Bcl-2 with high affinity. In this study, the contribution of each disulfide in the folding and function of ScTx-Bax proteins was investigated. Eight ScTx-Bax proteins were synthesized, each presenting a different combination of disulfides, and their

ability to bind Bcl-2 *in vitro* was evaluated using fluorescence polarization. It was determined that the number and position of disulfide bonds had significant implications on the folding and activity of ScTx-Bax proteins. Three analogs: ^{Flu}ScTx-Bax, ^{Flu}ScTx-Bax$^{\Delta,8\text{-}26,12\text{-}28}$ and ^{Flu}ScTx-Bax$^{\Delta\Delta,8\text{-}26}$ were found to fold into structures reminiscent of wild-type ScTx. Among the folded ScTx-Bax proteins, only ^{Flu}ScTx-Bax$^{\Delta\Delta,8\text{-}26}$ bound Bcl-2 with high affinity. Three other analogs: ^{Flu}ScTx-Bax$^{\Delta\Delta\Delta}$, ^{Flu}ScTx-Bax$^{\Delta,3\text{-}21,8\text{-}26}$ and ^{Flu}ScTx-Bax$^{\Delta\Delta,12\text{-}28}$ were found to bind Bcl-2 with high affinity despite showing no evidence of folded structure. This study underscores the importance of structural dynamics in influencing favorable BH3:Bcl-2 interactions and further validates ScTx-based ligands as potential modulators of anti-apoptotic Bcl-2 function.

P089 To Develop and Use Novel Chemical Methods for Efficient Synthesis of Insulin-like Peptides for SAR Studies

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Insulin and insulin-like peptide hormones have a diverse range of physiological functions.[1] Their clinical importance is highlighted by the fact that insulin, until today, remains the primary treatment for diabetes (particularly of type 1), and human relaxin-2 is in the clinic for the treatment of acute heart failure. Insulin-like peptides possess a very complex structure with 2 chains and 3 disulphide bridges. Currently these peptides are produced by recombinant gene expression technology which has known problems and limitations, particularly in the folding of non-native peptide analogs. In significant breakthroughs in the last 5 years, my team has developed novel chemical methods[2-4] that enabled us to carry out details SAR studies of these peptides leading to the generation of simplified peptidomimetics that target the G protein coupled receptors, RXFP1, 3 and 4.[5-8] My novel compounds offer safer and cheaper treatment options as they are target-specific and easier to prepare compared with native molecules.

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YI-P090 The Cyclic Peptide Alphabet

Parisa Hosseinzadeh,[a] Vikram Mulligan,[a] Matt Shortridge,[a] Gaurav Bhardwaj,[a] Timothy Craven,[a] Stephen Retti,[a] David Bakera,*
[a]*Department of Biochemsitry, University of Washington, Seattle*

We report the computational design of a large set of small (6-14mer) cyclic peptides consisting of D- and L- amino acids that are structured through backbone hydrogen bonds. The design process ensures that ALL possible closed conformations with desired hydrogen bonding threshold have been sampled, a feast that is achievable due to the small size of these peptides. The designed peptides have been extensively studied through a variety of computational metrics and some (currently 6) have been confirmed structurally through NMR. Analyzing the structures within these peptides suggests enrichment of rare turn types and a variety of previously underexplored conformations (more than 1000 distinct designs that are predicted to be structured with high confidence compared to less than 50 structures available in PDB and CSD combined). Moreover, these designed peptides provide an attractive starting library for rational design of peptide-based drugs. The use of D-amino acids and the cyclization process provides the stability required for such drugs and the structured nature of the peptides ensures minimal entropy cost upon binding to targets of interest.

YI-P091 Photolabile Backbone Amide Linker for the Solid-Phase Synthesis of Cyclic Peptides and Thioesters

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Since its introduction, the *B*ackbone *A*mide *L*inker (*BAL*) has proven to be a valuable tool in the solid phase peptide synthesis of C-terminally-modified and cyclic peptides.[1,2] Completed sequences are generally cleaved from the BAL linker under strongly acidic conditions rendering it incompatible with acid sensitive moieties. Another limitation of the BAL strategy is the acylation of the BAL-linked secondary amine. Miranda *et al.* developed a photolabile acyl transfer auxiliary (Hnb) to improve "difficult" coupling sequences. Hnb possesses an *o*-hydroxybenzylamine moiety that promotes an intramolecular *O* → *N* acyl transfer.[3] In an effort to synthesize cyclic peptides and thioesters using SPPS, we sought to develop a photolabile BAL linker (*p*BAL) that possesses orthogonality to acid labile groups and incorporates an ohydroxybenzylamine moiety to aid in "difficult" couplings of the first amino acid to the linker **(Hcnb) (Figure 1)**.

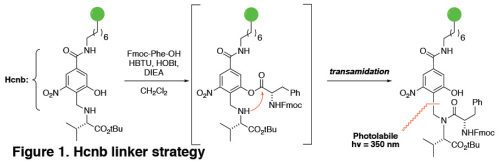

Figure 1. Hcnb linker strategy

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YI-P092 Lineal and Polyvalent Peptides Derived from Bovine Lactoferricin (17-31). Design, Synthesis and Evaluation of their Antibacterial Activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923

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Resistance to antimicrobial agents is a public health problem worldwide[1]. Cationic antimicrobial peptides (AMPs) are potential molecules to develop new therapeutic agents against infections caused by resistant pathogens[2]. In this work, peptides derived from LfcinB 17-31 (^{17}FKCRRWQWRMKKLGA^{31}) were designed, purified and characterized. A peptide library was constructed by systemically removing the flanking residues (N or C-terminal) of the RRWQWR sequence that corresponds to minimal antimicrobial motif[3]. For this research, it was also included (i) a peptide containing an Ala instead of Cys ([Ala^{19}]-LfcinB 17-31) and (ii) polyvalent peptides containing the RRWQWR sequence and a non- natural amino acid (aminocaproic acid). For each peptide the antibacterial activity was tested against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. We established that peptides LfcinB 17-26 (^{17}FKCRRWQWRM^{26}) and LfcinB 17-25 (^{17}FKCRRWQWR^{25}) presented the bigger activity against $^{E.\ coli}$ ATCC 25922 and $^{S.\ aureus}$ ATCC 25923, respectively. By the other hand, polyvalent peptides, the dimer $(FKARRWQWRMKKLGA)_2KA_{HxC}$, and tetramer $((RRWQWR)_2KAhxC)_2$, showed the highest antibacterial activity, indicating that the multiple copies of the sequence increase the activity. Our results suggest that the design of lineal and polyvalent sequences is a versatile strategy to identify potential sequences to developing the last generation of therapeutics agents.

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YI-P093 Fluorine Changes the Proteolytic Stability of Peptides

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Rapid digestion by proteases limits the application of peptides as therapeutics. A promising strategy to increase the enzymatic stability of peptides is the modification with fluorinated amino acids.[1]

We present a systematic study on the effects of fluorinated amino acids on the proteolytic stability of a peptide that was designed to comprise the substrate specificities of the proteases α-chymotrypsin, elastase, proteinase K and pepsin.[2,3] We sidespecifically incorporated fluorinated 2-aminobutyric acid analogues, trifluoroisoleucine and hexafluoroleucine at different positions of this peptide by SPPS, and studied the proteolytic stability towards the above-mentioned enzymes by an RP-HPLC assay. Furthermore, each cleavage product was identified by mass spectrometry. In surprisingly few cases we observed an increase in proteolytic stability, which points to specific interactions of fluorinated residues with the enzyme binding sites.

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P094 Antimicrobial Peptides Produced by a New *Enterococcus* Strain

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In recent years, a large number of infectious diseases have been difficult to treat with traditional antibiotics mainly due to the development of drug resistance. Bacteriocins, which are antimicrobial peptides that are ribosomally synthesized by bacteria, are potential alternatives to conventional antibiotics to be used against multi-drug resistant pathogens because of their high potency (picomolar and nanomolecular activity) and high specificity[1]. *Enterococcus canintestini* 49, a new *Enterococcus* strain isolated from a dog's intestine, was investigated for the production of antimicrobial compounds. Herein we describe the isolation, characterization, and identification of the bacteriocin, enterocin NKR-5-3D, which was previously isolated from a different strain, *Enterococcus faecium* NKR-5-3[2]. Enterocin NKR-5-3D was isolated through a series of hydrophobic interaction chromatographic techniques and characterized by MALDI-TOF and LC-MS/MS mass spectrometry. It showed homology to the inducing peptide IP-TX, which has been reported to have no antimicrobial activity[2]. Enterocin NKR-5-3D, however, was found to be active against *Listeria monocytogenes*, *Lactococcus lactis*, and *Enterococcus faecalis*. DNA extraction of the strain and a posterior genome sequencing was also carried out. The results revealed that *E. canintestini* 49 has the gene cluster that encodes for the production of five bacteriocins, enterocins NKR-5-3A, -B, -C, -D, and -Z, and three additional putative novel bacteriocins. Further work on the characterization of the other putative bacteriocins, and genetic analysis to examine what proteins are produced in response to transcriptional induction by enterocin NKR-5-3-D will be pursued.

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P095 Development of a Multi-Step Peptide Purification Process for Exenatide

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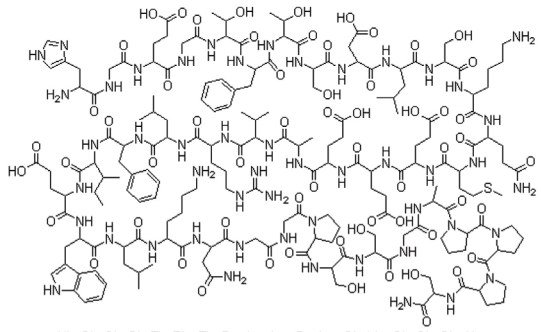
Purification of crude synthetic peptide mixtures often employs a multi-step chromatographic purification process. The first step removes most of the undesired components, followed by a different step to "polish" the material to the desired purity level. If applicable, a single step process can produce significant time and cost savings provided the single step can achieve the necessary purity while maintaining a desirable yield and throughput. A multistep process using the same stationary phase, can provide savings of time and costs.

The work presented here demonstrates the development of a multi-step purification process on a single stationary phase for a commercially significant crude synthetic peptide mixture, Exenatide (**Figure 1.**). The focus is on the initial development work, including the screening of multiple conditions to evaluate which steps will produce material of suitable purity. The investigated parameters include eluent pH, buffer components, and organic solvent composition.

Figure 1. Chemical Structure for Exenatide



His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH2

P096 HPLC Enantioserapration of EMOC Protected Amino Acids Using Chiral Stationary Phase under Reversed Phase Mode

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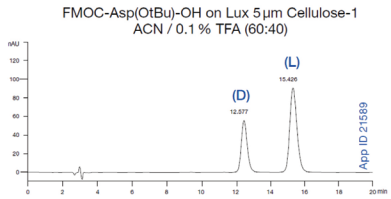
N-Fluorenylmethoxycarbonyl (Fmoc) α-amino acids are important building blocks for the solid phase synthesis of peptides.[1] After the development of Fmoc/tBu strategy[2] for solid phase peptide syntheses, Fmoc α-amino acids have become the raw materials of choice for the preparation of synthetic peptides.

Using this methodology, long peptides (up to 100 amino acids residues) can be prepared in a few days with high yield from micro molar (mg) up to molar scale (kg). As the number of amino acids residues increases the final purity and overall yield of the peptide produced is directly affected by the chemical and chiral purity of the protected amino acids used.

Currently, for the most common commercially available Fmoc protected α-amino acids, the expected enantiomeric purity is > 99.0% enantiomeric excess (ee) for the L form and sometimes the purity required must be >= 99.8% ee. This level of precision can only be achieved by very few analytical techniques, chiral HPLC being one of them. The main advantages of chiral HPLC analysis over other techniques are speed, detection level and ease of use. HPLC is also used on a regular basis by the peptide chemists to analyze purified fractions as well as peptide purity.

In this presentation, we will report the chiral separation of the most common 19 Fmoc protected α-amino acids derivatives under reversed phase separation mode using polysaccharide-based chiral stationary phases.[3] All Fmoc α-amino acids analyzed in this study are baseline resolved with an analysis time below 25 min in isocratic conditions. The order of elution as well as the enantiomer identification are also reported.



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YI-P097 Coupling Reagents: Oxyma Overcoming Benzotriazoles

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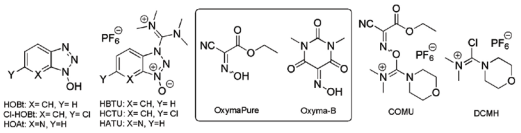
Just a few years ago coupling reagents were totally dominated by the hydroxybenzotriazole family, such as HOBt, Cl-HOBt, and HOAt with carbodiimides and HBTU, HCTU, and HATU as stand-alone reagents. The identification of the potential explosively of benzotriazole derivatives, turned the attention to develop new benzotriazole-free reagents. Later, our group proposed OxymaPure and it showed superior performance to HOBt and even in some cases superior to HOAt. Furthermore, we developed the morpholinium based uronium salt which in conjunction with OxymaPure render COMU.[1] Herein, the last developments of the Oxyma family will be discussed.

Thus, Oxyma-B which takes advantage of a special orientation of the carbonyl moiety which can play an assisted basic catalyst role enhancing the nucleophilicity of the amino function during the coupling, makes Oxyma-B a superior additive to minimize racemization.



Potassium salts of OxymaPure and Oxyma-B helps to avoid the leaking of the growing peptide from the CTC-resin and their combination with EDC.HCl, the greenest carbodiimides, showed a superior performance than DIC. New solvents have been investigated that allows COMU to be kept in solution without significant destruction. COMU can be formed in situ from OxymaPure and DCMH or other stand-alone reagents, facilitating its use in automatic synthesizers. Fmoc-Oxyma are excellent reagents for a safe introduction of the Fmoc group.

As conclusion, it is possible to say that the Oxyma family is the reagent of choice for driven the formation of the peptide bond.

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YI-P098 Solid-phase Peptide Synthesis by using Green Solvents

Y. E. Jad,[1] A. Kumar,[1] G A. Acosta,[2], T. Govender,1 H. G. Kruger,[1] A. El-Faham,[3,4] B. G. de la Torre,[1] and F. Albericio[1,2,3]
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SPPS is the method of choice for peptide synthesis. It allows the use of excess reagents to reach a quantitative yield and the excess reagents can be easily removed by several washing from the resin. However, the amount of consumed solvents is a drawback. In this regards, the commonly employed solvents are DMF (the mostly used), NMP and DCM. In fact, solvents are the major component in all reaction mixtures representing 80-90% of the non-aqueous masses as concluded in a survey by GSK at 2007 about the materials used for the manufacturing of APIs. Several selection guides for greener chemistry were concluded that DMF, DCM and NMP are hazardous chemicals.

Herein, we are presenting studies about substituting DMF by greener solvents. Initially, 2-MeTHF and CPME were evaluated for peptide coupling reactions and 2-MeTHF showed promising results. Then, we applied EtOAc and IPA along with 2-MeTHF for full green SPPS (GSPPS) with the objective of totally avoiding DMF from the synthesis. We concluded from that study that the use of 2-MeTHF for coupling and Fmoc removal steps with EtOAc washing steps is the best protocol in combination with ChemMatrix resin and DIC/OxymaPure. However, we found that Fmoc removal is problematic during SPPS of a peptide that tend to aggregate which required performing the Fmoc removal at 40 °C. Therefore, another independent study was achieved for that purpose where others green solvents were evaluated for the Fmoc removal step.

We can conclude that there are green alternatives for the hazardous solvents and in several cases with even superior results.

YI-P099 Synthetic Proteins: DNA-mediated Assembly of Artificial Proteins

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Designed peptides can form artificial proteins and even in a few cases nano-scale objects. Oligonucleotides have been intensively used for nanotechnology including so-called DNA origami, to create structurally advanced objects such as DNA-based nanoboxes. In contrast, formation of nano-scale objects and artificial proteins from peptide-oligonucleotide conjugates as building blocks, with combined involvement of two separate self-assembly principles, has previously not been realized.

We combined two orthogonal self-assembly principles, an oligonucleotide triple helix and a coiled coil protein domain, in the construction of peptide-oligonucleotide conjugates with molecular weights of 8.5-10.5 kDa. The resulting nano-scale assemblies were characterized by UV-melting, gel electrophoresis, CD spectroscopy, small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM). These studies revealed the formation of the desired triple helix and coiled coil domains to give a heterotrimeric protein mimic of 30 kDa at concentrations of 3.6 and 7.2 μM. This showed a compact and well folded structure. Remarkably, SAXS demonstrated formation of a well-defined, compact dimer of heterotrimeric units of 60 kDa at POC concentrations of 50μM, which was confirmed by TEM. The results validate the use of orthogonal self-assembly principles as a new paradigm for *de novo* protein design and for assembly of nano-scale objects. The dimerization of the heterotrimer at higher concentration points to a third level in the self-assembly, which may aid in design of higher-ordered nano-assemblies. By shortening the peptide to a 3-heptad structure we obtained a monodisperse trimer. In ongoing studies these concepts are expanded to organize small proteins in new nano-assemblies.

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YI-P100 Combatting Antibiotic Resistance with Synthetic Peptides: Inhibition of a Major Bacterial Drug Efflux Pump

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Bacterial multidrug resistance has become a prominent global health concern. One of several routes by which bacteria evolve resistance is through upregulation of multidrug resistant efflux pumps. A major contributor is a family of protein efflux pumps termed resistancenodulation-cell division (RND) superfamily. RND pumps function as a trimer and are capable of extruding a broad range of toxins, including potent antibiotics, before the drugs can exert their toxic effects on the bacterial cell[1]. Mutagenesis studies have elucidated a key interaction between transmembrane (TM) helices TM1 and TM8 within the membrane-embedded RND pump[2]. Our goal is to synthesize membrane-insertable peptides designed to competitively disrupt this interaction and thereby inhibit protein efflux function. We hypothesized that a peptide mimicking full length TM1 will adhere to its endogenous binding partner TM8 and thus competitively disrupt proper oligomerization. In initial studies, we found that an Nterminal acetyl-Ala-Sar3-tagged and C-terminal Lys_4- tagged synthetic peptide mimicking full-length TM1 displays measurable efflux inhibition. In order to optimize the synthetic peptides for maximal efficacy, we have undertaken to shorten the peptides to target the specific helix-helix interaction motif of the key TM1-TM8 interaction. As well, synthetic peptides of TM1 and TM8 have been labelled with fluorophore moieties dansyl and dabcyl in order to utilize FRET studies and other biophysical techniques to further characterize the helixhelix interaction motif. In the absence of new classes of antibiotics, these peptide drugs may become useful as critical adjuvants to boost the activity of current antibiotics.

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P101 Conotoxin Φ-MiXXVIIA from the New Superfamily G2 Employs a Novel Cysteine Framework that Mimics Granulin and Displays Anti-apoptotic aActivity

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Conotoxins are a large family of disulfide bond rich peptides that contain many unique cysteine frameworks that target a broad range of ion channels and receptors. We recently discovered the 33-residue conotoxin Φ-MiXXVIIA from Conus miles that contains a novel cysteine framework comprising three consecutive cysteine residues and four disulfide bonds and is the first member of a new superfamily G2. Regioselective chemical synthesis confirmed the disulfide bond connectivity and the structure of Φ-MiXXVIIA was determined by 2D NMR spectroscopy. Intriguingly, the cysteine rich motif displays a unique topology containing two β-hairpins with a structure that resembles the C-terminal granulin domain. Similarly to granulin, Φ-MiXXVIIA promotes cell proliferation (EC_{50} 17.85 μM) while inhibiting apoptosis at an EC_{50} of 2.2 μM. We discovered several Φ-MiXXVIIA-like peptides across different vermivorous cone snails with homologous signal peptides that define a new conotoxin superfamily. The novel structure and function of Φ-MiXXVIIA expands the repertoire of disulfide-rich frameworks and the structural and functional diversity of conotoxins.

YI-P102 Expanding the Structural Diversity of Peptide Macrocycle Phage Display Libraries by Cyclizing Peptides with Two Chemical Bridges

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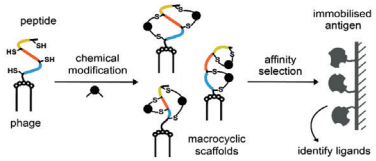

Successful screening campaigns depend on compound libraries that have a large size and a high structural diversity. Phage display allows screening of libraries comprising billions of different cyclic peptides in short time and with little effort. In reported phage display libraries, the scaffold diversity has been limited to only a few different macrocycle backbones. We have developed a new strategy for cyclizing phage-encoded peptides with two chemical bridges, affording hundreds of different macrocyclic scaffolds (Figure). Screening such libraries has yielded bicyclic peptide ligands with higher binding affinity and better proteolytic stability.

P103 Synthesis of *N*-amino Peptide Foldamers from Enantiopure α-hydrazino Acids

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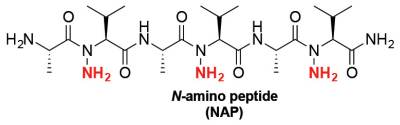
Backbone *N*-methylation of α-peptides has been widely employed to enhance the bioavailability and bioactivity of parent sequences. Heteroatomic peptide amide substituents have received less attention due, in part, to the lack of practical synthetic strategies. In an effort toward conformationally defined and proteolytically stable peptidomimetics, we sought access to diversely substituted backbone aminated peptides. Electrophilic amination of L-amino esters in aqueous media provides a direct route to α-hydrazino acids corresponding to 19 out of the 20 canonical proteinogenic amino acids. *N*-Amino peptides (NAPs) retain native side chains, which reduces the number of accessible ø and ψ torsions. The *N*-amino group in NAPs also offers a handle for H-bonding or subsequent chemical diversification. Our comparison of NAPs to *N*-methylated peptides by NMR demonstrates the trans amide-substantiating effect of the *N*-amino group and highlights the unique conformational impact of backbone amination relative to *N*-alkylation.



YI-P104 Peptides Synthesised or Attached to Hyaluronan-based Materials via SPPS

Sergej Karel[1], Martin Flegel[2], Romana Sulakova[1], Roman Frycak[1], Jana Sogorkova[1], Kristina Nesporova[1], Martina Hermannova[1] and Vladimir Velebny[1]
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Polysaccharides such as alginate[1] chitin[2] or cotton[3] were used as carrier supports to improve methods for Solid Phase Peptide Synthesis (SPPS). Peptide synthesis protocol was developed based on the use of biocompatible and biodegradable hyaluronan (HYA) materials as the carrier support. Our approach allowed to synthesize peptides either step by step on the HYA carrier or bind them to the surface of carrier and employ these constructs (without detachment) directly for the study of biological effects. Short peptides influencing cell adhesion (RGD like peptides) were chosen for this study. The behaviour and certain physical properties of such prepared HYA materials have been described. Fmoc synthetic protocol, particular reaction condition as well as analytical methods used for the synthesis monitoring, attachment of RGD peptides to HYA fibers and the basic *in vitro* biological effects of RGD-HYA constructs are also mentioned.

Peptides with biological, antimicrobial, cell adhesion modulating or hormonal activity were chosen for biological study. Biodegradable hyaluronan textile support were coated with these peptides and the biological effects of these conjugates were proved *in vitro*.

Fibers or textile bearing these peptides could be later exploited and employed as the wound dressing or tissue scaffold in various biomedicine applications advantageously for instance

as repairing patch for body organs. This could help to avoid the need of subsequent often painful or difficult removal of carrier.

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P105 Spectroscopic and Enzymatic Characterization of Fluorescent Peptide-based Substrates of Inflammatory Caspases

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Inflammatory caspases are implicated in inflammatory, autoimmune and autoinflammatory disorders. Currently available assays all use peptides that are capped with a fluorophore, such as 7-amino-4-methylcoumarin, on the C-terminal side. To explore the possibility that alternative fluorogenic peptides may exhibit enhanced assay properties, we have designed, synthesized, and characterized several novel fluorogenic peptides containing coumarin derivatives as side chains within a peptide sequence. The fluorescence quantum yields of these peptides were obtained and compared to commercially available C-terminal capped peptide substrates. Biochemical assays were also performed to kinetically characterize the new substrates.

P106 Multi-functional, Branched Peptides for the Treatment of Crohn's Disease

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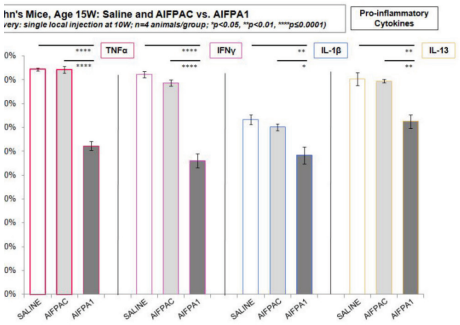
Crohn's disease (CD) is a chronic inflammatory bowel condition typically effecting the small intestine and is characterized by intense abdominal pain and irritation of the intestines accompanied by devolving intestinal integrity and function. Current treatment options for CD patients include biologic/ pharmacologic anti-inflammatory therapies that are associated with deleterious side effects. Late-stage treatment options for CD involve broad, untargeted, anti-inflammatory medications that even carry an increased risk of cancer. The Sharma Laboratory has recently demonstrated the effectiveness of usingpeptide amphiphiles (PAs) expressing anti-inflammatory (AIF) epitopes to regenerate functional bladder tissue while reducing inflammatory processes. The ability of PAs to self-assemble intosupramolecular structures, creating high density localization of peptide sequences, as well as other covalently bound molecules, provides a unique platform for applying this type of system to CD *in vivo*. The current project utilizes AIF peptides to create multifunctional PAs to modulate the inflammatory milieu *in vivo*. An established mousemodel of ileitis capable of mimicking human CD was used to test the resulting AIF-PAs. Results show significant decreases in key inflammatory events in mice injected with the AIF-PA.



YI-P107 A Structural Basis for Aza–Glycine Stabilization of Collagen

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Collagen is an essential protein in mammals, providing structure to skin, bones, cartilage, and the extracellular matrix. Native collagen is characterized by the variable amino acid sequence XYG. The variable X and Y positions in the XYG tripeptide are typically occupied by proline and hydroxyproline, respectively. Conversely, glycine is strictly conserved, and glycine mutations can propagate structural instability and collagen–related disease. This hallmark sequence promotes collagen's self–assembly into a distinctive triple helical supramolecular structure. However, it has been shown that certain synthetic modifications can lead to useful and intriguing biomechanical properties in collagen model peptides (CMPs). Previous studies in our lab have shown that the novel substitution of aza–glycine (azG) for glycine (G) in synthetic CMPs can impart new hydrogen bonds to the triple helix, leading to unprecedented thermal stability and faster folding kinetics.[1] In this study, we explored the generality of this effect by performing an aza–glycine substitution on an alternative arginine–containing collagen sequence: (POG)3PRG(POG)4 to (POG)3PRazG(POG)4. This single amino acid substitution substantially increased the thermal stability (ΔT_M = +9.7 °C) and folding rate of this collagen triple helix. Furthermore, this azapeptide was crystallized and its structure was determined to 1.1 Å resolution using X–ray diffraction, confirming the presence and stabilizing influence of enhanced hydrogen bonding provided by aza–glycine. Overall, this study illustrates that azaglycine substitution is an accessible tool for synthesizing modular, hyperstable CMPs, even in applications where alternative peptide sequences are desired.

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P108 Effect of Racemic Amyloidogenic Peptides on Phosphorylation of Focal Adhesion Kinase

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Amyloid fibrils are elongated, insoluble structures of 7-10 nm in diameter found in extracellular plaques. Previously, we screened the synthetic peptide library derived from the laminin-111 sequence, and identified five amyloidogenic peptides, A119, A208, AG97, B133, and B160. These peptides shows cell attachment activity and neurite outgrowth activity. Furthermore, D-enantiomers of A119, A208, and B133g peptide also form amyloid-like fibrils and show biological activities. Generally, it has been shown that equimolar mixtures of enantiomeric amyloidogenic peptides co-assemble into stronger fibrils than those from each enantiomer, do not co-assemble into two-component cross β sheets but rather self-sort to form distinct L- and D-enantiomeric fibrils, or fail to form fibrils altogether. We evaluated the amyloid-like fibril formability and biological activities of racemic mixtures of the five amyloidogenic peptides. We evaluated amyloid-like fibril formability using Thioflavin T (ThioT). The results of ThioT assay suggested that the racemic mixture of A119 peptide promoted stronger amyloid-like fibril formation than those from each enantiomer, and that the racemic mixture of AG97 peptide failed to form amyloid-like fibrils. The D-enantiomer of A119 peptide is reported to promote phosphorylation of focal adhesion kinase (FAK), but L-enantiomer of A119 peptide is not. The racemic mixtures of A119 peptide did not promote phosphorylation of FAK. Although further studies are needed, the effects of racemic amyloid fibril on signal transduction are suggested to be different from enantiomeric amyloid fibrils.

YI-P109 Development of TRH Analogue as Antiepileptic Agent and its Nano-encapsulation for Effective Nasal Administration

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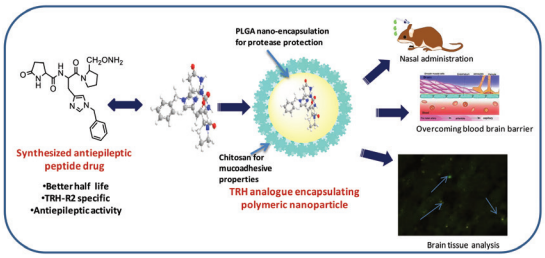
Thyrotropin releasing hormone (TRH), is a tri-peptide which functions as a neuroendocrine hormone by increasing TSH, leading to an elevation of thyroid hormone levels. Besides this hormonal activity, TRH has also been shown to exert a broad spectrum of CNS stimulatory actions that have attracted great attention for potential therapeutic applications such as CNS trauma, epilepsy, depression, cognition impairment, spinocerebellar degeneration, Alzheimer's disease and motor neuron disease. However, in common with other peptide-based drugs, the efficacy of TRH is compromised by its instability and hydrophilic nature. In the present study a TRH analogue was synthesized which exhibits significant anti-epileptic properties with higher stability. Considering the promising efficiency of nasal drug delivery route for the administration of various classes of drugs, we further developed a system for CNS delivery of TRH analogue through nasal route. The chemically synthesized TRH and its analogue were subjected to encapsulation using polymers, PLGA and chitosan, to form polymeric nanoparticles via solvent evaporation double emulsion method so as to impart stability for intra-nasal administration. These encapsulated polymeric nanoparticles were further characterized and studied using various physico-chemical techniques. Intranasal delivery as well as antiepileptic activity of synthesized TRH analogues encapsulated polymeric nanoparticles was studied in animal model.



YI-P110 A Novel Tri-Peptide Elicits Cardioprotective Effects in Myocardial Ischemia/Reperfusion Injury

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Currently, there are no pharmacologic treatments that have been shown to clinically improve cardiac function and reduce infarct size in patients with myocardial infarctions. Preliminary studies have shown that a novel tri-peptide (Phe-D-Arg-Phe-Amide, MW=468) attenuates diaphragm skeletal muscle atrophy in experimental cardiac bypass models when given as pretreatment. The aim of this current study was to determine the effects of this tri-peptide in myocardial I/R injury. The tri-peptide (50μM, n=3) was administered prior to I (pretreatment) in isolated perfused rat hearts subjected to I(30min)/R(45min) and compared to untreated control I/R hearts (n=4). Left ventricular developed pressure (LVDP) was measured using a pressure transducer inserted into the left ventricular cavity. The heart tissue was then assessed for infarct size using triphenyltetrazolium chloride (TTC) staining. The tri-peptide pretreatment group had significant recovery of post-reperfused LVDP by 58±9% of initial baseline LVDP compared to untreated control I/R hearts which only recovered to 30±4 of initial baseline LVDP ($p<0.01$). Furthermore, the tri-peptide pretreatment hearts had significantly reduced infarct sizes of 22±2% compared to control I/R hearts which had infarct sizes of 42±3% ($p<0.01$). These data suggest that this novel tri-peptide may be a rational approach to improve cardiac function and reduce infarct size in cardiac transplant recipients or when given as pretreatment prior to balloon angioplasty in heart attack patients.

This study was supported by the Center for Chronic Disorders of Aging, Division of Research, and the Department of Bio-Medical Sciences at Philadelphia College of Osteopathic Medicine.

YI-P111 Synthesis of Boron Clusters Modified with a Cell-Penetrating Peptide and Their Properties

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We synthesized boron clusters, a mercapto-closo-undecahydrododecaborate ($[B_{12}H_{11}SH]^{2-}$ $2Na^{+}$, BSH) modified

with a short arginine peptide (1R, 2R, 3R) via a maleimide linker for Boron neutron capture therapy (BNCT). The short arginine peptides containing a maleimide linker were prepared by solid-phase peptide synthesis, before thioether linkage of a thiol group of BSH and a maleimide group of the peptide in solution. BSH itself could not enter into cell, while the BSH modified with 3R as a cell-penetrating peptide was successfully delivered into cell. In a mouse brain tumor model, BSH modified with 3R successfully penetrated cell membranes of glioma cells *in vitro* and *in vivo*. Furthermore, to monitor the pharmacokinetic properties of these peptides *in vivo*, we also synthesized BSH and BSH-3R modified with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA); DOTA is a metal chelating agent for labeling positron emission tomography (PET) probe with ^{64}Cu. We administered BSH-DOTA-^{64}Cu and BSH-3R-DOTA-^{64}Cu to the tumor model through a mouse tail vein and determined the drugs' pharmacokinetics by PET imaging. BSH-3R-DOTA-$_{64}$Cu showed a high uptake in the tumor area on PET imaging. We concluded that BSH-3R is the ideal boron compound for clinical use during BNCT and that in developing this compound for clinical use, the BSH-3R PET probe is essential for pharmacokinetic imaging.

YI-P112 Designing Cell-material Interactions by Controlling the Chemistry of the Surface of Degradable Porous Scaffolds

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Tissue engineering has emerged as a promising tool for development of material that can replace natural tissue or organs in order to maintain or improve their function.[1] The biomaterial acts as a scaffold in the body, providing mechanical support while regenerating new tissue. Ultimately, the scaffold should degrade in a controlled manner producing non-toxic degradation products that can be eliminated by the body.[2]

The overall aim of this research project is to develop porous, degradable scaffolds with surface modifications allowing for soft tissue regeneration. The goal is to fine-tune the properties of the polymeric material to allow for ultimate cell-surface interaction, while maintaining specific mechanical strength so that it can be processed by 3D printing without being degraded. We will base the development of functional scaffolds on aliphatic polyesters, due to their mechanical and degradable properties as well as biocompatibility.

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YI-P113 Development of Synthetic Methods for Chloroalkene Dipeptide Isosteres and Their Applications

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It is highly important to enhance the activity of peptides by changing a part or whole of these molecules. Alkene-type dipeptide isosteres (ADIs) are expected as peptidomimetics due to a high structural homology with natural dipeptides.[1]

Recently, we have developed a synthetic method for trisubstituted (Z)-chloroalkenes adjacent to a asymmetric carbon.[2] As extension of the method, we have designed and developed a synthetic strategy of chloroalkene dipeptide isosteres (CADIs) due to expecting to have structural and chemical homology.[3,4] Treatment of (*E*)- or (*Z*)-γ,γ-dichloro-α,β-enoates with lower-order organocopper reagents obtained corresponding (L,D)- or (L,L)-type CADIs in high yield and diastereoselectivity. Furthermore, we will report an application study of CADI for peptidomimetic by introduction into a bioactive peptide.

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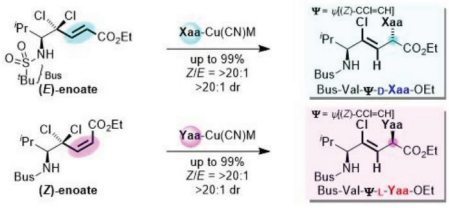

P114 Self-assembly of Short Peptides in the Presence of Metals Produces Catalytic Amyloids

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Enzymes fold into unique three-dimensional structures, which underlie their remarkable catalytic properties. The requirement that they be stably folded is a likely factor that contributes to their relatively large size (> 10,000 Dalton). However, much shorter peptides can achieve well-defined conformations through the formation of amyloid fibrils. To test whether short amyloid-forming peptides might in fact be capable of enzyme-like catalysis, we designed a series of 7-residue peptides that act as zinc-dependent esterases and copper-dependent oxidases. The metal ions help stabilize the fibril formation, while also acting as cofactors to catalyze ester hydrolysis and phenol oxidation. The catalytic repertoire of the fibrils is broad and includes difficult to hydrolyze substrate (e.g. paraoxon). Moreover, we have demonstrated that catalytic amyloids are capable of facilitating tandem reactions. These results indicate that prion-like fibrils are able to not only catalyze their own formation — they also can catalyze chemical reactions. Our results also have implications for the design of self-assembling nanostructured catalysts including ones containing a variety of biological and non-biological metal ions.

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P115 Autocatalytic Backbone N-methylation Hallmarks a Distinct Family of Ribosomally Encoded Peptides

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N-Methylated backbone amides alter the physicochemical properties of peptides and are critical for the activity and stability of blockbuster drugs like the immunosuppressant cyclosporin A. In nature, backbone N-methylations have not been observed in proteins, but only in peptides not synthesized by the ribosome. Here, we show that omphalotins, cyclic backbone N-methylated peptides from the fungus *Omphalotus olearius*, are ribosomally synthesized and post-translationally modified peptides (RiPPs). Expression of a single gene, *ophA*, in *Escherichia coli* revealed auto-α-N-methylation activity of the encoded protein. Remarkably, the sequence destined to be the nematotoxic natural products is encoded in the C-terminus of OphA, and we demonstrate that OphA catalyzes iterative α-N-methylation of this sequence. Thus, OphA is the first observed case of backbone N-methylations as post-translational modifications (PTMs) and the first RiPP precursor found to be capable of catalysis or self-modification. The omphalotins represent, therefore, a new RiPP family, one of the few so far identified in fungi. We also report that OphA can methylate engineered peptide sequences exchanged for the core omphalotin sequence, providing a straightforward and convenient biotechnological strategy to produce custom peptides with backbone N-methylation.

YI-P116 Long Acting Glucagon in a Preclinical Detting

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Genetic code reprogramming has facilitated successful implantation of diverse, versatile chemistry into peptides by incorporating a wide variety of non-standard amino acids. Assignment of non-standard amino acids to the codon table by intentional misacylation of tRNA can lead mRNA-templated translation of non-natural polypeptides by the ribosome. Flexible *in vitro* translation (FIT) system, using small aminoacylation ribozymes, i.e. 'flexizymes', has paved the way to effectively aminoacylate tRNAs with any non-proteinogenic alternatives. The unique FIT system combined with the remarkable tolerance of the ribosome for non-standard amino acids demonstrated that hundreds of uncommon substrates could be incorporated into polypeptides. However, incorporation of synthetic building blocks that can effectively bias the structure and folding of peptides still remains a challenge. By utilising the protein translation machinery and genetic reprogramming techniques, can we directly encode 'folding information' in the codon table?

Here we report that aromatic oligoamide foldamers, a well-known class of synthetic molecules with particular capability for folding and structural modularity, could be incorporated as the initiators of translation. Foldamers with sizable helical structures (> 1kDa) could be translated through the narrow ribosome exit tunnel by controlling their conformational flexibility. Moreover, macrocyclic peptides stapled with aromatic foldamers were successfully synthesized via a covalent thioether bond formation between N-terminal chloroacetyl group and a cysteine side chain. These foldamer scaffolds represent one of the most exotic building blocks that can be incorporated by ribosome, as their inherent, robust folding propensity may stabilize the conformation of short or medium-sized peptides.

YI-P117 Enhancing Protein Stability by Introducing Pseudoallylic Strain in Reverse Turns

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The rules for stabilizing protein secondary structures like α-helix and β-sheets are well established; however, the relatively shorter secondary structure motif, β-turn, despite its critical role in protein folding has received much less attention possibly due to the lack of any repeat features as in helices and sheets. Thus, we were motivated to design stable β-turns and test their efficacy in stabilizing proteins. As the natural repertoire of turn inducing motifs are limited, we focused our attention towards utilizing the pseudoallylic strain ($A^{1,3}$ and $A^{1,2}$) demonstrated by Nmethylated amino acids to confer dihedral restriction in the reverse turn. Utilizing a water-soluble peptide sequence lacking any cross-strand stabilizing interaction, we validated the efficacy of the $A^{1,3}$ and $A^{1,2}$ strain in inducing stable, monomeric β-sheet structure. It was observed that the $A^{1,3}$ strain strongly dictates the folding of the peptide than the $A^{1,2}$ strain, as discerned using CD and NMR. By amino acid screening at the *i+1* and *i+2* sites in the β-turn, we observed that the combined pseudoallylic strain could contribute to the β-sheet stability by 2.19 kcal/mol (ΔΔG).The most exciting observation was made when we grafted our engineered motifs onto a β-sheet mini-protein, Pin1 WW domain. All the Pin1 variants tested showed cooperative two-state transitions with thermal stability ranging between 69-78°C, which in turn, contributed to the increase in protein stability by 0.3-1.3 kcal/mol than the wild type. Our study provides the first example of a simple loop engineering strategy to stabilize β-sheet structures that has been translated into proteins and will find great utility in *de novo* protein design.

YI-P118 Novel Peptidomimetic GHSR-1a Agonists for PET Imaging of Prostate Cancer

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Contemporary diagnostic techniques for prostate cancer (PCa) have a limited ability to distinguish between benign and malignant disease. The Growth Hormone Secretagogue Receptor type 1a (GHSR-1a) is a potential biomarker for prostate cancer. We are generating ^{18}F-labelled peptidomimetics for the detection of GHSR-1a via PET imaging. A structure-activity study was carried out on a library of GHSR-1a-targeted peptidomimetics that were designed based upon previously known growth hormone secretagogues (GHS). These peptidomimetics were designed to contain the prosthetic

groups 4-fluorobenzoic acid (FBA) and 4-fluoropropionic acid (FPA), which can be readily [^{18}F]-radiolabeled before coupling onto the desired peptidomimetic. All compounds were tested using a competitive receptor-binding assay using HEK293/GHS-R1a cells, as well as evaluated with ACD/Labs to identify their cLogP. Two peptidomimetics, [1-Nal^4,Lys^5(4-FBA)]G-7039 and [Tyr^4,Lys^5(2-FPA)]G-7039, had comparable binding to GHS-R1a (IC50 = 69 nM and 0.283 nM respectively) as the natural ligand, ghrelin (IC_{50} = 4 nM). These compounds also had an acceptable cLogP and enhanced stability in human serum compared to that of natural ghrelin. Due to the use of two different prosthetic groups, different strategies have been developed for successful ^{18}F-labelling of both peptidomimetics, and data is finalized for one of the compounds. [1-Nal^4,Lys^5(4-FBA)]G-7039 had a radiochemical yield of 63%, a 98% radiochemical purity, and a molar radioactivity of 2-5GBq/μmol after a 140 minute synthesis. We have successfully synthesized two peptidomimetics that target GHSR-1a and can be radiolabelled for use as a PET imaging agent. Preliminary *in vivo* studies are planned with a murine cancer model for both peptidomimetics.

YI-P119 Stapled Ghrelin(1-20) Analogues for the Targeting of GHSR-1a

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The expression of the Growth Hormone Secretagogue Receptor type 1a (GHSR-1a) is elevated in a variety of diseases including prostate and ovarian cancer. The endogenous ligand for GHSR-1a is the 28 amino acid peptide ghrelin, which has an noctanoyl chain modification on Ser-3 that is important for binding to the receptor. The peptide can be truncated to 20 amino acids, as these residues contained an α-helical structure, based on computational structural predictions. However, truncation would lead to destabilization of the α-helix due to the lack of α-helical stabilizing residues and thus affect the binding to GHSR-1a. The objective of this project was to create cyclic ghrelin(1-20) analogues that stabilize the α-helical character of the peptide, by means of an *i, i+4* or *i, i+7* lactam bridge, and demonstrate that these stabilized peptides improve receptor affinity and protease stability. Twenty-two stapled peptides were synthesized and analyzed by circular dichroism (CD) spectroscopy, 2D-NMR spectroscopy, and competitive binding (IC_{50}) towards GHSR-1a. Stapling improved helicity in every case when compared to the linear sequence, which contained a random coil conformation under physiological conditions. The peptide with the greatest improvement resulted in a $[\theta]_{222}/[\theta]_{208}$ ratio of 0.84, and an IC50 of 7.85 nM. This IC50 is a 3.18-fold increase in binding towards GHSR-1a over its linear counterpart which had an IC_{50} of 25 nM. The lead analogues have been fluorescently tagged and preliminary tests have confirmed lead analogue binding to cells that have endogenous GHSR-1a expression and cells that have been stably-transfected to express GHSR-1a.

YI-P120 Development of an *In Vivo* Chemical Probe for Polycomb Chromodomains

Kelsey N. Lamb, Jacob I. Stuckey, Jacqueline L. Norris, Stephanie H. Cholensky, Hongda Zhu, Leaf Huang, Lindsey I. James, and Stephen V. Frye

The installation, interpretation, and removal of histone post-translational modifications (PTMs) by distinct classes of proteins represents a crucial mode of chromatin regulation. Lysine methylation (Kme) is one of the most abundant and better studied chromatin modifications, and dependent upon its location and degree of methylation, can be implicated in both active and repressed chromatin states. Polycomb repressive complex 1 (PRC1), recognizes the H3K27me3 mark through the chromodomain in CBX proteins (2,4,6,7,8), which appropriately positions PRC1 for deposition of H2AK119ub and eventually leads to a repressed chromatin state. The development of a peptidomimetic chemical probe for Polycomb chromodomains, UNC3866, capable of disrupting the surface groove binding mode of the chromodomain confirmed that peptide-like ligands are a potent and selective way to target this family of Kme readers. However, UNC3866 displays poor membrane permeability and short target residence time which, as of yet, has excluded its use as an *in vivo* probe. Here we report our efforts to improve the physiochemical properties and target residence time of our current chemical probe, UNC3866, while also exploring novel drug delivery systems to increase cellular and in vivo activity of our modified ligand. Initial studies in engineered cell lines and in a xenograft model suggest that our optimized ligand, UNC4976, displays a 50-fold increase in cellular efficacy and comparable activity to a clinically validated ovarian cancer treatment, paclitaxel. Further studies will aim to fully profile UNC4976 against CBX family members using biophysical assays, and quantitate its potency in a micelle formulation in engineered cell lines.

P121 New Concept of an Automatic User Friendly and Economical Peptide Synthesizer

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A plethora of peptide synthesizers is comercially available from multiple companies. However, a synthesizer which would combine simplicity, flexibility and economy of synthesis was still missing. Our solution combines the elegance and flexibility of synthesis in (potentially disposable) plastic syringes with efficiency of centrifugal liquid removal and simplicity of liquid handling resulting in very inexpensive completely automatic instrument. Synthesizer can handle simultaneously up to 18 10ml or 35 2ml reactors (syringes). Any individual syringe can be at any step of the synthesis removed from the synthesizer and individual operation can be performed manually. Any type of solid support can be used and both Fmoc and Boc synthesis can be applied in this machine since the synthetic compartment is separated from the environment and can be kept under constant flow of nitrogen. Synthesizer can be upgraded for automatic one-bead-one-compound library synthesis. Concept of the synthesizer and individual components are subjects of the patent application.
and can be kept under constant flow of nitrogen. Synthesizer can be upgraded for automatic one-bead-one-compound library synthesis. Concept of the synthesizer and individual components are subjects of the patent application.

P122 Functional Light-Sensitive Biomaterials

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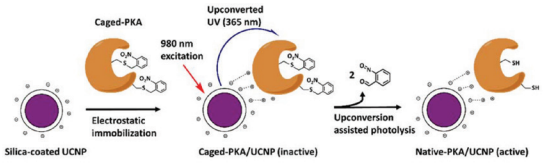
Photoactivable (caged) bio-effectors provide a way to remotely trigger on/off biochemical pathways in the living organism at a desired timing and location with a pulse of light. In the past 40 years, many caged/light-sensitive compound/material have been successfully synthesized. However, the phototoxicity of UV often limits the application of caged bioeffectors when applied in cellular or in vivo experiment. We recently have developed the first near infrared (NIR) photoactivable enzyme platform by immobilizing Protein kinas A (PKA) to upconversion nanoparticle. We have successfully photoactivated PKA using the upconverted NIR and consequently induced its downstream cellular response in the living cells With the similar concept, the upconversion-assisted channelrhodopsin opening (originally responsive to blue light) is also developed.

The caged-PKA/UCNP complex design and a conceptual description of upconversion-assisted PKA uncaging



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P123 Development of a 'Smart' Activity-based Probe for Monitoring Enzyme Activity in Living Cells

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Activity-based protein profiling (ABPP) is a powerful tool to study enzyme activity in complex biological systems. The use of chemical probes for ABPP, activity-based probes (ABP), enables quantitative biochemical analyses of specific enzyme classes. While currently available ABPs have proven to be useful for the applications in various biological samples including cells and tissue extracts, these probes use a non-responsive reporter moiety requiring extensive washing steps or another separation methods such as electrophoresis. In this work, we developed a 'smart' activity-based probe that can generate selective 'turn-on' signals upon binding to asparaginyl endopeptidase (AEP), a lysosomal cysteine protease. Confocal imaging experiments showed that our probe generated enhanced fluorescent signals in response to the activity of AEP in living cells. In addition, the modification of the general scaffold did not affect labeling efficiency and selectivity of the probe significantly when compared to a previously developed probe, which was confirmed by in-gel fluorescence. We believe that our 'smart' probe is particularly well suited for studying dynamic changes of enzyme activity in living cells, which is a major advantage to screen drug candidates in relevant disease model. The long-term goal of our research is to utilize this probe to elucidate the functional roles of AEP in the pathogenesis of Alzheimer's disease.

P124 Synthesis and CD Spectroscopic Studies of a New Class of Peptoid-Based Foldamers: N-substituted β2- or β3-Homoalanines

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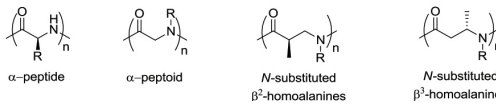
Peptoids (poly *N*-substituted glycines) have been widely studied as a desirable class of peptidomimetics. They are resistant to proteolytic degradation, easily synthesized and far more cell permeable than peptides. However, due to lack of backbone chirality and hydrogen bonding sources, peptoids are relatively flexible and do not form organized structure. As such, strategies to restricting the structural flexibility of peptoids are of great interest. Interestingly, it was demonstrated that peptoids are able to form defined secondary structures when they have α-chiral side chains. Inspired by the structures, we designed a new class of peptoid-based peptidomimetics (oligomers of *N*-substituted β2-homoalanines) as a new peptoid foldamer. The oligomers ranging from 2-mer to 8-mer were efficiently synthesized on solid-phase using pre-synthesized monomer building blocks. The purified oligomers were investigated by CD spectroscopy to suggest the existence of ordered folding structures. It is also noteworthy that readily available amines and alcohols can be utilized as side chains of the oligomers to provide a huge chemical diversity. In addition, we synthesized a modified version of oligomers (oligomers of N-substituted β3-homoalanines). The CD spectra of the oligomers provided different characteristic features compared to those of β2-homoalanines. More interestingly, the NMR spectrum of 2-mer provided a biased cis/trans ratio of amide rotamers, which supports the potential capability of the oligomers to form ordered folding structures. Further high-resolution studies will be conducted including X-ray and NMR studies to determine detailed secondary structures. These new peptoid structures will expand the scope of peptoid foldamers and act as a promising scaffold to design biologically potent oligomers.



P125 Rational Design of α-Helix Mimetics as Inhibitors of Anti-Apoptotic Bcl-2 Family Proteins

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Bcl-2 family proteins are key regulators of apoptosis and consist of two groups. Anti-apoptotic proteins (e.g., Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1) contain four Bcl-2 homology domains (BH1-4) and promote cell survival by inactivating pro-apoptotic proteins. The pro-apoptotic members are subdivided into two classes: multi-domain proteins (e.g., Bak, Bax) and BH3-only proteins (e.g., Bim, Bid, Puma, Noxa, Bad). The BH3-only proteins trigger a series of events, such as mitochondrial outer membrane permeabilization, cytochrome c release, and caspase activation, leading to programmed cell death by directly activating the multi-domain proapoptotic proteins or

by inhibiting the anti-apoptotic proteins. Overexpression of anti-apoptotic proteins is frequently observed in cancers and correlated with resistance to chemotherapies.

Structural studies show that the α-helical BH3 domain of pro-apoptotic proteins binds to anti-apoptotic proteins and mediates the heterodimerization of Bcl-2 family proteins. Thus, the BH3 domain is a key motif in regulating apoptosis, making it an attractive therapeutic target in treating cancers. In order to mimic the BH3 domain, we have developed new α-helix mimetics by using a N,N'-diphenyl-4,4'-biphenyldicarboxamide as a scaffold. This scaffold features a rigid and preorganized structure which projects its four substituents in a proper orientation as found in an α-helix, thereby reproducing the structure and function of the helix. By optimizing substituents and their positions in the mimetics, we were able to control their binding profiles for anti-apoptotic proteins, creating selective and pan-Bcl-2 inhibitors. These preliminary results suggest that these versatile mimetics would provide a powerful toolkit for dissecting the roles of Bcl-2 family members and for developing effective therapeutic candidates.

P126 Multifunctional Opioid Ligands with a New Biological Profile: MOR/DOR Agonist and KOR Antagonist Activity

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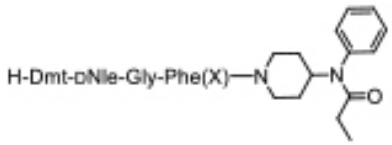
Chronic pain syndromes remain poorly managed due to inadequate efficacy of currently available drugs or undesirable side effects associated with doses or long term administration needed to obtain pain relief. There remains a critical need for novel therapies to enhance the efficacy and the safety of treatments for chronic pain. It is now well established that chronic pain induces up-regulation of the kappa opioid receptor (KOR) resulting in diminished analgesia from mu opioid receptor (MOR) agonists and undesirable adverse side effects including enhanced possibility of addiction. Known physiological adaptations should be considered in the design process in order to develop the most effective drugs for the disease state to be treated. For this reason, we have discovered multifunctional ligands with a new biological profile that incorporate agonist activity at the MOR and the delta opioid receptor (DOR) with antagonist activity at the (KOR) (Figure). These ligands exhibited strong analgesic effects along with a high potential to penetrate the blood brain barrier in in vivo tests and high metabolic stability in human plasma. These ligands open up the possibility of developing novel opioid drugs with clear therapeutic advantages for the treatment of chronic pain by additional modulation of KOR activity, because increased KOR activation can be blocked by the KOR antagonist function.



X=Cl: MOR/DOR agonist & KOR antagonist
X=F: MOR/DOR agonist & KOR partial agonist

YI-P127 On the Effects of Acylation of Cell-Penetrating Peptides in Nucleic Acid Delivery *In Vitro* and *In Vivo*

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Cell-penetrating peptides (CPPs) are a class of peptides that are able to carry cargo molecules like proteins and nucleic acids across cell membranes to facilitate their biological function. One way of improving CPPs is to modify them with fatty acids like. In this study we have investigated in detail how the length of saturated fatty acid tail influences the delivery of nucleic acids both *in vitro* and *in vivo*. For that we took a well described CPP, PepFect14, and varied its N-terminal acyl chain length from 2 to 22 carbons. To evaluate their delivery efficiency, the peptides were non-covalently complexed with nucleic acids at different peptide-to-nucleic acid ratios.

Our results show that there is a threshold of hydrophobicity after which the transfection efficiency starts to increase with each added carbon, whereas below this threshold there is no transfection of nucleic acids in cell culture. This effect was consistent also in in vivo experiments where the gene induction after systemic administration showed correlation with the length of acyl chain on the peptide further confirming the importance of the hydrophobic interactions in the CPP-based nucleic acid delivery platforms. The physicochemical characterization of the complexes showed that the peptides with longer acyl chains were able to form smaller and more tightly packed complexes with nucleic acids when compared to shorter acyl chain analogues. All together these data show how to design highly efficient CPPs for both *in vitro* and *in vivo* applications.

YI-P128 A Novel Peptide-decorated DM1-conjugated Nanotherapeutic Agent for Targeted Therapy against Lung Cancer

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DM1 is a thiol-containing maytansinoid that inhibits microtubule assembly and kills cancer cells at a sub-nanomolar concentration. However, its application in the clinic has been hampered by severe toxic side effects and poor efficacy.[1] To overcome this, targeted delivery of the toxin to the tumor site is greatly needed. We recently designed and synthesized a novel PEG-based, cholic acid/ DM1 hybrid telodendrimer (PEG^{5k}-CA_4-DM1), which self-assembled into well-defined nanomicelles (DM1-nano), for targeted therapy against non-small cell lung cancer (NSCLC). To further enhance the targeted delivery ability of DM1-nano to the tumor site, a peptide ligand LXY30 [cyclic cdG-Phe(3,5-diF)-G-Hyp-NcR] was decorated on the surface of DM1-Nano via copper-free Click reaction. LXY30

is a potent and specific ligand against $\alpha 3\beta 1$ integrin which is expressed at high levels on the surface of many cancer cells including A549 NSCLC cells.[2] The particle size, zeta potential and critical micelle concentration of LXY30-DM1-Nano were measured as 7.13 nm, 1.92 mV and 1 μM, respectively. The *in vitro* anti-cancer activity (IC50) of LXY30-DM1-Nano in A549 cells was measured and determined to be 1 μM. When loaded with a fluorescent dye (DiD), LXY30-DM1-nano was found to internalize inside A549 cells after 4 hours of incubation. *In vivo* anti-tumor efficacy study in nude mice bearing A549 tumor xenograft demonstrated that LXY30-DM1-nano showed better anti-tumor activity and less toxicity compared with free DM1 and non-targeted DM1-nano. In conclusion, the LXY30-DM1-nano offers a promising novel nanotherapeutic agent against lung cancer.

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YI-P129 *De Novo* Design Peptides Inhibiting Critical Protein-Protein Interactions in Cancer

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Inhibition of protein-protein interactions (PPIs) represents a significant challenge in terms of the composition and topography of such interfaces. Owing to a large surface area (>800 Å^2) and a general lack of hydrophobic pockets, protein-protein interactions have historically been considerably undruggable using small molecules. In this context, peptides are believed to be a class of bioactive agents capable of targeting PPIs considering their expansive surface and spacing of side chain making discontinuous, non-covalent contacts over a shallow and large proteins interface. Here we present a workflow to design peptides targeting critical protein-protein interactions in cancer. Peptide libraries were built on yeast surface display system and their bindings to target proteins were determined by labeling of yeast and proteins with two different fluorophores following quantitative screening through fluorescence-activated cell sorting (FACS). Leveraging the next generation sequencing techniques and bioinformatics tools, we quickly and confidently identified hit peptides from limit rounds of selection. Hit peptides were further characterized by series of biophysical and biological assays. In addition, a reversible structural complementation reporter assay was developed to probe desired protein: protein interaction. Preliminary results are shown and further peptides optimizing strategies will be discussed.

P130 High-affinity High-specificity Peptide Ligand LXY30 for Targeted Imaging and Therapy in Non-small Cell Lung Cancer

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Peptide ligands against cancer cell surface receptors have attracted great interest as vehicles for targeted delivery of imaging agents and anti-cancer drugs to enhance cancer diagnosis and treatment, respectively. We previously identified a potent and specific peptide LXY30 [cyclic cdG-Phe(3,5-diF)-G-Hyp-NcR] that bound to $\alpha 3\beta 1$ integrin on the surface of U-87 MG glioblastoma cells.[1] We recently tested the binding of LXY30 against a panel of non-small cell lung cancer (NSCLC) cell lines, malignant pleural effusion and peripheral blood mononuclear cells (PBMCs) from lung cancer patients. Flow cytometry showed LXY30 bound to most NSCLC cell lines and the tumor cells in the pleural effusion of NSCLC patients, but did not bind to PBMCs. LXY30 bound to $\alpha 3\beta 1$ integrin on the surface of lung cancer cells and entered the cells via endocytosis, as revealed by fluorescence microscopy imaging. Furthermore, in vivo optical imaging demonstrated the preferential uptake of the LXY30-Cy5.5 conjugate in tumors in several NSCLC xenograft mouse models including the subcutaneous and orthotopic xenografts of EGFR-mutant lung cancer H3255 (EGFR L858R), the subcutaneous xenograft of EGFR-mutant lung cancer H1975 (EGFR L858R/T790M) and A549, as well as a patient-derived xenograft (PDX) lung cancer model. All these lung cancers have high level of expression of the $\alpha 3\beta 1$ integrin on their surface. LXY30 is a promising cancer-targeting vehicle for targeted delivery of imaging agents and/or cancer therapeutic drugs to NSCLC and other $\alpha 3\beta 1$ integrin-expressing human tumors. Preliminary therapeutic studies of LXY30-drug conjugate in xenograft model of NSCLC will be presented.

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YI-P131 Antiproliferative Activity of Peptides Obtained from Glutelin Fraction of Pecan Nut (Carya illinoinensis) against SiHa Cell Line

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Pecan nut (Carya illinoinensis) is highly consumed world wide, and it is known to be a rich source of poli and monounsaturated fatty acids, also vitamins, minerals, and proteins[1]. Main source of proteins in walnut are storage proteins which are necessary for the development of the plant and are rich in apsaragine, glutamine, arginine, and proline[2]. Storage protein can be clasiffied accordirng to their solubility in differnt solvents. Glutelins fractions has been studied in amaranth, soy, andd recently in walnut (*Juglans regia L.*) it was discover that they contain encrypted peptides that deploy antiproliferative activity against cancer cell lines[3,4]. In the present study we extracted the glutelin fraction of *Carya illinoinensis* and analyze its

protein pattern. After that, fraction was precipitated to eliminate salts an other compounds that could interfere with afterward digestions with trypsin. Then, the peptides were separated with a 10kDa membrane. Later, three different concentrations of glultein tryptic digestions were tested against SiHa cell line and samples were taken at 0, 12, 24 and 48 hours to know the effect of them. Afterward, proteins were obtained from cells and a mass spectrometry analysis were performed to know the accumulation of different proteins during the treatment. The results indicated that a concentration of 50µg/mL at 48 hours has the highest antiproliferative effect, and increase in a dose-dependent manner. Also, MS/MS analisys was performed to know the different peptides in the glutelin digestion with the highest antiproliferative effect. Several peptides were identified and we believe a synergic effect occours rather than a single peptide effect.

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P132 The Effect of Force Field on the Structural Properties of Amyloid β(1-40) Dimer Explored by Replica Exchange Molecular Dynamics Simulations

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Alzheimer's disease is histologically marked by fibrils of Amyloid beta (Aβ) peptide within the extracellular matrix. Fibrils themselves are benign compared to the cytotoxicity of the oligomers and pre-fibrillary aggregates. The conformational space and structural ensembles of Aβ peptides and their oligomers in solution are inherently disordered and proven to be challenging to study. Optimum force field selection for molecular dynamics (MD) simulations and the biophysical relevance of results are still unknown. We compared the conformational space of the Aβ(1-40) dimer by 300 ns replica exchange MD simulations at physiological temperature (310 K) using: the AMBER-ff99sb-ILDN, AMBER-ff99sb*-ILDN, AMBER-ff99sb-NMR, CHARMM22*, and CHARMM36 force fields. All force fields result in sampled ensembles of conformations with radii of gyration and collision cross sectional areas for the dimer that are statistically significantly smaller than experimental results. All force fields, with the exception of AMBER-ff99sb-ILDN (8.8±6.4%) and CHARMM36 (2.7±4.2%), tend to over estimate the β-helical content compared to experimental CD (5.3±5.2%) with AMBER-ff99sb-NMR (41.3±12.9%) demonstrating the greatest degree of variance. The force fields also tend to under estimate the expected amount of β-sheet and over estimate the amount of turn/bend/random coil with the exception of AMBER-ff99sb-NMR. All force fields, with the exception AMBER-ff99sb-NMR, reproduce a theoretically expected β-sheet-turn-β-sheet conformational motif, however, only the CHARMM22* and CHARMM36 force fields yield results compatible with collapse of the central and C-terminal hydrophobic cores from residues 17-21 and 30-36, respectively.

P133 Cell Penetrating Peptides with Intrinsic Cell Selectivity

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Over the last years, we have developed cell penetrating peptides (CPPs) that are activatable both temporally and spatially to control the activity of CPPs in order to overcome uptake by non-target cells. We designed *e.g.* a UV-activatable CPP inactivated by means of constraining the peptide and 'hiding' it on the surface of a liposome.[1] Alternatively, we induced cellular uptake by the formation of a coiled-coil[2] or a disulphide bridge leading to the recombination of inactivated peptides into a functional CPP.[3] In addition we designed an enzyme activatable CPP.[4] For this a CPP was decorated with small peptide fragments to inactivate it. Cleavage of these peptides by enzymes restored the ability of the CPP to enter cells.

Now, we have found a strategy to modify a CPP such that it becomes intrinsically cell type specific. We have demonstrated this approach to work by applying minimal changes on the archetypal CPP Tat to induce differential uptake profiles between healthy mammalian and cancer cells, in which the modified CPP is taken up with high selectivity by the diseased cells. This is shown for the model cell lines HEK and HeLa and also for various patient-derived leukemic cell lines in distinction to healthy white blood cells that do not take up the modified Tat. We believe that our strategy to control CPP activity and selectivity can become a useful tool that can be employed for cell type specific diagnostics and delivery of drugs and other cargos.

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P134 Modulation of the Prostaglandin F2α Receptor with Azapeptides in an Approach to Inhibit Preterm Labor

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Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes inflammation, uterine contractions and cervical changes, which are key features of preterm labor. Although administration of $PGF_{2\alpha}$ inhibitors, such as indomethacin, have been shown to suppress preterm labor, their use is handicapped by complications to both fetus and mother.[1] Azapeptides employ semicarbazide residues as amino amide surrogates.[2] Aza-amino acyl proline analogues have been shown to safely modulate the $PGF_{2\alpha}$ receptor (FP) and to inhibit $PGF_{2\alpha}$-mediated uterine contractions by an allosteric mechanism involving biased G protein-coupled receptor signalling.[3] We will present recent advances in azapeptide FP

modulator structure-activity studies, which are designed to gain insight into their mechanism and further their potential as inhibitors of preterm labor.

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YI-P135 Ribosomal Synthesis of Thioamide Bond via Genetic Code Reprogramming

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Amide to thioamide backbone substitution has been utilized to study significance of certain hydrogen bonds in the formation of secondary structures, improve biological and pharmacological properties of oligopeptides and as minimalistic fluorescence quenching probes to study protein folding. For all such purposes, peptides having thioamide moiety were synthesized by chemical methods. In contrast, ribosome-mediated facilitation of thioamide moiety into the backbone of peptides and proteins has been challenging. It is only recently that Maini et al. successfully incorporated a thioamide-dipeptide, Phe-ψ[CSNH]-Gly, into full-length protein using mutant ribosomes in response to single codon (TAG).[1] Nevertheless, we were interested in exploring the possibility of thioamide bond (-CSNH-) synthesis by the wild-type ribosomes during translation.

We hypothesized that the ribosome should utilize an amino(thio)acyl-tRNA in a fashion similar to aminoacyl-tRNA to catalyze thioamide formation during peptide chain elongation. To prove this hypothesis, we synthesized an activated thionoester of alanine and prepared ala(thio)-$tRNA^{Glu}_{GGU}$ using flexizyme (dFx).[2] By reassigning Thr codon (ACC) to (thio)Ala, oligopeptides having one thioamide backbone modification (-Ala-ψ[CSNH]-Asp-) were successfully translated using a reconstituted cell-free translation system lacking threonine. Furthermore, we prepared *N*-pentenoyl-ala(thio)-tRNAfMet CAU and a cell-free translation system lacking 10- formyltetrahydrofolate (formyl donor) and methionine.[3] By reassigning AUG start codon, we also demonstrated thioamide bond formation during translation initiation. Experiments are underway to study the relative rates of thioamide bond synthesis by the ribosome. With these unprecedented results, it is now possible to incorporate thioamide moieties into the backbone of peptides and proteins using the wild-type ribosomes.

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P136 Lipidized Prolactin-releasing Peptide Analogs: A New Tool for Potential Treatment of Obesity, Diabetes and Neurodegeneration

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Obesity is an escalating epidemic, but an effective non-invasive therapy is still scarce. For obesity treatment, anorexigenic neuropeptides are promising tools, but delivery of peptides from the periphery to the brain is complicated by their low stability and limited ability to cross the blood-brain barrier. Recently, we have designed several lipidized analogs of neuropeptide prolactin-releasing peptide (PrRP) which is involved in regulation of energy homeostasis.

Our palmitoylated PrRP analogs showed high binding affinity and activation of PrRP receptor. Furthemore, peripheral administration of these analogs to mice and rats induced long-lasting anorexigenic effects and neuronal activation in the brain areas involved in food intake regulation. Several studies with mouse and rat models of obesity and (pre)diabetes (diet-induced obese mice, ZDF rats, Wistar Kyoto rats on high-fat diet) showed that repeated peripheral administration of palmitoylated PrRP31 lowered food intake, body weight and improved related metabolic parameters. Moreover, the treatment with palmitoylated PrRP improved glucose tolerance as shown by glucose tolerance test.

Our data suggest that lipidization of PrRP enhances its stability and possibly enables the peptide to cross blood-brain barrier after peripheral administration. Strong anorexigenic, body-weight-reducing and glucose-lowering effects make lipidized PrRP analogs attractive candidates for anti-obesity and anti-diabetic treatment.

Finally, our findings also supported the potential use of palmitoylated PrRP for the prevention and treatment of the Tau hyperphosphorylation, one of the hallmarks of neurodegeneration (Tau pathology) connected with obesity-related diabetes, and thus suggest neuroprotective properties of PrRP.

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YI-P137 Injectable Peptide Hydrogel-based Drug Delivery System for Pain Treatment

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Pain treatments are part of the major medical challenges since ineffective management thereof can result in a decline of normal daily functioning and life quality. To provide sustained analgesia and avoid frequent administrations, which can result in inconsistent pain relief, extended-release (ER) formulations have been developed[1]. ER systems are designed to provide a slow release of opioids and a long duration of action, improving patient compliance. Thanks to their biocompatibility, biodegradability, cytocompatibility and their physically crosslinked properties, peptide-based hydrogels represent an important class of injectable hydrogels suited as matrices for controlled and slow drug release.

In this work, a new family of amphipathic peptides, forming supramolecular hydrogels suited for extended drug release, was designed[2-3]. All hydrogels were characterized at the macroscopic and microscopic level by rheology and transmission electron microscopy. In order to study their eventual therapeutic potential, the hydrogels have been used for entrapment and sustained release of opioid drugs. Based on the best physicochemical, mechanical, and noncytotoxic properties, selected hydrogels were investigated for in vivo release of opioids. Opioid administration by subcutaneous injection and subsequent testing in the tail-flick assay (acute pain model), showed sustained antinociceptive effects over longer periods of times (up to 96 h). Finally, in order to get some insights into the in vivo biostability of the hydrogel, nuclear imaging experiments were performed.

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YI-P138 Biosynthesis of New Sungsanpin-like Lasso Peptide

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Lasso peptides are a class of ribosomally-synthesized and post-translationally modified natural products with diverse bioactivities and a unique three-dimensional structure. The C-terminus threads through an N-terminal macrolactam ring.[1] The majority of lasso peptides were discovered by isolation from bacteria. This changed in 2008 with the report of the first lasso peptide isolated by a genome mining approach.[2] Sungsanpin[3] was isolated from a Streptomyces strain collected in Korea in 2012. It is the shortest lasso peptide and a new and structurally unique member of lasso peptide family. Through genome mining eight strains were tested for homologous production, trying to obtain biosynthetic Sungsanpin and analogues. One of these strains produced Chaxapeptin,[4] a similar lasso peptide to Sungsanpin isolated in 2015. Thereby, a functioning heterologous production system for Chaxapeptin in *E. coli* was established and cloned.

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P139 Fast and Efficient Automated, On-resin Synthesis of Disulfide-bridged Type-II Diabetes-related Peptide Amylin

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Type-II diabetes, caused by chronic insulin resistance and a progressive decline in pancreatic β-cell function, affects over 150 million people worldwide[1]. The 37-mer Human islet amyloid polypeptide (IAPP) (**Figure 1**) or amylin, is a major contributor to the amyloid deposits found in the pancreases of patients with type-II diabetes[2,3]. Amylin is highly hydrophobic and prone to aggregation, making it a difficult peptide to synthesize in sufficient purity and yield[4]. To our knowledge, the application of heat during coupling reactions of amylin synthesis has not been fully assessed and may provide an advantage for reducing on resin aggregation. Automated on-resin disulfide bridge formation in amylin using Tl(tfa)3, a mild oxidant, can provide better yields and purities of the desired cyclic products, compared to other methods.

H-KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH$_2$

Figure 1. Amylin structure

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YI-P140 Towards the First Total Synthesis of the Bicyclic Octapeptide α-Amanitin

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α-Amanitin (Fig. 1) is a highly toxic naturally occurring peptide, isolated from *Amanita* mushrooms. The affinity of RNAP II for α-amanitin is at least 103 times greater than that of shown for other polymerases. Adoption of this bicyclic peptide scaffold to develop unique and sensitive probes can ultimately lead to understanding and control of mRNA production through RNAP II inhibition.

Our group has been interested in developing the first total synthesis of α-amanitin for its extreme potency towards RNAP II and its possible application towards cancer therapy. Moreover, once the total synthesis of this toxin is available, synthesizing analogues of it might help to further understand its mechanism of action, and perhaps lead the way to more potent toxins.

Among the many challenges of this total synthesis is the synthesis of the residue on the third position, (2*S*,3R,4*R*)-α,δ-dihydroxyisoleucine. There is no reported synthesis of this small yet challenging molecule in the literature as of this date. The most accessible route for us to obtain the pure desired enantiomer of this residue was to synthesize a mixture of 4 stereo-isomers and isolate the desired enantiomer once it has been attached to the peptide.

Additionally, incorporating the tryptathionine bridge in the peptide required the synthesis of a hydroxypyrroloindoline (Hpi) moiety, which upon exposure to Savige-Fontana conditions, yielded the desired Trp-Cys sulfide-bridge.

In summary, a cleaner and a more convenient method for synthesizing an analogue of α-Amanitin, containing the unnatural amino acid dihydroxyisoleucine and the tryptathionine bridge has been devised and proven to possess bio-activity.

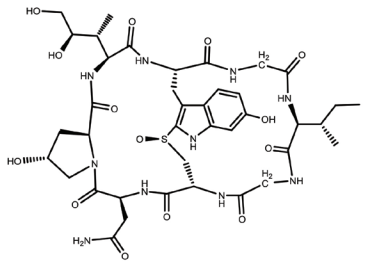

Fig. 1 – The Bicyclic Octapeptide α-Amanitin

P141 An Entirely SPPS-Based Synthesis of GBAP Analogues: Querying the Structure Activity Relationship in Enterococcus Faecalis Quorum Sensing-Dependent Pathogenicity

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The increasing prevalence of bacteria exhibiting multiple drug resistance, even to last resort antibiotics such as vancomycin, necessitates the development of alternative strategies to reduce the development of resistance. Many bacteria utilize quorum sensing (QS) to coordinate their pathogenicity. Their dependence on this communication pathway makes QS a viable alternative target for treatment of bacterial infections. Because attenuating QS-dependent pathogenicity is not directly bactericidal, the selective pressure to develop resistance is lessened while still potentially improving an infection's prognosis.

Enterococcus faecalis is an opportunistic pathogen responsible for the majority of all enterococci infections. It has also been implicated in spreading vancomycin resistance to other pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA). The fsr quorum sensing system in *E. faecalis* is responsible for triggering virulence factor production and the establishment of infections. Gelatinase biosynthesis activating pheromone (GBAP) is an 11-amino acid lactone-based macrocyclic auto-inducing peptide in *E. faecalis* that is responsible for activating the fsr QS circuit.

To aid in the study of the structure activity relationship (SAR) between GBAP and fsr QS, and to facilitate the production of libraries of GBAP analogues, we have developed an entirely solid-phase peptide synthesis (SPPS) of GBAP. This approach can accelerate peptide production, especially due to its compatibility with automation, and also avoids the multiple additional purification steps currently required to synthesize GBAP. We have used our method to produce bioactive GBAP and to generate two analogue libraries investigating the SAR of GBAP with fsr QS.

YI-P142 Protein Kinase C Epsilon Peptide Inhibitor Reduces Infarct Size and Improves Cardiac Function Following Myocardial Ischemia/Reperfusion (I/R)

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The generation of reactive oxygen species (ROS) during myocardial I/R contributes to post-reperfusion cardiac injury. The increase in ROS is attributed to activation of uncoupled endothelial nitric oxide synthase which is stimulated by protein kinase C epsilon (PKC ε) during reperfusion. We hypothesize that using a cell permeable PKC ε peptide inhibitor (PKC ε-) (N-myr-EAVSLKPT, MW=1054 g/mol, 5 μM, 10 μM or 20 μM) will improve post-reperfused cardiac function and attenuate infarct size compared to untreated isolated perfused rat hearts subjected to I(30 min)/R(90 min). Male Sprague-Dawley rats (275-325 g) were anesthetized with sodium pentobarbital (60 mg/kg) and anticoagulated with heparin 1000 units intraperitoneally. PKC ε- was dissolved in Krebs' buffer and infused during the first 10 min of reperfusion. PKC ε- treated hearts (10 μM and 20 μM) exhibited significant improvement in post-reperfused cardiac function at 90 min compared to untreated controls ($p<0.05$). The maximal rate of left ventricular developed pressure (+dP/dtmax) of PKC ε- treated hearts recovered to 56±5% (10 μM; n=8) and 50±3%; (20 μM; n=6) of baseline values. By contrast, the +dP/dtmax of untreated controls (n=8) and low-dose PKC ε- hearts (5 μM; n=6) recovered to 32±4% and 28±3% of baseline values, respectively. Interestingly, all PKC ε- hearts (5-20 μM) showed significant reduction in infarct size to 27-29±2% compared to untreated control hearts, which was 38±3% ($p<0.05$). The results suggest that PKC ε- effectively reduces infarct size, dose-dependently improves cardiac function and is a putative treatment that could aid in clinical myocardial infarction/organ transplantation patient recovery.

This study was supported by the Center for Chronic Disorders of Aging, the Division of Research and the Department of Bio-Medical Sciences at Philadelphia College of Osteopathic Medicine and Young Therapeutics, LLC.

YI-P143 The Peptide Transdermal Therapy against Melanoma with a Novel P53 Peptide Screened from P53-Overlapping Fragment Peptide Library

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Transdermal drug delivery system is pivotal in various fields from cosmetics to medicine, but the keratin layer is a major barrier for drug introduction into the skin. In the malignant melanoma therapy, topical application is a promising approach that can be used with the present medical treatment. In our previous researches, a peptide transdermal approach with a cell membrane- penetrating peptide (CPP, eleven-arginine; 11R) showed successful topical drug delivery with various physiologically active substances. Here, we screened for a new tumor-specific killing peptide with p53 template-overlapping fragment peptide library that can create many therapeutic candidate peptides against several melanoma cell lines. Therefore, a p53 fragment peptide library was constructed from the wild-type full-length p53 amino acid sequence comprising 22 amino acid peptides from the N-terminal p53; each fragment was formed by four amino acid shift from the previous fragment; ninety-four peptide fragments were created and fused with 11R as CPP. No.66-11R peptide inhibited the proliferation of five melanoma cell lines without causing damage to the normal primary cell line. Topical application of the No.66-11R using our peptide transdermal drug delivery method induced melanoma apoptosis in a mouse model of subcutaneous tumor.

YI-P144 Structure-activity Relationship of Novel Stable Lpidized Analogs of Prolactin-releasing Peptide

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Anorexigenic neuropeptides such as prolactin-releasing peptide (PrRP) have the potential to decrease food intake and ameliorate obesity. However, their delivery from the periphery to the brain is complicated by low stability and limited ability to cross the blood-brain barrier. Therefore, we have designed and synthetized lipidized analogs of PrRP. Palmitoylation of PrRP resulted in the stabilization of the molecule and enabled their central anorexigenic effects after peripheral administration.

In this study, PrRP analogs with different linkers for the palmitoylation at different positions were synthesized and evaluated. All these analogs showed a high affinity and receptor activation in cells with PrRP receptor as well as significant affinity to the neuropeptide FF-2 receptor, similar to natural PrRP. Moreover, binding affinity of lipidized PrRP analogs to the NPY receptor Y5 was found only two orders lower than native NPY, which may indicate antagonist effect of lipidized PrRP analogs. However, the activity to both off-target receptors and to rat pituitary RC-4B/C cells naturally expressing all above mentioned receptors was dependent on the position of palmitic acid attached to PrRP. Peripheral administration of several lipidized analogs to fasted mice and free fed rats induced strong and long-lasting anorexigenic effect. Furthermore, we found prolonged half-life of our analogs in rat blood plasma. In conclusion, significant affinity to several receptors involved in food intake regulation, strong anorexigenic effect and prolonged half-life in the blood make these analogs attractive multitarget candidates for anti-obesity treatment.

This study was supported by GACR 15-08679S and RVO:61388963.

YI-P145 Tunable Protein Release from a Peptide Hydrogel

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Hydrogels are useful vehicles for the localized delivery of therapeutic molecules, including proteins, and the rate at which these molecules are released from a gel is an important criterion for a given application. Previous work in the Schneider lab has shown that encapsulated proteins which display a similar net charge as the peptide hydrogel will be rapidly released from the gel due to electrostatic repulsion.[1,2] Alternatively, proteins and peptides that have opposing charges will have complementary electrostatic interactions and lead to the protein being retained within the gel with minimal release. Although these two modes of release can be useful for some applications, others may prefer sustained protein release over the course of a few weeks and such a mechanism has been difficult to design and achieve. The current work has focused on the development of a release platform that incorporates minimal changes to proteins of interest to achieve a range of release profiles from a single peptide gel.

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YI-P146 Control of Silver Nanoplate Formation by Oligomerization and Orientation of Biomineralization Peptides via Peptide and DNA

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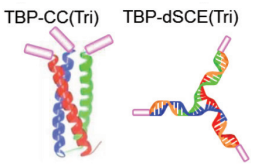

Fig.1. Structures of trimeric TBP with pSCEs and dSCEs

Various biomineralization peptides (BMPep) were reported, however these BMPeps formed disordered structures of inorganic materials. Recently, we reported the method for precise control of amyloid and palladium nanostructure by using self assembled peptides[1-3]. Here, we report the effect for silver nanoplate formation through oligomerization and orientation of BMPep via peptide and DNA as a “structure control element” (SCEs). For peptidic SCEs, we selected the tetramerization domain of tumor suppressor protein p53 and coiled-coil (CC) peptide. We also designed the DNA as a SCE (dSCEs). By conjugating a BMPep for silver, we synthesized oligomeric TBP-SCEs (Fig. 1). Scanning electron microscopy analysis has revealed trimeric and tetrameric TBP-SCEs predominantly formed hexa- gonal silver nanoplate. The results suggested that three interaction points of TBP peptides with silver induce the hexagonal nanoplate formation.

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YI-P147 Broad Spectrum Peptide Inhibition of Small Multidrug Resistant Efflux Pumps in Antibiotic Resistant Bacteria

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Antibiotic resistance bacteria have acquired multiple mechanisms to evade the lethal effects of current therapeutics. One such mechanism includes membrane embedded multidrug efflux pumps that can effectively expel an array of substrates, including common antibiotics and disinfectants, rendering these molecules unable to exert their biological function. Small multidrug resistant (SMR) efflux pumps consist of four transmembrane helices (TMs), with TMs 1-3 making up the substrate binding pocket and TM4 containing a binding motif to form an antiparallel homodimer, the minimal functional unit. This dependence on dimerization can be exploited to cause efflux inhibition via disruption of protein-protein interactions in the membrane. We have previously identified the TM4 sequence motif required for dimerization of the Archaea *Halobacterium salinarum* SMR (Hsmr)[1], and shown that the peptide acetyl-Ala-Sar_3-VVGLALINAGVVV-KKK-NH_2 (Sar = N-methylglycine), containing the Hsmr TM4 dimerization motif (underlined), is capable of reducing Hsmr-driven efflux of toxins[2]. We have recently noted that SMRs are ubiquitous across bacterial species with the TM4 binding motif being highly conserved among numerous 'top threat' bacteria including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis*. Accordingly, peptides containing the TM4 binding motif from various SMRs were synthesized and tested for functional activity. Notably, the TM4 peptide for *P. aeruginosa*'s SMR (acetyl-Ala-Sar3–LLGIGLIIAGVLV–KKK-NH_2) was found to significantly reduce efflux activity while resensitizing bacteria to previously non-lethal concentrations of toxins, including commonly used disinfectants. Further modifications to peptides to increase therapeutic potential will be described, including amino acid replacements to reduce toxicity, and hydrocarbon stapling via 2-(4'pentyl)alanine by Grubb's metathesis reaction to increase stability.

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YI-P148 Design, Synthesis, and Biological Evaluation of CXCR4 Ligands

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A combination of the CXCR4 inverse agonist T140 with N-terminal CXCL12 oligopeptides has produced the first nanomolar synthetic CXCR4 agonists. In these agonists, the inverse agonistic portion provides affinity whereas the N-terminal CXCL12 sequence induces receptor activation. Several CXCR4 crystal structures exist with either CVX15, an inverse agonist closely related to T140 and IT1t, a small molecule; we therefore attempted to produce another CXCL12 oligopeptide combination with IT1t. For this purpose, a primary amino group was introduced by total synthesis into one of the methyl groups of IT1t, serving as an anchoring point for the oligopeptide graft. The introduction of the oligopeptides on this analog however yielded antagonists, one compound displaying high affinity. On the other hand, the amino-substituted analogue itself proved to be an inverse agonist with a binding affinity of 2.6 nM compared to 11.5 nM for IT1t.

Molecular dynamics simulations based on the recent crystal structure reveal details accounting for the differences between IT1t and the amino-substitued analogue and provide preliminary results for the design of CXCR4 agonist using the IT1t scaffold1.

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P149 Tether-functionalized Stapled Peptides for Wild-type and Mutant Estrogen Receptors

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Hydrocarbon stapled peptides are typically designed to replace two *non-interacting* residues with a constraining, olefinic staple, but there is some work that indicates that the tether of a stapled peptide can interact with the surface of a protein. In some cases, stapled peptides have been designed so that they replace interacting, hydrophobic amino acids. In contrast to this approach, we are developing a design strategy to incorporate hydrophobic groups directly on the tether of a stapling amino acid to better mimic the replaced interacting amino acids. Our data suggest that this may be a way to enhance affinity, proteolytic stability, and conformation. In this work, we will present examples of tether-functionalized stapled peptides that inhibit the estrogen receptor/steroid receptor coactivator interaction, an important target in hormone-responsive breast cancer. Our *in vitro* and *ex vivo* work has been guided by molecular dynamics and x-ray crystallography, including for recently described estrogen receptor mutants that are implicated in endocrine-resistant metastatic breast cancer. Selective antagonists for these mutant estrogen receptors are currently unknown, and this work could provide a new tool to study these clinically important mutations to estrogen receptor.

YI-P150 Development of *N*-Sulfanylethylanilide-based Traceable Linker for Purification and Selective Labeling of Target Proteins

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Identification of target proteins of bioactive compounds has been essential for drug discovery and chemical biology. In recent years, traceable linkers (TLs) that enable purification and selective labeling of target proteins have been developed for facilitation of target identification (Figure 1A). We envisioned that N-sulfanylethylanilide (SEAlide) unit developed for N-S acyl-transfer-mediated preparation of peptide thioester should serve as a cleavable unit for TL (Figure 1B). In this study, we will discuss the application of the SEAlide unit for the development of TL.

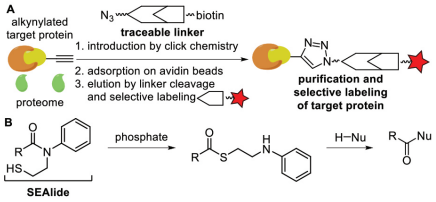

Figure 1. (A) Strategy of traceable linker. (B) Phosphate catalyzed activation of SEAlide unit.

YI-P151 Design of Apelin 13 and 17 Analogs for Cardiovascular Disease Treatment

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Apelin 13 and apelin 17 are natural proteolytic fragments of apelin (APLN), an endogenous 77 amino acids peptide. In the cardiovascular system apelin peptides act as regulatory hormones which regulate a vast range of physiological processes including cardiac contractility, blood pressure, and flow[1,2]. These two peptide isoforms of apelin show powerful binding to the apelin receptor APJ. This particular receptor has been widely studied in the last two decades as a promising target for cardiovascular health and therapy[3]. Due to the increasing pharmaceutical interest in apelin peptides, here we present the chemical synthesis of analogs of apelin 13 and 17 by means of organic synthesis in combination with solid phase peptide synthesis (SPPS). Optimal analogs will retain full or enhanced biological activity and will be metabolically stable toward natural mammalian proteases without inhibiting them. Bioassays coupled with mass spectrometry analysis and High-Performance Liquid Chromatography (HPLC) are used to evaluate the robustness and durability of these analogs, while *in vitro* Lagendorff isolated heart techniques and *in vivo* studies in mouse models will help to examine the efficacy of the new analogs.

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P152 Synthetic Approaches to Phosphinic Peptide Analog Inhibitors of Metalloaminopeptidases

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The synthesis of phosphinic peptide analogs typically involves a multistep preparation of P1 and P1' building blocks, which are combined in a phospha-Michael or amidoalkylation reaction, and subsequently N- and/or C-elongated. Substantial efforts have been also dedicated to the development of alternative approaches that allow more comprehensive variation of the side-chain substituents.[1] We have recently elaborated synthetic pathways leading to either individual target compounds (e.g. starting from Morita-Baylis-Hillman acetates) or the building blocks prepared for parallel derivatization. The final products were designed as inhibitors of selected metalloaminopeptidases, including human aminopeptidase N, APN, and endoplasmic reticulum aminopeptidases, ERAPs.[2,3]

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YI-P153 Rational Design of Structured Cyclic Peptides to Bind Targets of Therapeutic Interest

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Peptides represent an attractive class of molecules for drug development, and one that is potentially able to combine the advantages of small-molecule drugs (easy large-scale production, facile administration, and potential barrier permeability) with those of antibody therapeutics (mutability for desired properties, large binding surfaces for high target specificity, and standardized production methods). The conformational flexibility of small peptides represents a disadvantage, however, since a peptide must order itself, paying a considerable entropic penalty, to bind its target. This limits affinity. We have developed computational methods, using modifications to the Rosetta software suite, to enable us to rationally design peptides with rigid conformations. These peptides are stabilized by intrinsic conformational preferences of D- and L-amino acid building-blocks, N-to-C cyclization, internal covalent cross-links, and non-covalent amino acid side-chain interactions. When designed to bind to a target of

therapeutic interest, such peptides can be pre-organized in a binding-competent conformation, permitting much higher-affinity binding to the target. Here, we present our design methodology, and its successful application to the creation of rigid peptide scaffolds in the 6 to 60 amino acid size range. We also present the application of this technology to the design of inhibitors of the New Delhi β-lactamase 1 (NDM1), an enzyme that is responsible for antibiotic resistance in certain pathogenic bacterial strains. Finally, we summarize ongoing work to generalize the approach to permit design with a broader range of natural and artificial building-blocks permitting new functionality, including Nmethylated amino acids intended to facilitate membrane penetration.

YI-P154 Interaction between L- and D-peptides in the Coiled-coil Regions of Jun and Fos Proteins

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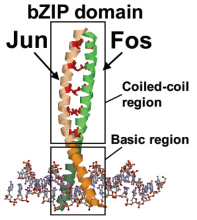

Oligomerization is essential for its function and regulation for many proteins[1]. In evolutionary process, proteins have gained precise mechanism of its function via modulating of oligomer formation. AP-1 transcriptional factors, Jun and Fos, form heterodimer via coiled-coil (CC) region and bind specific DNA sequence via basic region. Jun and Fos regulate cell differentiation, cell growth and apoptosis. Recently, peptides have received a lot of attention again as important target to control protein-protein interactions. However, the interaction state between natural proteins and D-peptides has remained obscure. Here, we report the interaction between L- and D-peptides derived from Jun and Fos proteins[2]. We synthesized L- and D-peptides of Jun and Fos, and analyzed the interaction of L/D-Jun and Fos peptides in combination. The results showed that the peptides of Jun and Fos Furthermore, the wild-type bZIP peptide of Fos and the chimeric bZIP Jun peptide consisting of L-basic region and D-CC region bound to the specific DNA sequence, indicating that they formed heterodimer. These results showed that the enantiomeric CC region peptides of Jun and Fos proteins can form the specific heterodimer. Our study clearly demonstrated the interaction between L/D enantiomeric polypeptides.

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P155 Comparison between 4-tert-butyl-benzhydrylamine-resin (BUBHAR) and MBHAR for the Use in the Boc-chemistry Peptide Synthesis

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The recently proposed BUBHAR[1] was compared with MBHAR in terms of efficiency for overcoming the difficult coupling reaction observed between Gln and Asn residues in the sequence of the antidiuretic Desmopressin peptide [3Mpa*-YFQNCP($_D$-R)G-amide]. Evaluations of this acylation step and the overall synthesis yields of the entire sequence in these two resins were made by considering parameters such as the dynamics of peptide chains within peptide-resin beads exactly at this coupling position. The average volume (in percentage) of peptide-resin bead occupied by DCM or DCM/DMF as measured by a microscope revealed very low bead solvation in MBHAR (near 35% and 45%, respectively) than in BUBHAR (about 75% and 85%, respectively). Accordingly, there was no need for Gln-Asn recoupling reaction in this latter polymer as occurred with MBHAR. Interestingly, this strong electron donor and hydrophobic (tert-butyl) group-attaching resin also showed increased peptide-resin solvation degree in polar aprotic solvents such as DMF, NMP or DMSO. In agreement with these and other findings, a higher synthesis yield of this peptide model was obtained with BUBHAR, thus reinforcing earlier results[1] which have suggested that this novel polymer would replace advantageously the worldwide used MBHAR.

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*3Mpa = 3-methoxybenzylpropionic acid.

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P156 Radiopeptides as Biomarkers for the Alzheimer's Disease

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The worldwide prevalence of Alzheimer's disease (AD) is estimated at 35 million[1]. However, the diagnosis of early AD in the clinical practical remains difficult. We have demonstrated that peptide fragments based upon the Aβ-amyloid peptide present in AD were labeled with the $^{99m}Tc(CO)_3$ radioisotope[2]. The aim of this study was to evaluate the capacity of these fragments as potential biomarkers for AD. The results showed that all the radiopeptides (10^{-4}M) showed radiochemical yield between 87-94%. The stability studies showed that the peptides were stable up to 2 hours in human serum at 37ºC. Using brain homogenate of transgenic mice was obtained binding specificity to amyloid deposits around 50% for all peptides. Furthermore, all the $^{99m}Tc(CO)^3$-peptides showed a binding percentage to plasma proteins of around 40% which is highly compatible with clinical applications. The studies of *in vivo* biodistribution showed an uptake of the compounds in the transgenic mice brain (% ID/g) at 5 min around 4% instead of 0.6% when healthy mice were used. Our results suggested that the use of these radiolabeled peptides offers tremendous hope for the diagnosis assays of the Alzheimer's disease.

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P157 Higher-order Assembly of Coiled-coil Trimers into Banded Microstructures

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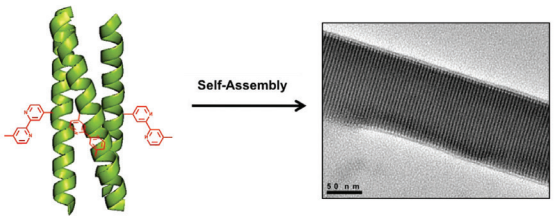
The successful application of synthetic biomaterials lies in their ability to mimic naturally occurring biological molecules and systems. The challenge however, has always been to generate materials with hierarchical assemblies down to the atomic level that have precisely tailored chemical heterogeneities and external stimuli-responsiveness. Self-assembling peptides have recently emerged as a potential avenue for the creation of novel biomaterials because they are materials based on natural building blocks. The knowledge about their sequence-structure relationship coupled with the ability to design and synthesize *de novo* peptides has sparked an interest in the use of coiled-coil peptides as biomaterials in various fields; tissue engineering, drug delivery, regenerative medicine and bio-sensing to name a few. Our approach utilizes a GCN4 leucine zipper sequence-based coiled-coil trimer that has been radially functionalized with aromatic ligands to build hierarchical assemblies. These higher-order assemblies, which take the form of banded rectangular nanosheets, are formed via aromatic-aromatic interactions. Their dimensions and overall shape vary with the strategic placement and number of aromatic ligands on the monomer backbone. Their assembly was observed to be reversible and can be controlled by adjusting the pH of the solvent medium. Aside from their prolonged stability, as well as their ease of formation in an aqueous medium, the ability to form these structures under physiological conditions render them favorable for biological applications.



P158 Engineering Glucose Responsiveness into Insulin – Profile of MK-2640

Ravi P. Nargund,* Niels C. Kaarsholm, Songnian Lin, Lin Yan, Pei Huo, Theresa Kelly, Margaret van Heek, James Mu, Margaret Wu, Ge Dai, Yan Cui, Yonghua Zhu, Ester Carballo-Jane, Vijay Reddy, Peter Zafian, Shuai Shi, Valentyn Antochshuk, Sandra C. Souza, Wolfgang Seghezzi, Mark Erion, and David E. Kelley
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Insulin therapy remains a cornerstone for the treatment of diabetes. Despite its widespread use in the clinic, attaining aggressive glycemic targets has been hampered by insulin's well recognized narrow therapeutic index and as such the margin is very small between an optimally efficacious dose and one that causes hypoglycemia. Despite decades of research, little progress has been made in creating "smart" insulins that can alter insulin action, upward or downward in response to rises or falls of blood glucose, mitigating risk for hypoglycemia and bolstering control of hyperglycemia. In this presentation, we disclose a novel approach to engineering "smartness" by designing insulin analogs that have a dual affinity for the insulin receptor (IR) and for the mannose receptor C-type 1 (MR). MR functions to clear endogenous mannosylated proteins and glucose, a low affinity ligand for MR can compete with such binding. This principle was exploited to endow novel insulin analogs with a glucose responsive characteristic by appending saccharide units with appropriate affinities for MR. In experimental conditions, as plasma glucose was lowered stepwise from 280 mg/dL to 80 mg/dL, progressively more analog was cleared via MR, reducing its availability for binding to IR, thereby conferring glucose responsiveness. Of the many analogs that were prepared and characterized, MK-2640 showed a favorable preclinical profile. It was selected for clinical testing. In this presentation, the *in vitro* and *in vivo* preclinical properties of MK-2640 will be presented and the potential of glucose responsive insulin for improving efficacy and safety of insulin treatment of diabetes will be discussed.

P159 H-Bonding Ability of (Z)-Chloroalkene as an Amide Bond Isostere: Catalytic Reaction Study and Theoretical Study

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Amide bond isosteres have emerged as important bioisosteres of amide bond and chemical tools in the fields of chemical biology, medicinal chemistry and organocatalyst chemistry.[1] In this study, we will present the synthesis of the peptidomimetic of Miller's peptide catalyst[2] containing the Pro-ψ[(Z)CCl=CH]-D-Val-type (Z)-chloroalkene-type amide bond isostere utilizing our developed synthetic methodology via 1,4-asymmtric induction,[3] and the investigation of the H-bonding ability of the (Z)-chloroalkene moiety as an H-bonding acceptor in the asymmetric epoxidation with a carbamate substrate as an H-bonding donor. Our studies revealed that the chlorine atom of the (Z)-chloroalkene moiety can work as an H-bonding acceptor that can interact with the carbamate proton via H-bonding interaction. Furthermore, ab initio molecular orbital calculations of the geometries and interaction energies of water complexes with a chloroalkene compound will be discussed.

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YI-P160 Development of Methodology for Preparation of Peptide Thioesters via On-rein N–S Acyl Transfer Using N-Sulfanylethylanilide Peptide

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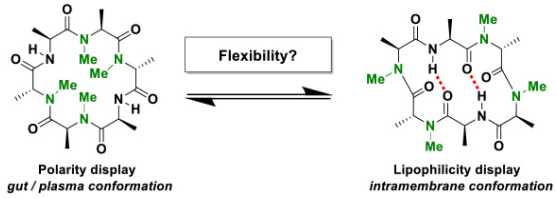
Native Chemical Ligation (NCL) is a powerful method for chemical synthesis of proteins. In this protocol, a peptide thioester as a key substrate chemoselectively reacts with an N-terminal cysteinyl peptide. Because Fmoc-based solid-phase peptide synthesis (SPPS) requiring piperidine treatment for

Fmoc removal is not applicable to straightforward preparation of highly electrophilic peptide thioesters, a wide variety of chemical devices allowing for the preparation of thioester under Fmoc conditions have been investigated. In this context, we have demonstrated that the N-sulfanylethylanilide (SEAlide) peptide easily obtainable using Fmoc SPPS can be converted to the corresponding thioester via N–S acyl transfer. Here, on-resin conversion of the SEAlide peptide to the thioester under acidic conditions was also investigated; however, epimerization of the C-terminal amino acid was encountered. In this study, epimerization-free N–S acyl transfer of the SEAlide peptide will be presented.

YI-P161 Optimal Scaffold Flexibility Permits Backbone Polarity Exclusion during Passive Membrane Permeation in bRo5 Peptide Macrocycles

Matthew Naylor, Mason Handford, Andrew Ly, R. Scott Lokey
University of California, Santa Cruz



Interest in the pharmaceutical development of beyond-Rule-of-5 (bRo5) peptide macrocycles has exploded as the therapeutic potential for this compound class is revealed. Although such molecules are abundant in the natural product literature, and examples like cyclosporine A have been administered for decades, medicinal chemists have been reticent to pursue these structures for perceived ADMET liabilities. Recent synthetic efforts in our laboratory and others have cast doubt on this universal liability, suggesting that both water solubility and cell membrane passive permeability can be attained with a careful balance of polarity, lipophilicity, and appropriate structure.

This balance is an essential design consideration when exploring frontier challenges in this chemical space, such as the characterization of chameleonicity — the ability of a cyclic peptide to attain differential conformations in aqueous or lipophilic conditions toward passive membrane permeation. To date, few design principles have emerged to strategically synthesize this behavior, which is the confluence of many interconnected peptide features (ring size, stereochemistry, sidechain sterics, amide methylation, esterification and others). Findings will be presented regarding the conformational flexibility of cyclic peptide backbones and its influence on the ability to attain both aqueous solubility and cell permeability. Series of flexibility-adjusted compounds were synthesized (representing both chameleonic and non-chameleonic cyclic peptides) and studied for consequences on ADME properties (aqueous solubility, membrane permeability, and metabolic stability).

YI-P162 Novel Peptides Derived from Dengue Virus Capsid Protein Translocate Reversibly the Blood-brain Barrier through a Receptor-free Mechanism

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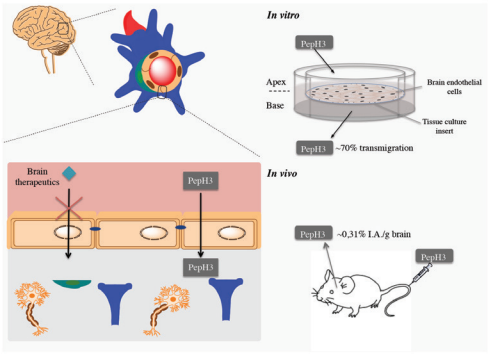
The delivery of therapeutic molecules to the central nervous system is hampered by poor delivery across the blood-brain barrier (BBB). Several strategies have been proposed to enhance transport into the brain, including invasive techniques and receptor-mediated transport (RMT). Both approaches have several drawbacks, such as BBB disruption, receptor saturation and off-target effects, raising safety issues. In the quest to find new drug delivery systems we have studied specific domains of Dengue virus type 2 capsid protein (DEN2C) that can be used as trans-BBB peptide vectors. These peptides have characteristics commonly found in cell penetrating peptides (CPP), such as being cationic and lipophilic. Their mechanism of translocation is receptor-independent and consistent with adsorptive-mediated transport (AMT). One peptide in particular, named PepH3, reaches equilibrium distribution concentrations across the BBB in less than 24 hours in a cellular *in vitro* assay. In addition biophysical studies with pepH3 showed that this peptide interacts with anionic membranes, similarly to what is found in brain endothelial cells. Importantly, *in vivo* biodistribution data with radiolabeled peptide derivatives shows high brain penetration. In addition, there is fast clearance from brain and high levels of excretion, showing that PepH3 is a very good candidate to be used has a peptide shuttle taking cargo in and out the brain.



P163 A Useful Protection for Synthesis of Peptides with 3-nitrotyrosine.

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Neurodegenerative diseases are influenced by oxidative stress including oxidation of proteins with generation of 3-nitrotyrosine (HNit-OH).[1]

In order to understand biophysical function of nitration, we carried out selective synthesis of nitrated peptides and proteins. We have found that Fmoc-Nit(Bzl)-OH is a good building block for SPPS of Nit containing peptides by Fmoc/tBu strategy.2 Bzl is removed rapidly with trifluoracetic acid in dark. The cleavage of Bzl from Fmoc-Nit(Bzl)-OH proceeds via pseudo-first order mechanisms with activation barrier 32 kcal.mol^{-1} and rate k= 15.3 s^{-1} at 20 oC. This rate is more than 2,000,000 times faster than that for cleavage of benzyl from Tyr(Bzl). We have explained the facile cleavage of benzyl group from nitrotyrosine containing peptides by neighbouring group assistance using density functional theory calculations.

We have compared the synthesis of alpha-Synuclein (118-140) peptides using Fmoc-Nit-OH and Fmoc-Nit(Bzl)-OH. Fmoc-Nit(Bzl)-OH is the best choice for SPPS especially in case of segments with higher content of Nit. E.g. completely nitrated alfa-Synuclein (118-140) was obtained only with Fmoc-Nit(Bzl)-OH.

This work was supported by Czech Science Foundation (reg. Nos. 14-00431S and 17-00121S).

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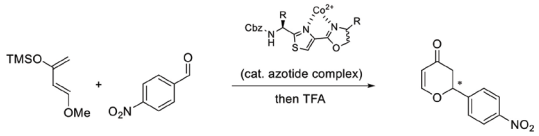
YI-P164 Peptide-Based Ligands for Asymmetric Catalysis

D. S. Nielsen, C. B. Jacobsen, M. Meldal, F. Diness
Center for Evolutionary Chemical Biology, Department of Chemistry, University of Copenhagen, Denmark



Azotides are peptide derivatives with thiazol(in)es and/or oxazol(in)es incorporated into the backbone. These heterocyclic constraints are found wide spread through Nature where they induce structure in molecules with important biological activities.[1] Some azotides display potent and selective affinity in complex with mammalian proteins[2] while others can act as ionophores that reversibly complexes and binds specific metal ions[3]. The inherent chirality of azotide-peptides coupled with their ability to bind metal ions makes an intriguing argument to explore azotides as ligands for enantioselective metal-catalysis. Here we present the evaluation of thiazole-oxazolines as ligands for a metal catalyzed hetero Diels-Alder reaction. Various chiral amino acid thiazoles were functionalized with D- or L-serine derivatives to yield novel enantiopure bicyclic azotide ligands. Conversion rates were high (>95%) with optimized ligands yielding the product in excellent enantiomeric excess. These results highlight the versatility of azotides in chemical and medicinal research and provide a novel entry to environmentally friendly ligands for asymmetric catalysis.

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YI-P165 Development of a Highly Selective and Cell Permeable Inhibitor for Protein Phosphatase-1

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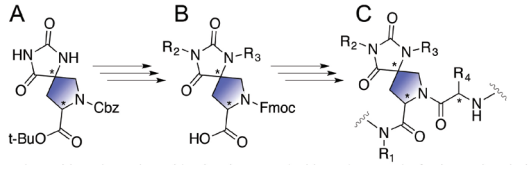
Protein Phosphatase-1 (PP1) is a ubiquitously expressed phosphatase responsible for the majority of dephosphorylation events in the processes such as cell cycle, glycogen metabolism etc. Although the roles of various phosphatases in these processes have been delineated using conventional biochemical and genetic methods, specific roles of PP1 and other phosphatases have remained elusive. Development of a chemical tool would aid in understanding the specific roles of PP1 in these processes with a greater confidence level. Attempts towards this end have been unsuccessful due to the structural homology of the catalytic subunits of PP1 and other phosphatases in the same protein family such as PP2A and PP2B. In this study we found that an inhibitory stretch derived from Inhibitor-2, an endogenous proteinaceous inhibitor of PP1, can be developed as a chemical tool for PP1. We adopted a library based design strategy to incorporate various unnatural and natural amino acid substitutions in the inhibitory stretch to enhance its potency, cell permeability and metabolic stability. We obtained an inhibitor with a low nanomolar IC50 value based on *in-vitro* phosphatase assay. The inhibitor was found to be highly selective to PP1 both *in-vitro* and in cell lysate. The peptide also has the ability to penetrate mammalian cell membrane as visualized using confocal microscopy and FACS. Through this study we have achieved a single peptide carrying the characteristics of multiple categories of PP1 inhibitors. These characteristics would make this a suitable chemical tool for studying the effects of PP1 in a temporal manner in various cellular pathways.

YI-P166 Synthesis of Enhanced Proline Based Peptides and Peptidomimetics

Justin D. Northrup* and Christian E. Schafmeister
Temple University



New amino acids and novel peptidomimetics are valuable tools not only for increasing design space, but also for exploring new interactions. Our lab has recently developed a

unique peptidomimetic, which was derived from our common spiroligomer hydantoin feedstock (*A*). The imide and amide of the hydantoin can be uniquely functionalized utilizing a simple, single-pot alkylation in DMF at room temperature (*B*) followed by a protecting group swap. In doing so, we have successfully decoupled the generation of a stereocenter from the introduction of functional groups, a difficult task in the synthesis of new amino acids. Using this alkylation reaction, we have incorporated over 25 functional groups onto the hydantoin of these enhanced proline derivatives, highlighting the wide range of chemical diversity of this approach. Furthermore, these enhanced prolines are easily assembled into larger peptides and peptidomimetics via standard SPPS (*C*). The ease of their synthesis and incorporation into SPPS will facilitate the rapid discovery of new scaffolds and potential metal-ligands for catalytic applications.

YI-P167 Natural Product-like Peptides against a Membrane Transporter

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Peptides combine properties of small molecule drugs and biologics, and are thus interesting drug leads. Typically, discovery of novel peptides is achieved through natural product mining or screening of synthetically generated naïve libraries. The Suga lab has pioneered a unique *in vitro* platform for peptide discovery that allows ready generation of peptides containing artificial amino acids. Up to 10^{13} peptides can be analyzed routinely per selection. In a first step, specific tRNAs are preloaded with unnatural amino acids through an artificial ribozyme, known as a Flexizyme, that catalyzes amino acylation. These amino-acyl tRNAs are then supplemented to a customized in vitro translation reaction. A puromycin linker covalently attaches each peptide to their coding mRNA, enabling peptide identification. Through affinity panning with an immobilized target protein, binders can be isolated and analyzed through sequencing. Major hallmarks of the FIT-RaPID technology (flexible *in vitro* translation — random non-standard peptide integrated discovery) include cyclization through thioether formation and incorporation of unnatural building blocks, such as D-amino acids. Here we demonstrate the generation of cyclic peptidic binders against an ABC transporter. Membrane targets are particularly interesting for peptide discovery since no translocation through the membrane is required. In depth analysis of the generated binders shows binding constants (K_D) in the lower nanomolar range. These results indicate that ABC transporters are interesting targets for peptidic inhibitors, which might prove useful for development of new drugs.

YI-P168 Lysine to Arginine Mutagenesis of Chlorotoxin Enhances its Cellular Uptake and Potential for Use as a Drug Delivery Vehicle

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Cell-penetrating peptides are short membrane-permeating amino acid sequences that can potentially serve as vehicle to deliver drugs into cells. However, they lack specificity. Chlorotoxin (CTX) a disulfide-rich peptide isolated from the venom of the scorpion *Leiurus quinquestriatus* has several biomedical properties, including the ability to bind preferentially to cancer cells. Here, we focus on its ability to internalize into cells and whether it can be improved through conservative substitutions. Mutants of CTX were made using solid-phase peptide synthesis and internalization into human cervical carcinoma (HeLa) cells was monitored by fluorescence and confocal microscopy. By increasing the number of arginine and tryptophan residues, we showed that arginine residues facilitate the cellular uptake of CTX. Particularly, a double mutant CTX[K15R/K23R] showed a two-fold improvement in uptake compared to CTX. We have also demonstrated that the uptake was influenced by the fluorescent dye attached to CTX with Cy5.5™ having a larger effect compared with Alexa Fluor® 488. In this study, we have showed that conservative amino acid substitutions can enhance the potential of CTX as a vehicle for delivery of molecules into cells.

This work was supported by the National Health and Medical Research Council, Australia (NHMRC; APP1010552) and the Comisión Nacional de Investigación Científica y Tecnológica, Chile (FONDECYT 3160140).

P169 Development of Chlorotoxin Peptide-Based Matrix Metalloproteinase-2 Inhibitors

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Matrix metalloprotease-2 (MMP-2) is a member of gelatinase class of MMP family and it is an important drug target that is used in the treatment of cancer and autoimmune diseases since it has high activity in the cancer of skin, prostate, bladder, breast, lung, and ovary.[1]

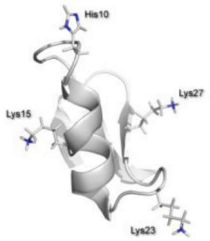

Figure 1. Three dimensional structure of chlorotoxin and its four critical positions.

MMP inhibitor discoveries are generally based on the zinc metal coordination chemistry of small organic molecules or synthetic peptide molecules. Organic molecules are not selective and inhibit various structurally similar members of MMP family since they can enter the active site and coordinate to zinc easily.[2] Synthetic peptides, on the other hand, are more target-specific but lack the inhibition potency of organic molecules.

In this work, the development of a novel, peptide-based, selective and potent inhibitor will be discussed. Inhibitor design is based on the peptide CLTX which is a selective MMP-2 inhibitor with 36 amino acids (4 kDa) originally isolated from the scorpion venom.[3] According to our preliminary work, the strategy for inhibitor design in this work is based on the coordination of four critical positions (H10, K15, K23 ve K27) on CLTX to zinc ion in MMP-2 active site via 20 thiol and azido alanine modifications with different carbon chain lengths. The inhibition effect (K_i) of these CLTX modifications will be compared and discussed as well as thermodynamic characterization of CLTX-MMP2 interaction by SPR will be presented.

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YI-P170 Chemical Synthesis of the N-terminal Palmitoylated Sonic Hedgehog Protein

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Chemical protein synthesis has become a potent tool for the synthesis of proteins, especially those with posttranslational modifications which are not easily obtained by DNA recombinant methods. The main reaction in chemical protein synthesis is native chemical ligation (NCL)[1,2].

A key step in NCL is the synthesis of C-terminal thioester peptides. In our group, thioester peptides are obtained following the N-acylurea approach, which afford N-acyl-benzimidazolinone (Nbz) peptides as thioesters precursors using Fmoc-SPPS[3,4]. We have further developed a new derivative of the *o*-aminoanilide linker which is acylated with *p*-cyanophenylchloroformiate. The use of this new linker allows the presence of a cysteine and a thioester precursor in a same peptide fragment, which makes possible to use this linker in kinetic controlled ligations
.
The methodology described above was applied to synthesize an analog of Sonic Hedgehog protein (SHh). This analog owns a palmitic residue at the N-terminal position, and a biotin residue substituting the natural cholesterol moiety at the C-terminus[5]. SHh displays important biological functions during embryo development, while in adults its aberrant signalling has been related to different cancers. From a biological point of view SHh is a potential therapeutic target.

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P171 Cellular Uptake and Cytotoxicity of Engineered SMACN7-Octaarginine Peptide Conjugates

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The second mitochondria-derived activator of caspase (Smac/DIABLO) is a recently identified, novel pro-apoptotic protein, released from mitochondria into the cytosol in response to apoptotic stimuli. Smac promotes apoptosis in cell by eliminating the caspase-inhibitory properties of the inhibitors of apoptosis proteins (IAP), particularly XIAP. Smac mimetics have several intrinsic limitations (e.g., poor cell permeability and poor in vivo stability and bioavailability) as potentially useful therapeutic agents. Therefore, our laboratory has focused on developing a number of Smac conjugates with the aim of improved binding affinities, cell-permeability, and in vivo stability and biocompatibility. A heptamer sequence AVPIAQK (SmacN7) that contain binding sequence was targeted. SmacN7 peptide was fused with a well- established cell penetrating peptide octaarginine (Arg8) to achieve cell penetration. In order to increase therapeutic efficacy of the bifunctional sequence, the peptide conformation was locked via cyclization, which is known to cause reduction of polarity, increase in proteolytic stability.[1] The biological properties of designed peptides in terms of cell-permeability cytotoxicity and apoptotic efficiency have been studied. Significant contribution of cyclization on cytotoxicity and apoptosis have been demonstrated.

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YI-P172 Resolution of *rac*-α-allylalanine via Continuous Preferential Crystallization (CPC)

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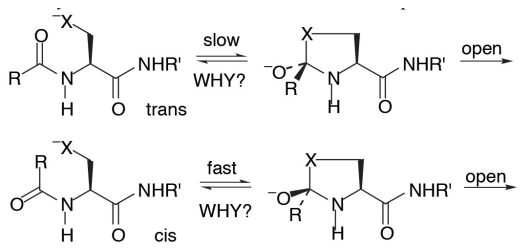
Non-proteinogenic α-methyl amino acids have gained significant interest due to their ability to enhance metabolic and chemical stability, as well as modulate hydrophobicity in peptide therapeutics. Most often, α-functionalized amino acids are produced by asymmetric synthetic and enzymatic resolution. While these approaches can produce the targets in high optical purity, production at large scale is challenging. We have developed a new method to resolve *rac*-α-allylalanine via continuous preferential crystallization (CPC), allowing separation of the (S)- and (R)- enantiomers in high enantiomeric excess (ee > 98%) and good mass throughput. Having established the α-carbon stereocentre *via* crystal separation, functionalization of the pendant alkene affords a wide array of chiral α-methyl amino acids on large scale.



P173 Approach Control. Stereoelectronic Origin of Geometric Constraints on N-to-S and N-to-O Acyl Shifts in Peptides

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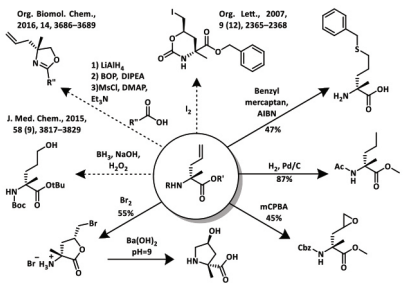
Intramolecular N-to-S or N-to-O acyl shifts in peptides (X = O, S) are of fundamental and practical importance, as they constitute the first step in protein splicing and can be used for

the synthesis of thioester-modified peptides required for native chemical ligation. It is observed that a cis amide reacts more rapidly than a trans, a difference that is often attributed to groundstate destabilization of the cis. Here we argue that the reactivity difference is instead a stereoelectronic effect arising from the ease of approach of the nucleophile to a carbonyl group. DFT calculations on model amides support this explanation in terms of the angles of approach. Implications for homoserine, and homocysteine and for aldol condensations will be presented.



P174 Remarks on the L1C3 Chimera: Grafting a Caspase Micro Domain onto Scorpion Peptide Boosts its Cytotoxic Activity and Shifts its Antimicrobial Spectrum

K.S. Gomes[1], P.V.M. Reis[1], A.A.F. Carvalho[1], V.M. Lima[1], E.M.L. Batista[1], J.M. Resende[2], D.A. Gomes[1], V.L. Santos[3], De Lima, M.E.[1], E.M. Souza-Fagundes[4,*], A.M.C. Pimenta[1,*]
[1]Departamento de Bioquímica e Imunologia, [2]Departamento de Química, [3]Departamento de Microbiologia, and [4]Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; [*]Co-seniorship

Lunatin-1 is a 13mer peptide from scorpion *Hadruroides lunatus* venom with pronounced antimicrobial activity, especially against clinical isolates of *Staphylococcus aureus* 8 and 20 (including biofilm forms), and *Candida* spp. Lunatin-1 has also shown antitumor activity against leukemic cells HL60, Jurkat, and THP1 (IC50 values of 43.42±0.10, 78.43±0.33, and 80.14±0.30 µM, respectively), and breast tumor cells MDA-MB-231 and MCF-7 (IC50 values of 49.78±0.18 and 44.57±0.19 µM, respectively). In HL60 cells, Lunatin-1 induced DNA fragmentation, phosphatidylserine exposure, and nuclear morphological changes such as condensation, fragmentation, and nucleus size reduction. Caspases 3, 8, and 9 were activated with 3 hours of treatment, suggesting cell death caused by pro-apoptotic mechanisms. Lunatin-1 was also able to significantly increase ROS and LDH release, as well as the percentage of cells with mitochondrial membrane potential loss. Confocal time-lapse imaging was used to confirm FITC-labeled Lunatin-1 ability to enter HL60 cells. Taking advantage on a sequence similarity between Lunatin-1 and caspases 3 and 9, we constructed a chimera peptide, named L1C3, by grafting caspase 3 amino acid residues onto Lunatin-1 original sequence. L1C3 was active against HL60, MDA-MB-231, MCF-7, and HCT-116 (IC50 values of 24.53±0.14, 24.25±0.15, 23.10±0.06, and 23.38±0.10 µM, respectively). In comparison to Lunatin-1, L1C3 partially lost the ability to inhibit the visible growth of *S. aureus* 8, whilst became able to inhibit *Escherichia coli* 67, and *Acinetobacter baumannii* 9 and 34. The molecular targets and mechanisms involving both antitumor and antimicrobial activities of Lunatin-1 and L1C3 are currently under investigation.

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YI-P175 Sequence Rich in Arg and Trp Derived from Lactoferricin B: A Challenging for Protecting Groups Removal

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In solid phase peptide synthesis (SPPS), for lateral chain protecting group removal several experimental protocols have been reported, this is due to the peptide sequences diversity[1]. It has been described that Arginine rich peptides require longer times to carry out successfully the cleavage process even when Pbf group is used as protecting group at the guanidine group[2,3]. Bovine Lactoferricin (LfcinB) is an antimicrobial peptide, rich in arginine and tryptophan residues. It had been demonstrated that short derived LfcinB peptides can present similar or even better antibacterial activity. Additional if the sequence is presented as a dimer or tetramer the result can be enhanced[4]. For this research, a palindromic lineal peptide (RWQWRWQWR) derived from LfcinB and its dimer were synthesized by Fmoc/tBu-SPPS over Rink amide resin. These sequences present difficulties in the Pbf group removal, and the optimal experimental conditions for the protecting groups removal were evaluated, i.e. (i) ratio of cleavage cocktail/ resin-peptide and (ii) reaction times. For all tested protocols, the products were monitored by RP-HPLC and it was possible to establish the optimum time, cleavage cocktail composition and ratio to peptide-resin. For both peptides, it was possible to obtain principal specie corresponding to expected product making easier the purification process.

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YI-P176 Synthesis of C-2 Substituted Tryptophans from Fluoropyrroloindolines for Crosslinking Peptides under Mildly Acidic Conditions

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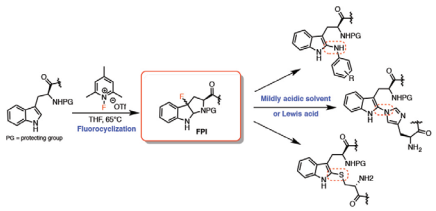
Crosslinks to C-2 of tryptophan are found in various cyclic bioactive natural product peptides and are challenging to install under mild conditions during late stage synthesis. The fluoropyrroloindoline (FPI) core was utilized as the main precursor towards C-2 substituted tryptophans. The scope of C-2 substitutions was investigated and mildly acidic conditions were developed making this approach applicable



to the late stage synthesis of cyclic peptides containing the aforementioned crosslinks.

P177 Fabrication of Cell-penetrating Peptide-conjugated Gold and Gold-silver Alloy Nanoparticles and Their Applications in Detection and Multicolor Imaging of Cancer Cells

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Because of their many advantages, such as small size, minimal immunogenicity, good biocompatibility, deep tissue penetration, lack of toxicity, and low cost, peptides are attracting increasing attention as targeting ligands for nanoparticles in the development of new diagnostic and therapeutic materials for nanomedicine.1,2 Among many different kinds of peptides, cell-penetrating peptides (CPPs) are of particular interest due to their ability of crossing cell membranes without causing significant lethal membrane damage. Here, we report the conjugation of the first CPP, trans-activator of transcription (TAT), derived from human immunodeficiency virus-1 (HIV-1), onto surface of gold and gold-silver alloy nanoparticles physically prepared via the method of pulsed laser ablation of solid target in water. The TAT-conjugated gold and gold-silver alloy nanopaticles have been fabricated by using an approach of sequential conjugation, which offers the capability of precisely tuning the number of functional ligands bound onto the surface of nanomaterials, thereby optimizing both targeting ability and biocompatibility of the obtained nanoconjugates. After the fabrication of these nanoconjugates, they are incubated with human cervical cancer cells. Their applications in targeting cancer cells with a rapid binding kinetics via CPPs and high-performance multicolor dark-field light scattering imaging of live cells have been demonstrated.

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YI-P178 Peptide Metabolism: High Resolution Mass Spectrometry Tool to Investigate Peptide Structure and Amide Bond Metabolic Susceptibility

Tatiana Radchenko[a,b] , Alison Bateman[c], Christopher Kochansky[c], Fabien Fontaine[b], Luca Morettoni,[d] Ismael Zamora[a,b]
[a]Pompeu Fabra University, Dr, Aiguader 80, Barcelo na Spain; [b]Lead Molecular Design, S.L, Vallés 96-102 (L27), Sant Cugat del Val lés, Spain; [c]Merck & Co, 770 Sumneytown Pike, West Point, PA, 19486-0 004, USA; dMolecular Discovery Ltd, London, UK

Several in-silico approaches have been developed such as PeptideCutter to predict peptide cleavage sites for different proteases. Moreover, several databases exist where this information is collected and stored such as MEROPS. Despite these new methodologies there are still some limitations in their usage: inability to handle unnatural amino acids and cyclic peptides. The aim of this work is to develop a new methodology to analyze the mass spectrometry driven experimental data to find those metabolites, then determine their structures, database all the results in a chemistry aware manner and finally to compute the peptide bond susceptibility by using a frequency analysis of the metabolic liability.

This approach uses ultra performance liquid chromatography with high resolution mass spectrometry to obtain the analytical data from incubations of peptides with different enzyme matrices. Metabolite identification was performed on 13 commercial peptide compounds and 4 positive substrates for the four selected proteases (serine and aspartic). The peptides were incubated for three hours with five time points being taken during the experiment. The compounds were diverse with respect to linear and cyclic structure, containing natural and unnatural amino acids and also ranged in molecular weight. The analysis of this data set resulted in 45 metabolites that were annotated in the database. The frequency analysis revealed 26 site of cleavage and the Trp-Ser being the most frequently cleaved bond for all cases. Selectivity was identified for pancreatic elastase and trypsin/chymotrypsin because the Ser-Tyr and Leu-Ser were revealed as a most frequently cleaved bond, respectively. These results were in agreement with previous studies.

P179 Improved Purity, Yields, and Total Synthesis Time of Alzheimer's Disease Related Peptide: Human β-amyloid (1-42)

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Gyros Protein Technologies, Tucson, AZ 85714

Humanβ-amyloid (1-42) (Figure 1) is a major component of the plaque deposits found in Alzheimer's disease (AD) patients' brains[1]. β-amyloid is in constant demand for the continued research into the pathology, diagnostic tools, and potential therapeutics for AD. Conventional solid phase peptide synthesis (SPPS) of β-amyloid (1-42) has been reported to be difficult due to the high hydrophobicity of the C-terminal region and the sequence's predisposition to on-resin aggregation[2]. Recent synthetic advances such as induction heating and optimal coupling reagent combinations were applied for the automated synthesis of β-amyloid using shorter cycle times resulting in improved crude purity and yields.

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
Figure 1: β-amyloid (1-42) structure

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YI-P180 Multivalent Display of Functional Split Proteins on β-Sheet Self-Assembled Fibrils

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Department of Chemistry, University of Rochester, Rochester, NY 14627-0216

Multivalent display of large proteins on self-assembled peptide nanofibrils is difficult due to the steric bulk of proteins often impeding self-assembly of peptide monomers to which they are attached. Alternative methods are described herein to employ a split-protein strategy for displaying large proteins along the fibril axis of β-sheet self-assembled Ac-(FKFE)2-NH2 fibrils. A short affinity motif peptide derived from the split protein was attached through solid phase peptide synthesis to the termini of self-

assembling amphipathic Ac-$(FKFE)_2$-NH_2. Upon coassembly of this affinity tagged self-assembly peptide with Ac-$(FKFE)_2$-NH_2, the affinity motif was displayed along the fibril axis without hindering self-assembly due to its short sequence length. The complementary split protein segment was then introduced post-assembly to reconstitute the whole protein resulting in multivalent display of proteins on the Ac-$(FKFE)_2$-NH_2 nanofibrils. Two split protein systems were used to demonstrate the efficiency of this method of functionalization of self-assembling peptide fibrils, ribonuclease S' (RNase S') and split green fluorescent protein (GFP). Biological activity of the two proteins was measured post assembly and complementation to ensure reconstitution did not inhibit expected functionalization. Multivalent RNase S' and GFP nanofibrils exhibited protein activity similar to their wild type counterparts.

YI-P181 Progress towards D-Protein Therapies against PD-1 for Cancer Immunotherapy

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Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Disruption of interactions between PD-1 (programmed cell death protein 1) and its ligands PD-L1 and PDL-2 have led to two FDA-approved antibody therapies against a wide range of cancer types, with additional clinical data suggesting that this type of therapy will be efficacious against an even larger range of cancers. However, like all antibody therapies, there are significant downsides to these therapies, including serious potential side effects and the possibility of severe immune responses. One strategy to ameliorate these disadvantages is to use mirror-image protein therapies. Since mirror-image proteins use unnatural D-amino acids, these proteins are resistant to proteases and non-immunogenic. Development of mirror-image protein therapies requires chemical synthesis of the D-protein, phage display to produce a protein therapeutic effective against the D target, then chemical synthesis of the D-protein therapeutic. In this presentation we will describe our efforts to develop mirror image therapeutics monobodies based on a fibronectin type III domain to serve as immune checkpoint inhibitors by binding to PD-1.

YI-182 Cyclic Bivalent Ligands: Mu/delta Opioid Agonists

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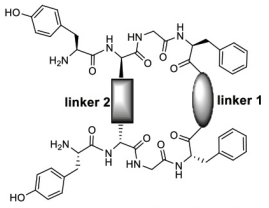
Chronic neuropathic pain is difficult to treat due to serious side effects such as tolerance and addiction caused by long-term administration of medications. There is a need to develop novel ligands for the treatment of prolonged pain, whilst having control and minimal side effects. It has been known that the mu and delta opioid receptor agonists can bring about synergistic analgesic effects along with reduced side effects. Therefore, pursuing novel bifunctional activity, a series of cyclic bivalent ligands, in which two enkephalin-like tetrapeptides (Tyr-DXxx-Gly-Phe) are linked at position 2 and the C-terminus as shown below, were designed and synthesized by standard solution phase synthesis using N^{α}-Boc-chemistry. Due to the high constraint, these analogues were anticipated to possess increased potency and stability. The structure-activity relationship of these analogues will be discussed in detail.



Cyclic bivalent ligands

YI-P183 Mouse MyostatinProdomain-derived Inhibitory Peptides for Treatment of Muscle Atrophic Disorders

C. Rentier[a], M. Saitoh[a], A. Nakamura[a], T. Shimada[a], S. Sashida[b], K. Takayama[a], Y. Negishi[b] and Y. Hayashi[a]
[a]Department of Medicinal Chemistry and [b]Department of Drug Delivery and Molecular Biopharmaceutics, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392, Japan

Myostatin, a protein of the TGF-β1 superfamily, is responsible for the negative regulation of skeletal muscle growth. Based on these properties, a myostatin inhibition strategy appears as an attractive therapeutic approach towards several muscle atrophic disorders, including Duchenne muscular dystrophy. Recent findings by our group described for the first time approaches involving small peptides inhibiting myostatin[1,2]. In particular, The N-terminal substituted peptide (1, XRQNTRYSRIEAIKIQILSKLRL-NH2, where X is a 2-naphthyloxyacetic group) improved the IC_{50} of the non-modified original peptide (X = Trp) by a factor 3 (from 3.53 ± 0.25 μM to 1.19 ± 0.11 μM).

The present work deals with further optimization of peptide **1**. A panel of substitutions and backbone restraints were investigated in an attempt to tune the inhibitory activity of the synthetic peptide. Moreover, the synthesis of peptides incorporating several modifications to the original peptide at the most important positions as previously determined by Ala-scan3 was performed. This led to the obtaining of a peptide **2** with an *in vitro* sub-micromolar IC50 of 0.32 ± 0.05 μM, a value 11 times lower than that of the original peptide, and 4 times more potent that the previously described most efficient inhibitor **1**. Additionally, peptide **2** significantly increased the *in vivo* weight of tibialis and gastrocnemius muscles in mice.

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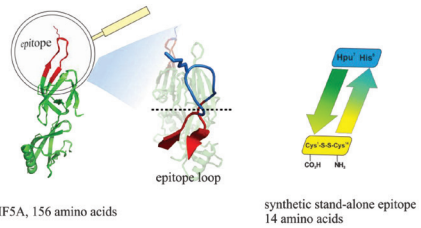
YI-P184 Cyclic β-hairpin Fragments of Eukaryotic Translation Initiation Factor 5A (eIF5A) Preferentially Target Phagocytic Peripheral Blood Mononuclear Cells

Jan Reutzel[1], Dennis M. Klee[1], Sukwinder Singh[2], Hartmut Hanauske-Abel[2], Curtis Dobrowolski[3], Jonathan Karn[3], Armin Geyer[1]
[1]Fachbereich Chemie; Philipps-Universität Marburg, Germany, geyer@staff.uni-marburg.de; [2]Departments of Microbiology, Biochemistry, and Molecular Genetics; Obstetrics, Gynecology & Women´s Health; Pediatrics; New Jersey Medical School of Rutgers University; Newark, New Jersey, USA; [3]Department of Molecular Biology and Microbiology; Case Western Reserve University, Cleveland, Ohio, USA

The hydroxylated form of the eukaryotic translation initiation factor 5A (eIF5A-OH) is overexpressed in several classes of major diseases, such as leukemia and HIV-1. This strictly *intracellular* protein of 156 amino acids carries its unique K50-derived; functionally essential posttranslational modified amino acid (hypusine) within a β-turn motif that is conserved among all eukaryotic life forms. Previous work suggested that

extracellular eIF5A, irrespective of hypusine formation, might act as an enhancer of apoptosis.[1] Guided by the eIF5A crystal structure, we hypothesized that fluorescently labelled, structurally defined peptides of the evolutionarily invariant ß-turn region should probe for apoptotic eIF5A function(s). We herein report the first synthesis of cylic β-hairpin fragments of native, unmodified eIF5A by locking the ß-turn epitope with a well-designed set of hydrophobic interactions.[2-4] The stabilization of the ß-turn motif containing just six unmodified amino acids resulted in a peptide that, even at 1 µM, binds to freshly isolated human mononuclear blood cells (PBMCs) in a cell type- and cell function-specific manner (phagocytic monocytes/macrophages <<< non-phagocytic T cells < non-phagocytic B cells). This selective uptake increased with time only in the phagocytic PBMCs (100-fold overnight) and indicates that even without the hypusine residue; our peptides hold the prospect of drug delivery preferentially to monocytes/macrophages, a prime challenge in the treatment of multidrug-resistant tuberculosis and HIV-1.[5,6] We propose that the secondary structure of the invariant eIF5A ß-turn at K^{50} directly relates to the uptake selectivity. Structural elucidation was performed by NMR spectroscopy, biological studies by flow cytometry and confocal microscopy.



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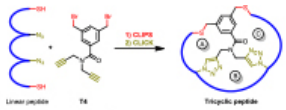
YI-P185 Facile Synthesis of Tricyclic Peptides via Tandem CLIPS/CLICK Cyclization

G.J.J. Richelle[a], H. Hiemstra[a], J.H. van Maarseveen[a], P. Timmerman[a,b]

[a]Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands; [b]Pepscan Therapeutics, Zuidersluisweg 2, 8243 RC Lelystad, The Netherlands



Scheme 1: Example of a tandem CLIPS/CLICK reaction to generate tricyclic peptides

Multicyclic peptides provide a very attractive molecular format for the design of novel therapeutics,[1] Therefore, novel routes for synthesis and HTS-screening of this fascinating class of compounds are desperately needed. A decade ago, we launched a novel scaffold-assisted peptide-cyclization technology platform, termed **"CLIPS"**, to generate in a one-step procedure a new class of bicyclic peptides able to act as potent inhibitors of hitherto undruggable therapeutics targets.[2,3] Following this, we now present a next-generation technology that combines both the **CLIPS** and **CLICK** technology in order to create yet another class of tri-, tetra-, and pentacyclic peptides. This lecture highlights our most recent data on synthesis, structure and biological activity.

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YI-P186 Fmoc Group Removal with Hydrazine Hydroxide for Solid Phase Peptide Synthesis

Andrea V. Rodríguez[a], Daniel Melo[a], Héctor Pineda[a], Zuly J. Rivera[a], Javier E. García[b]

[a]Chemistry Department and [b]Pharmacy Departmentof Universidad Nacional de Colombia. Bogota D.C.

The use of peptides as therapeutic agents has been the mainspring to study and finding efficient methods to synthesize them[1]. In the solid phase peptide synthesis (SPPS) the formation of amide bond have to be selective and therefore is necessary to use orthogonal protection schemes. According to the protecting groups used in SPPS there are two main strategies Fmoc/tBu or tBoc/Bzl. In the Fmoc/tBu strategy, as protect alpha-amine group is employed 9-Fluorenyl methyloxycarbonyl (Fmoc) group. The Fmoc group removal is usually performed using piperidine[2] or its derivatives[3], however, these substances are controlled due to be used in the illicit production of psychotropic drugs, which makes it difficult to market and import. Here in, we propose the use of hydrazine as the agent to eliminate alpha-amine group in Fmoc/tBu strategy. The Fmoc-Val-OH deprotection reaction was monitored by RP-HPLC and our results exhibits that Fmoc group was completely removed with 16% hydrazine hydroxide in 60 min. Thus, it was possible to establish the experimental conditions for remotion of Fmoc group in SPPS. Additionally, it was compare SPPS using 4-methylpiperidine or hydrazine like deprotecting agents.

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YI-P187 Synthesis of N-glycosyl Amino Acids using T3P® – Propane Phosphonic Acid Anhydridel

S. Pinzón[1], R. Fierro[1], C. Iregui[2], Z. Rivera[1], J. García[3], Andrea Veronica Rodriquez

Universidad Nacional de Colombia. Sciences faculty; [1]Chemistry department, [3]Pharmacy Department, [2]Veterinary faculty, Bogotá, Colombia

Carbohydrates are involve in many biological processes and glycoconjugates are frequently being used as antimicrobial drugs and are also emerging as anti-cancer candidates.[1] These discoveries have motivated the design of synthetic carbohydrates and its derivatives.[2] We have design, synthetized and isolated protected and unprotected N-glycosyl amino acids through a new and general synthetic strategy, using T3P® as coupling reagent. The coupling reaction offers advantages

because present fewer by-products compared to other coupling agents, high purity and yields. The methodology developed in our laboratory for binding carbohydrate to the amino acid and the purification methods can be consider as a green methodology, due to the use of a green coupling agent (T3P®), the reduction of organic solvents consumed and energy and time required. The methodology developed can be used to obtain building blocks for glycopeptide synthesis. The methods carried out reduce the use of polluting organic solvents, purifications were done using a faster and simple methodology. It also requires low consumption of organic solvents, which reduces the adverse effects that they can cause to living things and the environment.

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YI-P188 Peptides Derived from Lactoferricin B: Evaluation *In Vitro* of its Cytotoxic Activity Against HBT-132 Cells of Breast Cancer

Jorge Rodríguez[a], Daniel Martínez[a], Adriana Umaña[b], Giovanni Almanzar[c], Jaiver E Rosas[a], Zuly J. Rivera[b], Javier E. García[a]
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Bovine Lactoferrin (LFB) is a multifunctional protein with antimicrobial and anticancerigenic properties[1], this activity has been related to Lactoferricin B (Lfcin B) that is a peptide of 25 residues located at the N-terminal region of LFB[1-2]. It has been reported that the RRWQWR motif corresponds to the minimum sequence with antibacterial activity[3]. Here in we report the design, solid phase synthesis, purification and characterization of monomeric, dimeric, tetrameric and cyclic peptides, containing the RRWQWR motif. Additional, for each designed peptide, It is reported the cytotoxic activity against HBT-132 cells, which corresponds to a cell line derived from breast cancer. This research shows that peptides exhibit cytotoxic activity which is concentration dependent and the peptides with high cytotoxic activity presents action after 1 to 4 h of incubation. Our results show that designed polyvalent molecules (dimers and tetramers) improve the anticancer activity compared to linear peptides and cyclic peptides. The designed molecules can be considered as potential candidates for the development of drugs in treatments and therapies of breast cancer.

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YI-P189 Peptide Sequence Optimization within Reprogrammed Genetic Codes

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Compared to their linear counterparts, cyclic peptides can better evade degradation by proteases, pass cellular membranes and specifically bind protein targets. These 'drug-like' properties can be improved further by incorporating unnatural amino acids into the peptide chain, e.g. allowing for N-methylated backbones. Such highly artificial cyclic peptides can now be easily made on the ribosome using 'genetic code reprogramming'. Ribosomal peptide synthesis can be manipulated, reprogrammed, using 'flexizyme' — a ribozyme capable of charging any unnatural amino acid onto any tRNA. Fusing this technology with 'PURE' *in vitro* translation systems and mRNA display has allowed for the rapid discovery of cyclic peptides that can tightly bind any desired protein target. Here, we describe how the same combination of methods can be used as a tool for biophysical analysis. By coupling genetic code reprogramming with next-generation sequencing, many thousands of unnatural peptide sequences can be quantitatively evaluated in parallel. Using these methods, we show how existing or newly discovered peptides can be improved using unnatural amino acids.

P190 Controlling the Conformational Stability of Helix-loop-helix Peptide Foldamers

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Foldamers are unnatural oligomers that display a defined tendency for folding to specific, stable three-dimensional structures in solution. The rational control of the structural propensities of such molecules has already resulted in a number of important applications. The construction of short peptide foldamers constitutes a great challenge in peptide chemistry as it could lead to a better understanding of more extended structures (e.g.,proteins), as well as providing molecules exhibiting a wide variety of properties as antimicrobial, protein-protein interaction inhibition or gelating. Modulation of the peptide three dimensional structure could be achieved by incorporation of β-amino acids residue in peptide backbone. Recently, we have presented the tendency to form helices of the peptide foldamers containing both cis-β-aminocyclopentanecarboxylic acid (*cis*-ACPC) and β-amino acid residues combined in various sequence patterns (ααβ, αααβ, αβααβ and ααβαααβ)[2]. Moreover the helix handedness of ααβ-peptides could be controlled by sequence shifting.

Here, we present the conformational analysis of more advanced structures based on *cis*-ACPC such as helix–loop-helix. The structures rely on joining two ααββ-helices by a flexible linker (oligoglycines). CD as well as the NMR spectroscopy studies confirmed the formation of designed three-dimensional structures in solution.

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P191 A Novel Peptide for IgG Purification by Affinity Chromatography

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Therapeutic monoclonal IgGs are nowadays produced in large quantities. Protein A affinity chromatography is the standard methodology for their purification, but the harsh elution conditions produce leaching, thus contaminating the product of interest and reducing the operating life of the chromatographic matrix.

The therapeutic mAb Rituximab, provided by CMC Biologist (USA), was labeled with Texas-Red. A peptide combinatorial library was synthesized on HMBA-ChemMatrix resin by the Divide-Couple-Recombine method. After library screening, colored fluorescent beads were isolated and analyzed by MALDI TOF MS/MS. One of the peptide selected was re-synthesized in a larger quantity and immobilized on agarose. The generated resin was able to completely adsorb IgG from CHO cell supernatants while all the contaminants passed through without interacting with the chromatographic matrix. In conclusion, an efficient Affinity Chromatogaphic method based in a simple peptide allows the purification of the mAb Rituximab in a single step at low cost.

YI-P192 Purification of Recombinant Human Growth Hormone Using a Short Synthetic Peptide

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Recombinant human growth hormone (rhGH) is used for the treatment of several pathologies, most of them related to growth. Although there are several expression systems used for its production, the milk from transgenic cows is one of the most interesting due to the high levels of rhGH achieved (5 g/l). We have designed and synthesized a short peptide using Fmoc chemistry and studied its ability to purify rhGH from milk once immobilized on a Sepharose support. The adsorption isotherm for the rhGH-peptide system showed a dissociation constant of 3.1×10^{-6} M, more moderate than those of antibodies, which are in the order of 10^{-9} M. Once performed the chromatography between our generated peptide-resin and spiked milk with rhGH, we analyzed with SDS-PAGE the wash and elution fractions and observed that rhGH was purified with over 90% purity in a single step. This proves that short peptides are an efficient and economical tool for the purification of therapeutic proteins from complex mixtures.

YI-P193 Antibacterial Activity Against Multiresistant Bacteria of (p-BthTX-I)2 and its Derivatives Obtained from Plasma Degradation

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p-(BthTX-I)2 [$(KKYRYHLKPFCKK)_2$][1] is a non-lytic and non-toxic antimicrobial peptide derived from the C-terminal region of Bothropstoxin-I. Despite the therapeutic potential, peptides have some limitations, as proteolytic degradation. In this context, this work analyzed the serum stability of peptides p-BthTX-I and its disulfide linked dimeric form [$(p\text{-BthTX-I})_2$]. After, antimicrobial activity against a variety of bacteria, including resistant and clinical was analyzed. The serum degradation profile of the $(p\text{-BthTX-I})_2$ obtained by HPLC analysis showed a rapid degradation of the peptide with the formation of a stable product after 30 minutes of incubation, which remained in solution even after 30 h. The mass spectrometry analysis showing that the main product is the peptide without four lysine residues. To identify this compound, the peptides des-Lys^{13}-$(p\text{-BthTX-I})_2$; des-Lys^{12},Lys^{13}-$(p\text{-BthTX-I})_2$; des-Lys^1-$(p\text{-BthTX-I})^2$ and des-Lys^1,Lys^2-$(p\text{-BthTX-I})_2$ were synthesized and characterized. Comparison among the HPLC profiles showed that the stable peptide obtained from degradation proteolytic is the peptide without four lysine residues on its C-terminal region [des-Lys^1, Lys^2-$(p\text{-BthTX-I})_2$]. The activity of $(p\text{-BthTX-I})_2$ and des-Lys^1,Lys^2-$(p\text{-BthTX-I})_2$ against multiresistant bacteria were performed. Both peptide showed activity against S. epidermidis ATCC35984; *S. aureus* ATCC25923; *S. aureus* SA16; *S. aureus* SA33; *S. aureus* SA88; *S. aureus* SA90; *E. faecium* VRE16; *E. faecium* HSJRP8; *K. pneumoniae* ATCC700603; *K. pneumoniae* BAA1705; *K. pneumoniae* NDM-1; *E. coli* ATCC25922; *E. coli* ATCC35218 and *E. coli* CA4. In conclusion, this study showed a non-lytic peptide that probably is the responsible for biological activity of the peptide $(p\text{-BthTX-I})_2$. These peptides are promising prototypes for new drugs aiming the treatment of multidrug resistant infections.

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YI-P194 Synthesis and Conformational Analysis of N-Amino Peptides

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The introduction of backbone amide substituents has a profound impact of the conformation and stability of host peptides. Recently, we reported the synthesis of diverse chiral α-hydrazino acids that can be chemoselectively incorporated into a growing peptide chain on solid support to afford N-amino peptides (NAPs). We reasoned that the propensity of these residues to participate in hydrogen bonding could be exploited to enforce intramolecular interactions that yield stabilized extended structures. Here, we describe the synthesis and conformational analysis of β-hairpin model peptides featuring backbone amination. NMR, CD, and thermodynamic analysis demonstrate the unique ability of NAPs to stabilize β-sheet conformation. Sheet-like structures featuring intraresidue C_6H-

bonds were observed in the X-ray crystal structure of a NAP dimer, which to our knowledge represents the first structure of this type reported for N-substituted peptides. These results suggest that backbone amination promotes β-sheet folding by controlling both local backbone geometries and longer-range intermolecular interactions.

YI-P195 Detection of Protease Activity by Fluorescence Alteration of Pyrene Using Bispyrene Peptide Substrates

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Pyrene have been used for the detection and imaging of protein and nucleic acid targets because the excited-state pyrene molecules have a unique emission property, excimer fluorescence (460–480 nm). This fluorescence is emitted when two pyrene molecules are spatially proximal. However, the applications of pyrene excimer signaling to the detection of protease activity are rare.[1,2]

In this study, we designed and synthesized pyrene excimer-based peptide substrates. They consist of a pair of 1-pyrenebutyric acid (Pba) and one or two substrate peptides. Two types of substrates were designed: short-type substrate and long-type substrate. The short-type substrate is comprised of one Pba-conjugated substrate peptide and Pba on each edge of a diamino compound as the core respectively. The long-type substrate is composed of two Pba-conjugated substrate peptides. Proximate two pyrene moieties form excimer in the substrates, and the substrate emits excimer fluorescence. After proteolytic cleavage, the pyrene excimer formation dissociates, and the excimer fluorescence decreases. This excimer fluorescence change allows for the detection of protease activity.

Fluorescent spectral data revealed that the proximate two pyrene moieties in the substrate induced excimer fluorescence before the addition of protease. According to the result of monitoring the change in excimer emission during proteolytic degradation, the kinetic parameters were successfully calculated.

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P196 Direct Transformation of Peptide Hydrazide into N-Thz Thioester Peptide

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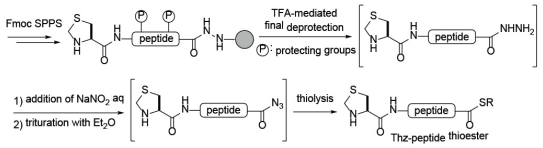
Native chemical ligation (NCL), featuring the use of peptide thioesters and N-terminal Cys peptides, is amongst the most useful ligation method for chemical protein synthesis.[1] To synthesize more than 100-residues of proteins by NCL, N-Cys- or thioester-cryptic intermediates are required to selectively ligate multi segments. N-Thiazolidinyl (Thz) peptide thioesters are widely used as a key intermediate in C-to-N directed sequential NCL.[2] The use of peptide hydrazide as a precursor of peptide thioester has recently been attracting attention because of the easy operation and compatibility with Fmoc chemistry[3]; however, direct transformation of N-Thz peptide hydrazide into the corresponding thioester still remains difficult due to decomposition of Thz moiety during the conversion. In this study, we found the reaction conditions applicable to such conversions using $NaNO_2$-thioanisole-*m*-cresol-triisopropylsilane-H_2O in TFA. This conditions yielded the N-Thz peptide thioester directly from the corresponding hydrazide without decomposition of Thz moiety.



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P197 Advances in One-Pot Peptide Ligation Chemistry

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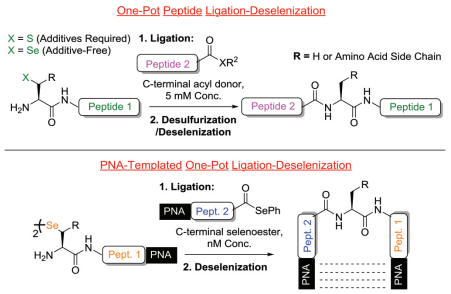
Native Chemical Ligation (NCL) is a powerful and widely applied method for the total chemical synthesis of homogeneous peptide and protein targets.[1] This method, which initially required a cysteine at the ligation junction, has been extended by the use of post-ligation desulfurization protocols to include non-proteinogenic amino acid building blocks bearing suitably positioned thiol auxiliaries. This one-pot ligation–desulfurization manifold has been successfully applied at a plethora of amino acid junctions.[2]

Despite such developments, NCL retains several limitations including the requirement for additives, a slow rate of ligation, and the necessity for reactions to be conducted in the mM concentration range. A recently discovered selenium-mediated variation of NCL, utilising peptides bearing an N-terminal selenocystine residue and a C-terminal phenyl-selenoester, has been demonstrated to facilitate ligation reactions in additive-free conditions and at an unprecedented rate.[3] This rapid one-pot chemoselective ligation-deselenization technique represents a significant advancement in the field of ligation chemistry and attention has now turned to expanding the technology to include the use of synthetic selenol-derived amino acids as selenocystine surrogates, two of which will be presented. Further, investigations aimed at addressing the concentration limitation of NCL through incorporation of PNA tags into the termini of peptide fragments, are currently underway. This



PNA-templated selenium-mediated ligation reaction proceeds at unprecedented concentrations, paving the way for the total chemical synthesis of previously inaccessible targets.

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P198 Targeted Delivery of Cyclotides via Conjugation to a Nanobody

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Most naturally occurring peptides have poor proteolytic stability, which limits their therapeutic application. Cyclotides are plantderived cyclic peptides that resist proteolysis due to their highly constrained structure, comprising a head-to-tail cyclic backbone and three disulfide bonds that form a cystine-knotted core. This makes them potentially useful as scaffolds onto which peptide sequences (epitopes) can be grafted. We target delivery of cyclotides via conjugation to a nanobody to confer specificity. We chose VHH7, an alpaca-derived nanobody that targets murine Class II MHC class II molecules, for conjugation of cyclotides and examined their immunogenic properties upon delivery to antigen presenting cells. Two cyclotides, MCoTI-I and MCoTI-I with a HA-tag epitope (YPYDVPDYA) grafted in loop 6 (MCoTI-HA), were tested. A conjugation strategy using sortase A and strain-promoted click chemistry provides a straightforward method for targeted delivery of cyclotides to antigen-presenting cells and could play a role in the development of peptide-based vaccines. More generally, these types of fusions might also be useful to direct cyclotides to other cell types, based on the specificity of the VHH fusion partner.

YI-P199 Modulating the Protein-protein Interactions between the $GABA_B$ Receptor and the KCTD Proteins by Peptide Inhibitors

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Protein–protein interactions (PPIs) are crucial for most cellular and biochemical processes and represent a promising class of drug targets γ-Aminobutyric acid (GABA) type B ($GABA_B$) receptors are G protein-coupled receptors for GABA, the main inhibitory neurotransmitter in the central nervous system. GABAB receptors are involved in cell growth and survival, as well as differentiation, migration and axonal guidance of nerve cells. Consequently, dysfunction of $GABA_B$ receptors has been implicated in neurological disorders such as epilepsy, spasticity, depression, anxiety, pain, schizophrenia and cognitive deficits. $GABA_B$ receptor signaling is modulated by the K^+ channel tetramerization domain (KCTD) proteins, which are soluble proteins that bind directly to the intracellular Cterminus of $GABA_B$ receptor subunit 2 ($GABA_{B2}$) to stabilize G protein binding. Here we are exploring the therapeutic potential of this interaction by characterization and development of peptide-based inhibitors. We have employed SPOT and microarray technology, which allows high-throughput synthesis and qualitative binding analysis of peptide inhibitors. We performed a systematic screen of the $GABA_{B2}$ C-terminal and defined the binding epitope. From this we identified shorter peptide inhibitors, which are currently being used as templates for the development of KCTD subtype specific super-binding peptides, which will serve as a tool compounds to modulate $GABA_B$ receptor mediated neuronal signaling.

YI-P200 Molecular Dynamic Simulation of Highly Potent and Selective Peptide Inhibitor of PACE4

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PACE4 is a member of proprotein convertases family of serin proteases. Experimental inhibition assays of certain peptide inhibitors with the structure of Ac-LLLLRXKR-NH_2 for this enzyme showed that P3 residue is needed to be β-branched.

Peptide's inhibitors of proteases form a β-sheet with the backbone of active site. It has been known that β-branched residues like Val and Ile in the structure of peptides and proteins is useful to induce the β-sheet secondary structure. In order to confirm the importance of presence of β- branched residues in the structure of our PACE4 inhibitors, we ran MD simulations of two peptide inhibitor warhead docked into a homology model of PACE4. We investigated the conformation of peptides in the active site during the simulation. We measured the phi and psi angles of P3 residue of inhibitors. The inhibitor with P3 Val was revealed the greater tendency for β-strand conformation than P3 Ala. We also calculated the β-sheet hydrogen bonding angles of the P3 residues with S3 Gly158 backbone. Val showed higher values which is in consistent with ideal hydrogen bonding in β-sheets. In summary, our investigations demonstrated the importance of branched residues in the P3 of PACE4 peptide inhibitors.

P201 The DNA Target Determines the Dimerization Partner Selected by bHLHZ Proteins AhRJun and ArntFos

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The molecular basis of protein-partner selection and DNA binding of the basic helix-loop-helix (bHLH) and basic region-leucine zipper (bZIP) superfamilies of dimeric transcription factors is fundamental toward understanding gene regulation. Because these families share structural similarities, we swapped bHLH and leucine zipper (LZ) modules between families to uncover how individual modules influence protein-partnering and protein:DNA complexation. We previously described ArntFos, a bHLHZ-like hybrid of the bHLH domain from bHLH/PAS protein Arnt and LZ from bZIP protein c-Fos, binding to the Arnt E-box site (TCACGTGA) as a homodimer. Here, we describe a heterodimer between ArntFos and AhRJun, a hybrid of the bHLH domain from AhR and LZ of

JunD. We designed AhRJun and ArntFos to heterodimerize, given the strong interaction between native AhR/Arnt and Jun/Fos, but the hybrids showed no preference for hetero- or homodimerization in Y2H assays.

However, adding a specific DNA target drove formation of a single dimeric protein species over others. EMSA showed AhRJun/ArntFos heterodimer binding the cognate DNA site XRE1 (TTGCGTG) at K_d 337 nM. Unexpectedly, the palindromic Arnt E-box drove binding of the AhRJun/ArntFos heterodimer (K_d 276 nM) — not the ArntFos homodimer — that does bind to Arnt E-box. However, the dimerization preference switched to ArntFos homodimer when variant Max E-box (CCACGTGG) was used. We conclude that the DNA sites themselves are the primary determinants of dimerization specificity for AhRJun and ArntFos, not the JunD and c-Fos LZs, a result that sheds light on the dynamics of protein/protein and protein: DNA interactions and structural modularity of bHLH and bZIP proteins.

P202 Identification of Peptidomimetic Inhibitors of Skp2

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Skp2 is frequently overexpressed in many human cancers and considered as a proto-oncogene. It is believed that Skp2 has at least two critical roles in tumorigenesis. As a component of the SCFSkp2 ubiquitin E3 ligase complex, Skp2 drives the cell cycle by mediating the proteasomal degradation of cell cycle proteins such as the tumor suppressors p27 and p21. In addition to the proteolytic function, Skp2 suppresses p53-dependent apoptosis by outcompeting p53 for binding to histone acetyltransferase p300, thereby perturbing p300-mediated p53 acetylation and leading to tumorigenesis. This Skp2 activity is independent of ubiquitin-mediated proteolysis and thus called non-proteolytic function. As a result, inhibition of Skp2 function (either proteolytic or non-proteolytic) is emerging as a promising and novel anti-cancer strategy.[1]

In this study, we used bead-based high-throughput screening method to identify such Skp2 inhibitors. We employed cyclic peptoids as a library source as they are shown to have conformational rigidity, good cell permeability and proteolytic stability. We constructed a one-bead one-compound (OBOC) combinatorial library of cyclic peptoids with a theoretical diversity of 1.13 x 10^7 (156) by split-and-mix method. The vast bead-based library was screened against Skp2 protein, resulting in the discovery of cyclic peptoids that binds directly to Skp2. The hit compounds were resynthesized and purified by HPLC. Further biochemical and biological studies on these compounds as Skp2 inhibitors were investigated. Our Skp2 inhibitors hold great potential as an invaluable probe to investigate Skp2 functions and as novel drug candidates for anticancer therapy.

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YI-P203 The Glaser Bioconjugation

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The copper-mediated hetero-Glaser coupling between alkynyl pep-tides and proteins and small molecule alkynes is reported. Super-stoichiometric amounts of copper(I) in the presence of an appropri-ate water-soluble ligand reliably forges a chemically inert 1,3-diyne linkage under benign conditions in aqueous solution. The general-ity of this method is illustrated by the conjugation of a sampling of nonpolar and polar small molecules onto a fully unprotectected GLP-1R agonist.

YI-P204 Understanding the Intrinsic Cell Selectivity of Cell Penetrating Peptides

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Cell penetrating peptides (CPPs) have been studied extensively over the last two decades as they may prove to be a promising lead in assisting the uptake of pharmaceutical drugs or drug-containing vehicles. A main issue with CPPs is the lack of intrinsic cell selectivity. Recent research in the Löwik group presents new possibilities to introduce cell selectivity in Tat by altering the side-groups of the amino acids.[1] Understanding of how this intrinsic cell selectivity arises and can be influenced is presently inadequate.

Here, we study the effect of the cargo of the CPP on the intrinsic cell selectivity in HEK and HeLa cells. Furthermore, the role of the glycosaminoglycans (GAGs) on the cell surface is explored.

We believe that a better understanding of the intrinsic cell selectivity of CPPs will help in the development of new and improved CPPs for drug delivery

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P205 High Temperature Treatment Improves Therapeutic Peptide Physical Stability

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Peptides are becoming increasingly prevalent as therapeutic agents in the pharmaceutical industry. Physical instability of peptides poses a significant risk to the successful development of a peptide drug product. Peptide A is observed by dynamic light scattering to initially exist as an oligomer in solution with a narrow pH window for optimal physical and chemical stability. Indeed, when the solution pH is < 8, the peptide forms an entangled network of fibrils resulting in the formation of a highly viscous solution over time. Peptide instability is also enhanced when it is combined with chemical preservatives that insure formulation sterility. As such, a means to stabilize Peptide A at a lower pH, where chemical stability is high, would be valuable to the preparation of this peptide for formulation development. Hydroxypropyl-β-cyclodextrin (CD), a commonly used stabilizing molecule, is believed to interact with the peptide through hydrophobic interactions however is not fully effective in the presence of preservatives. To enhance these hydrophobic

interactions between Peptide A and CD, solutions were incubated at temperatures where oligomer dissociation and peptide denaturation have been observed. Indeed, compared to solutions incubated at room temperature, solutions incubated at 90oC show no increase in Thioflavin T fluorescence over time consistent with the inhibition of fibril formation. Importantly, treatment of the solutions for ≤ 5 min at 90oC results in enhanced physical stability and minimal formation of peptide degradation products. Thus, high temperature treatment may be a viable route for enhancing peptide stability during formulation development.

YI-P206 Long Range Salt Bridges Mediated by Non-polar Amino Acids in a Alpha Helix Model System

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Brigham Young University

Protein salt bridges can contribute to protein structure when they are in close proximity, typically 4 angstroms. We describe a nonpolar cyclic mediated long-range salt bridge (13 angstroms) engineered into two coiled-coil model proteins. The opposing charges are engineered onto the solvent exposed face of a coiled-coil trimer at the i and i+8 positions while arenes, a non-arene, and a nonpolar amino acid are positioned at the i+4 position. Using double and triple mutant cycle analysis we show that this interaction can contribute up to -1.02 kcal/mol in stability to the folded protein.

YI-P207 Macrocyclic Analogues of Neurotensin 8-13: Design and Discovery of a Hit

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Neurotensin receptors (NTS1 and NTS2) belong to the G protein-coupled receptor (GPCRs) family and represent very interesting targets for the treatment of pain. Indeed, their activation has been shown not only to induce an analgesic effect very similar to morphine (although MOR-independent)[1] but do not seem to induce addiction[2], which is one of the main drawbacks of current opioid treatments.

However, neurotensin is a 13-residue peptide which shows pharmacological properties (extremely short half-life, low bioavailability etc.) that make it unusable as a drug. Since macrocycles have the potential to improve these properties[3], we decided to produce macrocyclic analogues of neurotensin 8-13 (shortest fragment of neurotensin which retains its full activity).

The development of a "hit" macrocycle (which adopts a conformation that allows satisfactory binding to the receptor) was achieved through an iterative approach:

1) Peptides were synthesized on solid phase and the introduction of alcene-containing unnatural amino acids allowed to macrocyclize via Ring-Closing Metathesis.

2) Binding of the macrocycles to the receptors was assessed through radiolabelled ligand displacement assay.

3) In sillico studies were accomplished (based on the neurotensin 8-13-bound NTS1 receptor crystal structure[4]) in order to rationalize the binding results and design the next generation of macrocycles.

This led to the discovery of a macrocycle with an affinity of 40 nM and 14 nM for NTS1 and NTS2, respectively. This "hit" is able to activate the G-protein signaling pathway as well as to induce the recruitment of β-arrestins following binding to NTS1 and exhibits an improved half-life in rat plasma compared to the neurotensin native peptide.

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P208 Evaluation of Histone H2A-H2B Dimer based on Protein Chemical Synthesis

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In eukaryotic cells, genome DNA forms nucleosome structure with four types of core histone proteins. Posttranslational modifications (PTMs) in histones regulate gene expression through recruiting histone chaperones, which control nucleosome condensation, histone transport and turnover. Although histones and their complexes show dynamic behaviors, few reports investigated direct effects of PTMs on the complexes in vitro and in cells. For conventional biological methods, it is difficult to prepare histone proteins containing a specific PTM or fluorescent dyes for the analysis.

Herein, we report chemical synthesis of histone proteins for tracking their structural changes. In this research, we focused on histone H2A and H2B, which form H2A-H2B heterodimer. H2A-H2B dimer is an attractive target for studying histone dynamics, because it is incorporated into the outermost part of nucleosome and therefore shows active turnover.

First, we synthesized histone H2A phosphorylated at Tyr57 through solid phase peptide synthesis and native chemical ligation. In PTMs of H2A and H2B, this phosphorylation seems to affect the stability of H2A-H2B dimer from crystal structure. The nucleosome and the dimer were reconstituted in vitro using the phosphorylated H2A. Their stabilities were estimated through thermal shift assay, and compared to that of canonical complexes. The result shows that the phosphorylation destabilize H2A-H2B dimer.

Also, FRET analysis by two fluorescent dyes is a well-known method for investigating protein interaction. We tried to synthesize histone H2B containing different dyes. We designed the position of the dyes to show FRET by forming H2A-H2B dimer, and introduced the dyes through orthogonal reactions.

P209 Novel Approach to Determine Prolactin Releasing Peptide Analogs via LC-MS using Monolithic Column

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Anorexigenic neuropeptides are promising tools for treatment of obesity, but their delivery from the periphery to the brain is complicated because of their low stability and limited ability to cross the blood-brain barrier. Our newly designed lipidized analogs of prolactin-releasing peptide (PrRP) can exert their central effect after peripheral administration due to the palmitoylation of the peptide.

Here, the main goal of this multi-component project was to develop a analytical method suitable for the determination of the stability of the novel PrRP analogs in mouse, rat, and macaque plasma. The appropriate liquid chromatography-mass spectrometry (LC-MS) method was developed and optimized. The results obtained were compared with the data measured via a commercial enzyme-linked immuno-sorbent assay (ELISA) kit. A final preparation strategy for plasma samples was optimized and consisted of simple dilution of the plasma samples followed by direct injection onto a short monolithic column in combination with MS detection. The developed analytical method was utilized for the determination of the stability of the prepared lipopeptides in plasma and also for the quantification of the lipopeptides in a preliminary pharmacokinetic study. The feasibility of the developed separation method was clearly demonstrated.

The study was founded by a specific university research grant of the Ministry of Education Youth and Sports of the Czech Republic, project MSMT no. 20-SVV/2016 and RVO: 61388963 of the Academy of Sciences of the Czech Republic.

P210 Carcinogenic Dietary Bioactive Peptides By-product from Commercial Food Processing in Instant Food Sold in Supermarket

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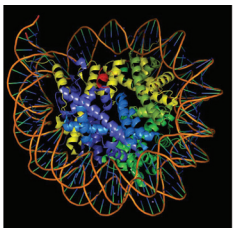
School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551



We identified that instant food commonly sold in supermarket contain bioactive glycated and glycoxidated lunasin peptides with predicted genotoxic effects on the consumer. These potential toxins may play a key role in the development of gastrointestinal (GI) cancers, which are highly prevalent in modern society, likely due to increasing consumption of commercially processed instant food. Importantly, these proteinaceous toxins were not present in fresh foodstuffs and are likely generated as byproducts of high-temperature food processing and/or fermentation.

Despite numerous epidemiological studies indicating a strong association of diet with human cancer risk, and reports that ingested aflatoxin and other mycotoxins can promote the development of liver cancer, the dietary molecules that influence host tumorigenesis remain poorly defined. GI cancers in particular are a major causes of death in modern society, but previous efforts to identify the dietary mediators of these malignancies have been inconclusive. While some of the most toxic molecules and venoms known to science are proteinaceous in nature, the study of toxic proteins in the diet has been restricted by the technical challenges associated with analyzing these molecules in complex food preparations. We have therefore spent over a decade developing advanced proteomic methods that enable the study of toxic bioactive peptides generated by high-temperature food processing, fermentation, and prolonged storage of food products.

Given the high burden of morbidity and mortality associated with cancer, together with evidence that up to 70% of cases can be attributed to dietary factors, the discovery of novel dietary proteinaceous toxins has genuine potential to significantly reduce rates of malignancy by enabling the development of new food safety regulations and interventions.

Molecular model of the advanced glycation end product derived from lunasin binding to the human nucleosome. The red ribbon represents the helical region of lunasin (PCEKHIM) which is modified by glycation/ glycoxidation (advanced glycation end products) and displays structural homology with a conserved region of chromatin-binding proteins. The conformation of the lunasin sequence was generated based on the peanut homolog 2S albumin (1W2Q). The human nucleosome shown in this model corresponds to the crystal structure of the core particle (2CV5).

P211 Uncovering Neurodegenerative Peptide/protein Modifications Identified a 'Vicious cycle' of Brain Tissue Damage Induced by Hypoxic-ischemic Brain Injury

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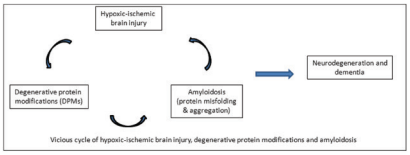
Human dementia is regarded as a 'proteinopathy' in which changes in brain protein structure and function promote neurodegeneration. Degenerative protein modifications (DPMs) are caused by non-enzymatic chemical reactions that induce changes in peptide/protein structure and function which promote disease initiation, pathological progression, and also natural ageing. These undesirable DPMs include oxidation, carbonylation, carbamylation, glycation, deamidation, racemization, etc which impart deleterious structural and functional changes on extracellular matrix proteins and long-lived cell types such as cardiomyocytes and neurons, leading to impaired overall organ function. Despite the obvious clinical importance of understanding DPM biology, the molecular mechanisms that mediate these modifications remain poorly understood largely due to the technical challenges associated with their study.



Over the past 10 years, we have focused on proteomic studies of hypoxic-ischemic brain injury, degenerative protein modifications (DPMs) and aggregation. We discovered for the first time a 'vicious cycle' of brain tissue damage induced by hypoxic-ischemic brain injury that critically dysregulated key enzymes mediating protein degradation and repair in the

affected tissues, leading to accumulation of DPMs damaged proteins and subsequent protein misfolding and aggregation, resulting in neurodegeneration and dementia. The results may critically support drug discovery efforts aiming to tackle this major public health problem.

P212 Development of Lipopolyplexes as Imaging Probes: Effect of Differing Modes of Targeting Peptide Display on Cellular and Tumour Uptake

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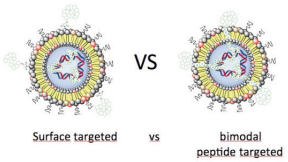
Early detection and diagnosis of diseases such as cancer are crucial to successful treatment. One area of growing importance is the delivery of genes coding for optical probes or biosensors, and we have previously developed a range of lipopolyplex-based nanoparticles comprising lipid, plasmid DNA and peptides specifically targeted to cell surface receptors on cancer cells.[1,2]



A variety of different approaches are possible for mounting the targeting moiety on the surface of the nanoparticle and packaging the cargo for effective delivery. However, the effects of different modes of display of the targeting peptide on the structure, transfection efficiency and tumor-specific delivery of the nanoparticle have not previously been studied. Here we demonstrate that lipopolyplexes formulated from bifunctional peptides with targeting and DNA binding properties are more effective in transfecting tumor cells than lipopolyplexes in which the targeting peptide is surface mounted by conjugation to the lipid coat. The synthesis, formulation, transfection and structural properties of the lipopolyplex architectures are reported and compared.

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P213 Semisynthetic Lantibiotics: Simplicity, Stability, Specificity?

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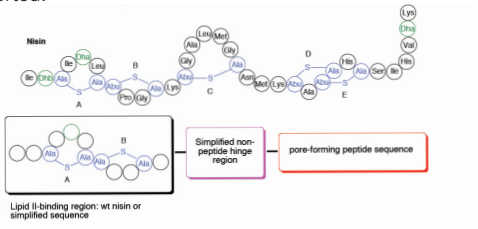
There is an urgent need to develop new classes of antibiotics. Certain lantibiotics have a novel mode of action and are therefore good leads for next generation antibacterial agents. The *N*-terminus (rings A and B) of nisin recognises and specifically binds lipid II, a lipid only found in bacterial membranes, and the C-terminus then form pores in the bacterial membrane. However, lantibiotics have complex structures, characterised by multiple thioether bridges, and are not easy to produce either synthetically or biosynthetically. They are unstable *in vivo* and *in vitro*, have reduced solubility compared to other peptides, and are susceptible to proteolysis.

We have designed and synthesised simplified lantibiotics to circumvent these problems. In these hybrid lantibiotics the complex C-terminus is replaced by simpler pore-forming peptides and connected to the lipid II-binding region, based on the sequence and structure of rings A and B of nisin, via flexible chemical linkages, replacing the hinge region. The synthesis and structural properties of the hybrid peptides, and their antibacterial action against a range of bacteria, will be reported.



YI-P214 Synthetic Study of the Selenocysteine-substituted Ferredoxin

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Ferredoxin (Fd), one of a protein of the photosystem I (PSI), is categorized as iron-sulfur protein. In the plant-type Fd, [2Fe-2S] cluster, composed of two iron and two sulfur atoms, acts as an electron carrier to generate a NADPH by ferredoxin NADP$^+$ reductase (FNR). In spite of extensive studies on Fd, how the electron is transferred from/to Fd is not understood in detail. To obtain further functional and structural information of Fd, we are trying to synthesize a selenocysteine-substituted Fd-1 of *T. Elongatus* by the ligation method. Some antioxidant enzymes use selenocysteine to reduce reactive oxygen species because of its lower redox potential than cysteine. Therefore, we thought that the selenocysteine-substitution enhances the electron donation activity of Fd. To establish a synthetic strategy for Fd-1, we first attempted to synthesize native Fd-1 by applying native chemical ligation method (NCL). The entire sequence of Fd-1 was divided into three at the N-terminus of two Cys residues and each of them was synthesized by the solid-phase method. Then these segments were condensed by the NCL method to obtain native Fd-1. The result of the synthesis of native Fd-1 and the segment of selenocysteine-substituted Fd-1 will be presented.

P215 Chemical Methods to Interrogate Cell-Signaling in streptococci

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Quorum sensing (QS) is a ubiquitous process in bacteria that governs many important symbiotic and pathogenic phenotypes including bioluminescence, biofilm formation, root nodulation, motility, and virulence. As such, QS has attracted considerable attention as a means to control bacterial behaviors — attenuate undesired phenotypes, and promote productive processes. The Streptococcus genus includes commensal and pathogenic Gram-positive bacteria, many of which are frequently found co-colonizing the same natural niches, such as the oral cavity, nasopharynx, and the throat. Streptococci species utilize QS circuits that are centered on a peptide signal termed the competence stimulating peptide (CSP). The CSP-induced QS

circuits govern competence, along with biofilm formation and virulence factor production, and may be involved in interspecies competition between streptococci. We aim to develop CSP-based QS modulators that would be applied to study different streptococci species and assess the role of QS in bacterial competition, and presented here our recent results on the *S. pneumoniae* and *S. mutans* systems

P216 Bivalent Ligands of the Chemokine Receptor CXCR4 with Polyproline Linkers and Their Anti-chemotactic Activity

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In our previous study, bivalent ligands with polyproline linkers were shown to have higher binding affinity for a GPCR, the chemokine receptor CXCR4, than the corresponding monovalent ligands.[1] Interaction of CXCR4 with its endogenous ligand, stromal-cell derived factor-1 (SDF-1)/CXCL12, induces various physiological functions involving chemotaxis. The relationship between the dimerization of CXCR4 and metastasis of cancer cells, in which CXCR4 is overexpressed, has also been proved. Bivalent CXCR4 ligands with polyproline linkers bearing a cyclic pentapeptide, FC131, were previously shown to have higher binding affinities for CXCR4 and to detect more highly the dimer state of CXCR4 on the cancer cell surface than the corresponding monovalent ligands. Bivalent ligands based on a 14-mer peptide T140 derivative with polyproline linkers have been designed and synthesized.[2] The binding affinity of these series of bivalent ligands is increased as the linker length increases up to the 12-mer proline linker. The T140-derived bivalent ligands with the 9- and 12-mer proline linkers showed the most effective inhibitory activity against chemotaxis on Jurkat cells, which is even higher than that of known CXCR4 antagonists in the monomer structure. The effective metastatic inhibition by bivalent CXCR4 ligands indicates the therapeutic potential against cancer.

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P217 Blood-Brain Barrier Shuttle Peptides: An Emerging Tool for Brain Delivery

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Brain delivery is one of the major challenges in drug development because of the high number of patients suffering from central nervous diseases (CNS) and the low efficiency of the treatments available. Although the blood–brain barrier (BBB) prevents most drugs from reaching their targets, molecular vectors — known as BBB-shuttles — offer great promise to safely overcome this formidable obstacle. Peptides which are experiencing a golden era are receiving growing attention because of their lower cost, reduced immunogenicity, and higher chemical versatility than traditional Trojan horse antibodies to be used as BBB-shuttles, as we have recently reviewed.[1]

In spite of their potential use for transport of small molecules, passive diffusion shuttles have limitations for transporting macromolecules (proteins, mAbs, nanoparticles).[2-5] This limitation prompted us more recently to focus on the use of peptides recognized by receptors as actively transported vectors.[6,7]

In this communication, I will review our latest results related to these peptides acting as actively transported proteaseresistant BBB-shuttles and their capacity to cross the BBB and enhance the transport of cargoes of distinct sizes and types that can not cross it unaided. Presenting unpublished results on their application in the field of delivery of macromolecules and/or nanoparticles as promising future therapy for CNS disorders that require to cross the BBB.

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YI-P218 Attacking Ras-driven Cancer: A Promising Display-matured Peptide

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*University of Cambridge; *Isogenica Ltd.*

Prior work has indicated that inhibition of Cdc42 interaction with its effectors can reverse Ras-driven oncogenesis.

We have used CIS display, in a collaboration with Isogenica Ltd., to develop a peptide that selectively binds to Cdc42 with nanomolar affinity and competes with ACK (a specific Cdc42 effector). After the first round of selection a 16mer cyclic peptide that binds with high nanomolar affinity selectively to Cdc42 was isolated. The initial library was designed to incorporate a disulphide bond into peptides and this structure is vital for high affinity binding in the enriched sequence. A cell penetrating sequence was added to the peptide to facilitate entry into mouse embryo fibroblasts conditionally expressing oncogenic K-Ras — an established *in vitro* cancer model. We found the peptide could inhibit signalling through ACK and the Erk signalling cascade (a key Ras transformation pathway).

This promising activity was transient in the initial experiment: To ameliorate the effect duration, we have established a method to chemically stabilise the disulphide bond that cyclised the structure. Maturation of this peptide by CIS display has increased binding affinity more than ten-fold and we will shortly apply the stabilised, higher affinity peptide in cell assays to assess its effect on proliferation and cell signalling.

Future work will investigate binding in more detail and mature the peptide further with a view to increasing the compound's therapeutic potential against Ras transformation.

YI-P219 Antifungal Activity of a Synthetic Cowpea Defensin and Application in Baking Dough

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Department of Food and Nutritional Sciences, University College Cork, Cork Ireland

Plant defensins are small, cysteine-rich antimicrobial peptides of the immune system found in several organs during plant development. A synthetic antimicrobial peptide, KT43C, a linear analogue of the native Cp-thionin II found in cowpea seeds, was evaluated for its antifungal potency. This peptide displayed antifungal activity against several contaminants of cereal products, such as *Fusarium culmorum* or *Aspergillus niger*. KT43C inhibited conidial germination and mycelium development without inducing morphogenic changes. In a membrane-mimicking environment, KT43C adopted a more helical conformation. This change enhanced the activity of the peptide inducing permeabilization of the hyphal membrane. In addition, with high concentrations of the peptide, an increase of the production of reactive oxygen species in the granulated cytoplasm was highlighted. Like native plant defensins, KT43C showed heat- and moderate salt-stability which make it a good candidate for applications in baked goods. In a challenge test against *F. culmorum*, the use of the peptide extended the shelf life of chilled dough (based on the number of fungal colonies) by 2 days. After 14 days of storage, the level of fungal contamination in the control dough was twice as high as in the treated dough. The toxicity of KT43C was also evaluated against mammalian blood cells. KT43C did not show haemolytic when applied at the concentrations that exhibit antifungal potency, up to 200 ug.ml^{-1}. These results highlight the potential for the use of synthetic antimicrobial defensins for shelf-life extension of food products.

YI-P220 Antifungal Ultrashort Peptide: Potential as Novel Food Preservative

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Antimicrobial peptides are essential components of the immune system of many organisms from insects to mammals. Due to their multiple mode of action, these peptides appear to be promising candidates to combat drug-resistant microbes. The identification of key determinants of peptide antimicrobial activity, such as cationicity and amphipathicity, has enabled the design of so-called ultrashort peptides (USPs). In this study, the amidated USP, Orn-Orn-Trp-Trp-NH2, described previously, showed growth inhibition and killing of several fungal contaminants of cereal products, such as *Fusarium culmorum* and *Penicillium expansum*, in addition to anti-yeast activity. This USP showed heat-stability up to 100°C for 1 h and pH resistance in the range 3-10, but is sensitive to cations increase.

The addition of a lipid tail at the N-terminal of the USP significantly increased its antifungal activity as well as its resistance in salt solutions and to proteolytic digestion. Several lipopeptides have been approved by the FDA in treatment of bacterial infections. The two peptides induce rapid membrane permeabilization of fungal hyphae and yeast cells. Differences in structural conformations were highlighted and correlated to antifungal potency. In order to foresee potential food applications, peptides were tested for their cytotoxicity towards human colonic cell lines and haemolytic activity *in vitro*. In contrast to the lipopeptide, Orn-Orn-Trp-Trp-NH2 does not show haemolytic or cytotoxic activity when applied at the concentrations that exhibit antifungal potency. Furthermore, the use of the peptide delays yeast growth in a challenge test performed in different commercial beverages.

YI-P221 ^{68}Ga Imaging of AR42J Rat Pancreatic Tumor Xenograft Bearing Mice with a DOTA-TATE Analogue Bearing a Tryptathionine Cross-link Inspired by α-amanitin

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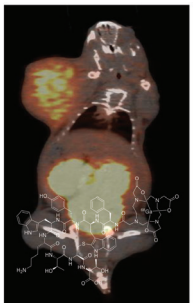

PET (positron emission tomography) is a growing field in molecular imaging. Peptide based, tumor specific, ligands coupled with radio prosthetics capable of bearing positron emitting isotopes, such as ^{68}Ga, are frequent agents of PET imaging. They offer high affinity and specificity, but can also have undesirable pharmacokinetic properties due to low lipophilicity and metabolic instability. Taking a cue from nature, we looked to the family of fungal amatoxins, which potently bind mammalian RNA Polymerase II through a highly constrained β-turn structure and resist proteolysis due to their unique intra-annular tryptophan-cysteine bridge and bicyclic structure. We sought to exploit these features in an attempt to design a more metabolically stable and drug-like Octreotate derivative. We synthesized the tryptathio-derivatives of Octreotate with varied stereochemistry at the cross-link, hoping to constrain the β-turn of the peptide in a conformation analogous to the native disulfide structure. Gratified that a compound with a 20nM Ki to Sstr2 was found, we proceeded to synthesize a further variant bearing the gallium chelator, DOTA. We were pleased to find this compound had a 13nM Ki and continued to an in-vivo imaging study in a mouse model. Labelling with ^{68}Ga afforded 1.4mCi of activity for a 20nmole sample. Upon injection of this radio-tracer into the model system, the tumor was clearly visualized. A modest tumor uptake value of 1.2% was mitigated by low gut and liver uptake, demonstrating a potentially promising platform for further elaboration.

P222 A Novel GIP Analog, ZP4165, Improves Glycemic Control and Positively Modulates Bone Turnover in Rodents

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Type 2 diabetes mellitus (T2DM) accounts for 95% of diabetes cases globally. Obesity and lack of physical activity are the most common causes and agonists of the glucagon-like peptide-1 receptor (GLP-1R) are commonly used for the treatment of T2DM due to their anti-hyperglycemic and body weight lowering actions. In contrast, agonists of the other incretin receptor,

glucose-dependent insulinotropic polypeptide receptor (GIPR), has not been pursued therapeutically, primarily because of an impaired insulinotropic action of GIP in diabetic patients and conflicting data on the role of GIP in rodent obesity. Recent research showing beneficial metabolic effects of GIPR and GLP-1R co-activation has however led to a renewed interest in GIP biology. Here we present the effect of a novel GIP analog, ZP4165, on glycemic control and on bone resorption (CTX) and formation (osteocalcin) plasma biomarkers in rodents. ZP4165 has been optimized for a prolonged half-life compared to native GIP by ablating the DPP-IV enzymatic cleavage site and incorporating a Lys17 acylation to mediate albumin binding. ZP4165 is a potent agonist on the human GIP receptor and has an extended half-life of 1.1 h in mice and 3.6 h in rats following s.c. administration. In a diabetic mouse model ZP4165 improved long-term glycemic control, which was further enhanced by co-administration with liraglutide. In addition, in normal rats ZP4165 acutely affected bone turnover with a net effect favoring bone formation. We conclude that ZP4165 has potential as a novel therapeutic for the management of glycemic control and potentially to reduce bone fracture risk of diabetic patients.

P223 Selective Precipitation of Gold Nanocrystals from Aqueous Solution Containing Platinum Ion Contaminants by Peptides

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In the last decade, the importance in recovery of precious metals from industrial liquid waste has been getting increased from the viewpoint of development of sustainable societies. One of the common processes for recovering precious metal ions from liquid waste would be extraction from aqueous phase to organic phase by organic capture agents specific for metal ions of interest. However, the process requires a large quantity of organic solvents and strong mechanical stirring, resulting in a high-energy consumption process.
Meanwhile, we are focusing on biomimetic precipitation of gold ions by reducing with peptides[1,2]. This method involves that peptides accommodate gold ions into the interior cavity of the self-assembled nanoarchitecture, followed by reduction to afford gold nanocrystals.

In this study, we examined to use the self-assembling peptides for selective metallic gold precipitation from an aqueous solution containing a mixture of $HAuCl_4$ and H_2PtCl_6. Formations of metallic nanoparticles were detected by UV-vis spectroscopy and elemental analysis of the precipitates were determined by EDS-FE-SEM. We found that the peptides precipitated gold nanocrystals more efficiently than platinum from a mixture of $HAuCl_4$ and H_2PtC_{l6}.

This study was financially supported in part by The Iwatani Naoji Foundation (Japan) and Joint Research Center for Science and Technology, Ryukoku University (Japan).

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YI-P224 N-terminal Macrocyclic Derivatives of Apelin-13

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The apelinergic system is an important actor in the regulation of both cardiovascular activity and energetic metabolism. The pathologic comorbidities in those two vital functions are widespread (hypertension — diabetes, ischemia — high LDL level), making this physiological system an attractive target for drug development.

To this aim, the C-terminus of the apelin peptide, a critical site for ligand functional efficacy, was modified extensively. However, the N-termini — a crucial part for affinity with increasing evidences about a metabolic action, is far less studied. In this project, we built a range of N-terminal apelinergic macrocycles with diverse types of linkers (amine, olefin, aromatic), based on pyr-apelin-13.

This chemical modifications allowed us to truncate 2 amino acids of N-termini (including the crucial residue Arg2) with less than 1-log loss in binding affinity for the APJ receptor, thus leading to the synthesis of shorter apelinergic analogues. As regards the signaling pathways, the majority of compounds showed a balanced profile between Gαi activation and β-arrestin-2 recruitment in BRET-based assays, indicating a minor implication of the N-terminal apelin fragment in those signal transduction pathways. With one exception, the macrocycle bearing an aromatic linker possessed a better efficacy in β-arrestin2 recruitment (EC50 = 37 nM vs 75 nM of pyr-apelin-13), which translated to a longer hypotension phase when administered intravenously to rats.

In conclusion, our study proposes a diverse macrocyclic pattern for the N-terminus of apelin. Because the length of the resulting peptides is shorter, in keeping the affinity and transduction profile, they represent an interesting tool to evaluate the role of full-length N-termini of apelin.

Acknowledgments: Programme canadien de bourses de la Francophonie (PCBF), Institut de Pharmacologie de Sherbrooke (IPS), Canadian Foundation of Innovation (CFI), Merck.

YI-P225 *In Vitro* Selection of Macrocyclic Peptides against THG-1 for Squamous Cell Carcinomas Therapeutic Lead Compounds

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Squamous cell carcinoma (SCC) accounts for a high-percentage of cases in many cancers including esophagus cancer (90% in Asia), cervical cancer (80-90%), and lung cancer (25-30%). There is continuing need for targeted therapy against SCC due to the patients' low 5-year survival rate. TSC Homologous Gene 1 (THG-1), according to our unpublished data, is a potential oncogene target for SCC treatment. Immunohistochemistry staining revealed that THG-1 was overexpressed in 92.6% (112/121), 80% (96/120) and

67.6% (23/34) of esophagus SCC, cervical SCC, and lung SCC specimens, respectively. Knockdown of THG-1 in SCC cell lines resulted in the reduction in cell proliferation, invasion and tumorigenesis. Through high-throughput proteomic analysis and *in vitro* experiments, we have evidence supporting the hypothesis that THG-1 promotes its oncogenic activity by means of protein-protein interactions (PPIs). THG-1 PPI antagonists will be helpful as lead compounds for drug development. Unfortunately, the majority of PPIs are considered "undruggable" by proteinaceous ligands due to technical difficulties in targeting intracellular molecules, specifically the large, relatively flat binding interfaces of the target proteins. Macrocyclic peptides have ideal size, affinity and specificity for targeting intracellular PPIs when compared to small molecules and antibodies. Using the Flexible *In vitro* Translation (FIT) system, we ribosomally incorporated a non-canonical amino acid to promote the macrocyclization of the peptides from a diverse peptide library. The Random non-standard -Peptide -Integrated Discovery (RaPID) system was applied for the selection of specific macrocyclic peptides as lead compounds against THG-1. Here, I discuss the importance of THG-1 in SCC, the generation of specific macrocyclic peptides against THG-1, and our on-going study of identified peptides as a lead compounds for disruption of THG-1 PPIs.

P226 Discovery of Privileged Peptide Sequences for the Wittig Reaction in Water by Selection on Genetically Encoded Peptide Libraries

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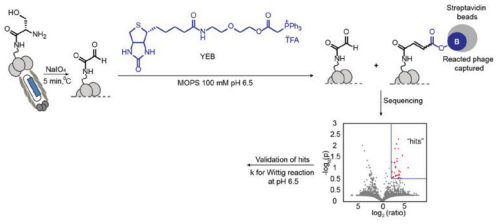
Genetically encoded peptide libraries are larger and easier to screen compared to classical combinatorial libraries; they are attractive for development of ligands in the pharmacological field,[1] as well as for discovery of catalytic peptides and chemical reactions. Peptide library displayed on phage is a powerful way to create and screen peptide libraries, since the genetic code of the phage allows easy determination of the DNA information that directly correlates to the displayed sequence.[2] We describe a method for selecting privileged substrates that undergo rapid Wittig reaction in water. A biotin tagged ylide reacts with orthogonal glyoxals generated after N-terminal serine oxidation of a population of 10^8 peptides displayed on phage. Capture and sequencing of biotinylated substrates stuck to beads yield a subpopulation of peptides with specific chemical features. These sequences are synthesized and rate constants are determined, demonstrating that they indeed undergo fast Wittig reaction in water. These results will allow us to get insight for the still debated mechanism of lithium salt-free Wittig reaction in water, as well as will confirm phage display as a valuable tool for selection of privileged substrates and evaluation of chemical reactivity.



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YI-P227 Crown Ether Peptides and Peptidomimetics: Scaffolds with Prospective Therapeutic Relevance

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Synthesis of bio-inspired molecules is a demanding area of bioorganic chemistry especially peptidomimetics and bioinspired nanoarchitectonics that have a potential involvement in therapeutics. Crown ether tethered peptidomimetics gives facilitation over others in metal interaction, which forms their therapeutic relevance. In this realm, we have synthesized, characterized and studied crown ether tethered molecules having pivotal importance. The crystal structure of dipeptide Boc-12-C-4-DOPA-Gly-OMe was studied by us in enantiomeric and racemic forms, which is the first crystal structure of any crown ring tethered peptide of any ring size. Two distinct crystal structures of this peptide were obtained where solvent played a crucial role in crystal packing. Further, biological studies were carried out with the chiral form of dipeptide that was potentially inducing cell cycle arrest and apoptosis in rat eggs in dose dependent manner. Moreover this dipeptide and some peptidomimetics have shown better inhibition activity against mushroom tyrosinase in comparison with kojic acid (reference compound).

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4. Patent filed–Title: TYROSINASE INHIBITORS Application No: EP15202739.7 (European Patent Office) Application Date: 24-Dec-2015.

P228 Rational Design of a Novel Thioester Equivalent N-Sulfanylethylaminooxybutyramide (SEAoxy)

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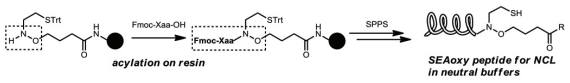
Preparation of a peptide-thioester or its equivalent is one of key points to succeed in native chemical ligation (NCL). Previously, we reported that the *N*-sulfanylethylanilide (SEAlide) peptide is an Fmoc-SPPS-compatible thioester equivalent that enables direct NCL under phosphate-buffered conditions via *in situ* intramolecular *N*-to-*S* acyl shift.[1,2] However, the poor nucleophilicity of an *N*-alkyl aniline amino group made direct SPPS of SEAlide peptides difficult. To overcome this disadvantage, here we report a novel crypto-thioester *N*-sulfanylethylaminooxybutyramide (SEAoxy) that can be synthesized by straightforward Fmoc-SPPS, including on-resin *N*-acylation of C-terminal amino acids. Moreover, SEAoxy peptide can be directly applied to NCL under neutral conditions. The method was exemplified by the syntheses of two bioactive peptides.[3]



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YI-P229 Exploration of Polo-Like Kinase 1 Inhibitors Targeting Intramolecular Protein-Protein Interaction

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Polo-like kinase 1 (Plk1) is a serine/threonine kinase that plays crucial roles in mitosis. Plk1 is overexpressed in many cancers and it is a clinically-relevant target for anti-cancer therapeutic development. A number of high affinity, ATP-competitive inhibitors of Plk1 have been reported that target the N-terminal kinase domain (KD).[1] However, structural similarities among ATP binding sites within the kinome make it challenging to develop selective kinase inhibitors. The Plk family is distinguished by the presence of unique C-terminal polo-box domains (PBDs), which recognize phosphoserine (pSer) and phosphothreonine (pThr) containing sequences, thereby serving to localize the enzyme to specific interaction sites. The PBD also modulates the kinase activity of Plk1 through intramolecular interactions with its KD. The majority of inhibitors designed to disrupt PBD-mediated protein-protein interactions derive much of their affinity from replicating aspects of phosphopeptide recognition within the canonical PBD. In this work, we have examined the effects of introducing additional interactions outside this region. This has resulted in the discovery of PBD-binding inhibitors, which can show up to two-orders of magnitude enhancement in their abilities to disrupt PBD-ligand interactions relative to current peptidomimetics.[2]

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P230 Phage Display and Selection of Lanthipeptides on the Carboxy-terminus of the Gene-3 Minor Coat Proteinn

Johannes H. Urban[1], Markus A. Moosmeier[1], Tobias Aumüller1, Marcus Thein[1], Tjibbe Bosma[2], Rick Rink[2], Katharina Groth[1], Moritz Zulley[1], Katja Siegers[1], Kathrin Tissot[1], Gert N. Moll[2], Josef Prassler1
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Ribosomally synthesized and post-translationally modified peptides (RiPPs) are an emerging class of natural products with drug-like properties and containing a high degree of chemical and structural diversity. To fully exploit the potential of RiPPs as peptide drug candidates, tools for their systematic engineering are required. Here we demonstrate the engineering of lanthipeptides, a subclass of RiPPs characterized by multiple thioether cycles that are enzymatically introduced in a regio- and stereospecific manner, by phage display. This was achieved by heterologous co-expression of linear lanthipeptide precursors fused to the widely neglected C-terminus of the bacteriophage M13 minor coat protein pIII, rather than the conventionally used N-terminus, along with the modifying enzymes from distantly related bacteria. Prior to phage assembly the precursor peptide fusions are translocated via the SEC pathway and span the bacterial inner membrane with the C-terminally fused peptide remaining in the cytoplasm. This ensures prolonged exposure of the precursor peptide to the modifying enzymatic machinery expressed in the cytoplasm of the producer cell, results in efficient post-translational peptide modification, and further enables the display of mature cyclic peptides on the phage surface. Biopanning of large C-terminal pIII display libraries readily identified artificial lanthipeptide ligands specific to urokinase plasminogen activator (uPA) and streptavidin.

YI-P231 Capturing Cysteine Proteases in Action using Novel Activity Based Probes

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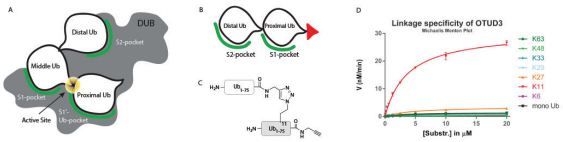

Ubiquitin (Ub), a 76 amino acid post-translational modifier, is at the centre of a large number of cellular processes. Ub can be coupled to another Ub molecule via any of its seven lysine residues to yield Ub chains. These differently linked poly-Ub chain types determine the outcome of specific Ub signalling. Deubiquitylating enzymes (DUBs) can reverse ubiquitylation by cleaving the iso-peptide bond between the C-terminal carboxylate of Ub and the substrate protein or next Ub module in poly-Ub chains. DUBs can have several modes of action, depending on the different types of binding pockets they possess guiding their Ub-linkage specific hydrolytic activity (fig. A). We were able to generate novel tools, based on synthetically prepared protease-resistant diUb molecules, that can specifically target more remotely situated binding pockets on DUBs (fig. B and C). These tools were used to study linkage-specific recognition of human OTU DUBs[1] (fig. D) as well as the SARS virus DUB PLpro[2].

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P232 Exploring Viral Proteins for Membrane-active Peptides

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Antimicrobial peptides (AMPs) and cell-penetrating peptides (CPPs) are membrane-active peptides, considered potential drug leads in the development of new antibacterial agents and drug delivery systems, respectively. Multifunctional proteins are abundant in viruses but have never been thoroughly screened for bioactive peptide sequences. Using bioinformatics tools we have evaluated the propensity of structural viral proteins, in a total of 272 proteins from 133 viruses, to comprise potential AMPs and CPPs sequences. Selected sequences, from a pool of potential AMPs and CPPs, were tested experimentally and validated the results, showing that viral proteins are a rich source for new bioactive peptide sequences. Given the proposed interplay between AMPs and CPPs activity, the antibacterial activity of selected viral protein-derived CPPs was evaluated against both Gram-positive and Gram-negative bacteria. Two viral CPPs, vCPP 0769 and vCPP 2319, displayed high antibacterial activity against all bacteria strains tested, proving to have a dual activity nature. The antibacterial mechanism of action of the two most active viral protein-derived peptides, vAMP 059 and vCPP 2319, was studied. Both peptides exhibit bactericidal activity against both Gram-positive S. aureus and Gram-negative *P. aeruginosa*, with bacterial cell death occurring within minutes. Additionally, both peptides induce bacterial membrane permeabilization and damage of the bacterial envelope on *P. aeruginosa* cells, which indicates that these peptides action is related to their ability to disrupt bacterial membranes.

YI-P233 Rational Design of Macrocyclic Peptides with Thioamide Modification Results in Super Active and Selective Integrin

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Thioamide is the strictest isostere of peptide bond where the carbonyl oxygen is replaced by a sulphur. However, this subtle modification is attributed to altered physico-chemical properties of the peptide. The utility of such 'backbone-modified' cyclic peptides as molecular scaffold has not been explored so far. For our studies, we selected cyclic penta- and hexa-alanine peptides template with fixed pattern of chirality. We observed that these mono thio-substituted cyclic peptides are conformationally homogeneous, display all-*trans* conformation with presence of classical β-II', γ, inverse γ and β-VI turns, suggesting the compatibility of thioamides with the standard turns. High-resolution solution structures of these thionated cyclic-ala peptides motivated us to employ these templates for spatial-screening of linear bioactive sequences. By rational design, we developed superactive small cyclic peptides as antagonists for the RGD-binding subfamily of integrins, a prominent class of cell adhesion receptors. We successfully developed integrin subtype selective thionated cyclic peptides that bind the αvβ3 and α5β1 integrins and show even higher selectivity and affinity than the drug cilengitide, which is an N-methylated analogue. Although N-alkylation represents a powerful tool to modulate the pharmacokinetic properties of cyclic peptide but the presence of *1,3*-pseudoallylic strain gives rise to *cis-trans* isomerism, and thus reduced population of the bioactive conformation. Contrary to that, thioamide modification imparts conformational rigidity and homogeneity to cyclic peptides due to enhanced *cis-trans* rotational barrier about the thioamide bond and owing to its compatibility.

YI-P234 Toward Solid Phase Peptide Fragment Ligation by Traceless-Ugi Multicomponent Reaction

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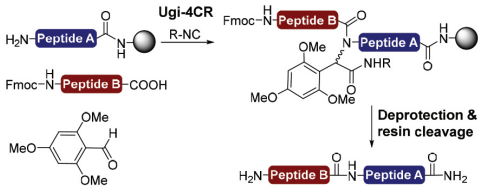
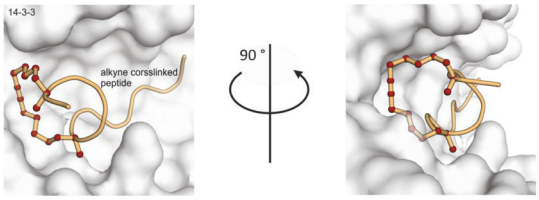
Recent progresses in ligation methodologies have allowed the convergent synthesis of several complex peptides and small proteins. However, most of these methods require special C- or N-terminal modifications, modified amino acids or a specific N-terminal residue at the ligation site. Based on a traceless Ugi multicomponent reaction strategy, we have developed a new method to couple peptide fragments on solid support. The approach uses a microwave-assisted on-resin Ugi four-component reaction to attach a carboxyl free peptide to a supported peptide bearing a free N-terminal amine via the formation of an Nprotected amide bond at the ligation site. Afterward, the generated backbone amide protecting group can be efficiently removed by microwave- assisted acidolysis with TFA to afford a fully deprotected peptide. The development of the traceless Ugi reaction approach and its use to prepare various oligopeptides will be presented.



YI-P235 Beyond Stapled Peptides – A New Approach for Constraining Peptide Secondary Structures through Hydrocarbon Crosslinks

K.Wallraven[a], P.M. Cromm[b], A. Fürsnter[c], T.N. Grossmann[a], *[a]VU University, Amsterdam, The Netherlands; [b]Max-Planck-Institute of Molecular Physiology, Dortmund, Germany; [c]Max-Planck-Institute for Coal Research, Mülheim/Ruhr, Germany*

Stapled peptides are well studied probes to elucidate biological systems. They comprise an α-methylated, hydrocarbon crosslink that stabilizes α-helical secondary structures. Such macrocyclized peptides are known to provide higher target affinities compared to their unmodified counterparts by reducing conformational flexibility. These peptides adopt the bioactive conformation already in their unbound state thereby reducing entropic penalty upon target engagement. Also, stapled peptides reveal increased proteolytic stability. Due to their excellent surface recognition properties, such modified peptides are expected to fill the gap between small molecule drugs and biologicals. Therefore, they are gaining increased interest as inhibitors of protein-protein interactions (PPIs).[1] However, peptide stapling is designed to stabilize α-helical structures only and thereby leaving a variety of secondary structures unaddressed. Although the crosslink aims for conformational restriction it is relatively flexible itself and remains unfunctionalized. Here we present a novel approach of ring-closing alkyne metathesis (RCAM) to constrain an irregular peptide secondary structure through an alkyne-containing hydrocarbon crosslink.[2] The alkyne moiety provides new steric and conformational features and serves as a platform for post-crosslinking functionalization.[3]



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YI-P236 Development of Potent Cyclic Peptide Inhibitors of PAD4 using the RaPID System

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[1]Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; [2]The Institute of Genetics and Molecular Medicine, The University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU, United Kingdom

PAD4, one of five members of the human peptidyl arginine deiminases, catalyses the deimination of peptidyl arginine to citrulline. It acts on numerous substrates including histones H1 and H3, influencing epigenetic gene regulation and pluripotency. Aberrant activity is implicated in many pathological conditions including cancers, atherosclerosis and inflammatory diseases, such as rheumatoid arthritis (RA) and systemic lupus. Recently, inhibition or deletion of PAD4 in mouse models has been demonstrated to reduce RA disease severity. As such it represents an important drug target.

Cyclic peptides represent an emerging class of natural product like drugs with diverse structures that can be easily adapted to target any protein of interest. To address the pharmaceutical need for potent and selective inhibitors of PAD4, we employed the RaPID system (Random non-standard Peptide Integrated Discovery), developed in the Suga laboratory. Through multiple round of enrichment using affinity panning with bead immobilised target protein, this system enables the isolation of potent binders of a protein of interest from a starting library of up to 10^{13} genetically-barcoded cyclic peptides. Using the RaPID system, we identified a series of nanomolar PAD4 binders whose inhibitory activity was characterised both i*n vitro* and in cells. The resulting peptides should provide powerful tools for further elucidation of the function of PAD4 in both normal development and disease and provide a basis for downstream drug development.

P237 ACE Inhibitory Activity of Protein Hydrolysates of Three Chinese Medicines

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Black soybean, sword bean and raw malt are traditional Chinese medicine with various physiological and biological activities. The proximal composition of these Chinese herbs was determined and the protein content of them accounted for 46.73 %, 31.95% and 10.27 %, respectively. Four different protein fractions were sequentially extracted according to the solubility and then were hydrolyzed by pepsin. The hydrolysate was passed through an ultrafiltration membrane to collect ultrafiltrate. Then the angiotensin I converting enzyme (ACE) inhibitory activity of the peptides was evaluated. Our result showed that peptides from different protein fraction indicated diverse inhibitory activity. In which, the raw malt albumin manifested the highest ACE inhibitory activity with inhibitory ratio of 34.24 ± 0.12 % followed by black soybean prolamin of 30.10 ± 0.09 %, at the final concentration of 0.01 mg/mL. On the contrary, peptides from raw malt glutelin enhanced the ACE activity by 10.50 %. The data strongly suggest that black soybean, sword bean and raw malt have potential, at some extant, to lower blood pressure. In additon, this study also provides useful guidace for the clinical use of these three Chinese herbs.

YI-P238 Application of β-Seleno-Leucine in One-pot Ligation-Deselenization Chemistry

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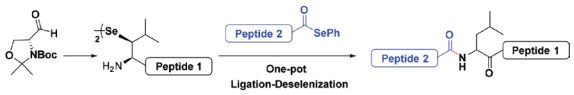

An efficient synthesis of a p-methoxybenzyl(PMB)-protected β-selenoleucine derivative from commercially available Garner's aldehyde is described as well as its incorporation into the N-termini of peptides via Fmoc-solid phase peptide synthesis (SPPS). Simple mixing of these selenopeptides with peptides containing C-terminal selenoester functionalities in aqueous buffer rapidly provides native amide bonds in 5 minutes to 3 hours in high yields without the use of any other reagents or additives.[1] Following the ligation event, an in situ deselenization rapidly affords native peptide product without any hydroxylation by-product. This method has been applied to synthesize an 83-mer human cytomegalovirus protein, UL22A, which selectively binds RANTES (regulated upon activation, normal T cell expressed and secreted) with high affinity.[2] This chemistry now serves as a promising new technology for the rapid one-pot synthesis of other natural proteins or biological and therapeutic interest.

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YI-P239 Functional Delivery of siRNA by Disulfide-Constrained Amphipathic Peptides

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Oligonucleotide agents that perturb expression of disease-related genes, including siRNA, have great potential for treatment of disease. Unfortunately, the promise of these agents is unrealized, in part, due to challenges with functional cellular delivery. We have shown that disulfide-constrained cyclic amphipathic peptides form a noncovalent complex with short-interfering RNA (siRNA), resulting in successful cytosolic delivery and knockdown of target gene products in both cell

culture and in vivo in mouse lung. Key design features which assist in functional delivery include reduction of the disulfide constraint upon cell entry and proteolytic clearance of the reduced peptide.

P240 Biosynthesis and Chemical Synthesis of Bioactive Peptides from Enthomopatogenic Bacteria

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To provide the symbiosis with their host nematodes, the enthomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* produce a broad range of natural products, including small molecular weight molecules and nonri-bosomal peptides.[1] One compound class are the bioactive and chemically diverse rhabdopeptides (RDPs),[2] which were originally identified in *X. nematophila*. However, RDPs or closely related compounds, like xenortides or mevalagmapeptide, occur exclusively in many *Photo-rhabdus* and *Xenorhabdus* strains. The structures of RPDs were identified from detailed MS^n fragmentation. Due to their interesting biosynthesis using a combination of iterative and flexible use of monomodular nonriboso-mal peptide synthetases and other mechanisms, a huge chemical diversity is created in single bacteria strains.[3] To obtain RDPs for bioactivity testing and additional structure confirmation, a chemical synthesis was devel-oped, combining backbone amide linking and on-resin methylation.

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YI-P241 Catching Proteases in the Act: Chemoselective Installation of Covalent Capture Probes in Deubiquitylase Targets

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Reversible protein modification by ubiquitin in humans is undertaken by over 600 ligases, and about 100 deubiquitylating enzymes, or DUBs. The dysregulation of protein ubiquitylation underlies diseases ranging from infection, inflammation, metabolic syndromes, neurodegenerative diseases, to cancers. Ubiquitin-targeted therapies benefit from knowledge of the specific disease-associated ligases and DUBs; however, the substrate-specificity of DUBs remains largely unknown. We have developed a chemical strategy to identify substrate-specific DUBs by the site-specific installation of a covalent capture probe in peptides derived from key targets of ubiquitin. The 21st amino acid, selenocysteine, was employed to ubiquitylate a peptide sequence from the ubiquitin ligase TRIM25, thereby enabling chemoselective conversion to the Michael-acceptor dehydroalanine (Dha) in the presence of multiple Cysteine residues (**Fig. 1**). The ubiquitylated TRIM25 probe effectively captured a DUB previously associated with TRIM25, thus validating our strategy. The development and application of our methodology will be discussed, and efforts toward expansion to full-length proteins will be presented.

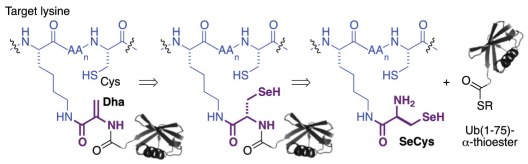

Figure 1. Scheme for the installation of dehydroalanine in targets of ubiquitin

YI-P242 Identification of Ice-binding Peptide Sequences from Genetically-encoded Phage Libraries

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Ice-binding peptides are small molecules capable of exerting control over ice nucleation. In this project, we aim to identify ice-nucleating glycopeptides. The identified peptides could be used in cryosurgery to nucleate ice in a spatially controlled manner.

Taking inspiration from an approach used in the literature for the purification of antifreeze proteins from a mixed solution, we developed a phage-display technique allowing for the selection of ice-binding peptides from a naïve library. In this method, a test tube chilled to -25°C is placed into a phage-containing solution. Ice builds around the test tube, integrating phage that display ice-binding peptides as it grows. Through multiple freezing rounds, we are able to narrow the selected population from 10^{12} pfu to 10^{2} pfu in five rounds. We present preliminary selection results from two different phage libraries. Here we demonstrate a selection method applicable in a challenging system where the target (ice) must be able to be distinguished from the surrounding liquid. In addition, we present a freezing platform capable of validating ice-nucleating properties. Our next steps will be to synthesize and evaluate the ice-nucleating properties of peptide hits to identify the peptides that exhibit a statistically significant influence on ice nucleation temperature.

YI-P243 Development of Cell-Permeable, Non-Helical Constrained Peptides to Target a Key Protein–Protein Interaction in Ovarian Cancer

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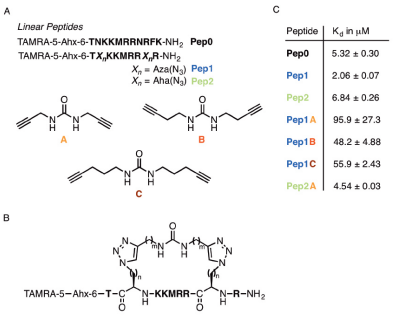
There is an urgent need for the development of novel therapeutics for ovarian clear cell carcinoma (CCC). The cancer is often resistant to platinum-based chemotherapy and there is a lack of current treatment options. An attractive target is transcription factor hepatocyte nuclear factor 1β (HNF1β), which is overexpressed in CCC. shRNA-mediated knockdown of

the target protein in five high- and low-HNF1β-expressing CCC lines has validated that HNF1β is a target for CCC. However, due to the high content of intrinsically disordered regions in HNF1β no drugs have been developed to date. The research approach adopted here was to target the transcription factor by nuclear import disruption. The nuclear localization sequence of HNF1β was verified by mutagenesis studies and was used as a starting point for inhibitor development. Guided by X-ray crystallographic data and molecular dynamics simulations,1 cell-permeable, non-helical constrained proteomimetics were designed and synthesized to target the HNF1β-Importin a protein-protein interaction.2 In this way, we developed the first reported series of constrained peptide nuclear import inhibitors and importantly this general approach may be extended to target other transcription factors.



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P244 Investigating the Unique Cysteine Framework found in Conotoxin α-SII

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Cysteine-rich α-conotoxins (α-CTs) targeting the nicotinic acetylcholine receptors (nAChRs) have been identified to be potential drug leads to treat chronic pain conditions. In contrast to most other α-CTs the peptide α-SII not only contains three disulfide bonds but also represents the sole member of cysteine framework II (CCC-C-C-C). To investigate the interplay of the structure and the pharmacological activity of α-SII, a high resolution 3D structure from solution NMR analysis revealed the presence of a characteristic 3_{10} helix known to have strong interactions with the ion channel. Furthermore, the additional disulfide bond was shown to connect the unusually extended termini. The affinity profile of α-SII was determined using electrophysiology, with the peptide showing a strong preference for neuromuscular nAChRs over neuronal subtypes. These studies will allow us to expand our knowledge about the interplay of the structure initiated by the cysteine framework and the pharmacological profile exhibited by α-CTs.

YI-P245 Development and Pharmacological Validation of Cyclic Peptides Synthesized using the Sonogashira Reaction

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Cyclization is commonly used in peptide drug design to increase the potency of the drug and help prevent enzymatic degradation of the peptide. Peptide cyclization usually occurs through disulfide and lactam bond formation. Both of these motifs are found naturally in peptides and proteins and may be broken down by enzymes, limiting the amount of active peptide to reach the site of action. Additionally, cyclic peptides are very flexible, allowing for many different conformations in solution. A cyclization site not recognized by enzymes that contains a more rigid structure may help "lock" the peptide into its bioactive conformation, increasing the potency and stability of the drug. The Sonogashira reaction, between an alkyne and an alkyl halide, creates a unique, rigid triple bond structure not found in biological systems. This study involves the development of an optimized method for Sonogashira cyclization in peptides using microwave-assisted solid-phase peptide synthesis (SPPS). Eighteen peptides were cyclized on resin using CuI and $Pd(PPh_3)_4$ in the presence of diethylamine and analyzed for cyclization efficiency by UPLC-MS. Dipeptide, tripeptide, tetrapeptide, hexapeptide, and octapeptide cyclizations containing a variety of amino acids and commonly used SPPS side chain protecting groups (*t*Bu, Boc, Pbf, and Trt) were tested. LC-MS analysis showed yields over 80% after the first run and single peaks after 4x20min reactions. To test the biological compatibility of the cyclization, peptide opioid agonists (TAPP, GATE3-8, DPDPE) and antagonists (CTP, CTOP, CTAP) were synthesized by the Sonogashira reaction, purified, and tested for biological compatibility with the opioid receptors.

YI-P246 Synthesis and Pharmacology of Dual Delta-kappa Opioid Receptor Peptide Agonists Featuring Multiple N-terminal Tyrosines

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Dual pharmacophore opioid peptides with high selectivity for the delta (DOR) and kappa (KOR) opioid receptors over the mu (MOR) receptors were designed by combining the pharmacophores of GATE3-8, a DOR-selective peptide, and dynorphin, a KOR-selective peptide. Recently, analgesic dual DOR-KOR agonists have been reported, which did not demonstrate reward or aversion in place conditioning studies, making them interesting leads in the treatment of cocaine and opiate addiction. We describe the synthesis and pharmacology of two series of peptide DOR-KOR dual agonists. The first series combined the pharmacophores of GATE3-8 and dynorphin with succinamic acid linkers of increasing length separating the pharmacophores; the general formula being Tyr-DAla-(pI)Phe-Glu-X-Leu-Arg-Arg-Ile-Arg-Pro-Lys-NH2, where X represents the distance between the N-terminal GATE3-8 pharmacophore and the C-terminal dynorphin pharmacophore. All analogs bound DOR and KOR with nanomolar affinities, and were selective

over MOR. The peptide with the longest linker showed no binding at the MOR, while maintaining nanomolar DOR and KOR binding. As linker length increased, the peptides bound KOR, though the strength decreased with. Opioid agonists require an N-terminal tyrosine for activity, so the effect of KOR affinity on proximity to the dynorphin pharmacophore was studied. This second group of peptides consists of GATE3-8 and dynorphin A combined into a single linear peptide with two N-termini. The effects on DOR and KOR affinity of multiple N-termini and their proximity to each pharmacophore were studied.

P247 Application of Phage Display for Zika Virus Immunogen Engineering

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Zika (ZIKV) and Dengue (DENV) viruses are mosquito-borne flaviviruses that circulate in similar geographical areas. ZIKV is linked to Guillian barré syndrome and microcephaly in babies born to ZIKV infected mothers and currently no approved vaccines for ZIKV exist. Recent studies demonstrated serological cross reactivity between ZIKV and DENV causing Antibody Dependent Enhancement of infection (ADE). ADE is a phenomenon where non-neutralizing antibodies increase viremia and pathology of disease by enabling antibody coated virus entry into FcΥ-receptor bearing cells. Antibodies mainly target the envelope (E) protein, which is important in viral entry. E has three domains DI, DII, and DIII. Antibodies against the lateral ridge epitope of ZIKV DIII are potently neutralizing and virus specific. A vaccine that focuses the humoral immune response toward the DIII lateral ridge could avoid cross reactive ADE. In order to focus the humoral immune response toward the ZIKV DIII lateral ridge, we are taking a phage display approach in which residues in non-neutralizing epitopes are masked by mutation in combinatorial libraries allowing limited variation. *In vitro* characterization is in progress.

P248 Highly Carbaborane-Loaded Neuropeptide Y Analoga as potential Boron Delivery Agents for Boron Neutron Capture Therapy

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Targeted delivery of drugs into tumor cells by a carrier system yields the opportunity to increase therapeutic efficacy and to minimize side effects. Recently, peptide ligands that target distinct G protein-coupled receptors (GPCRs) on the surface of cancer cells gained significant attention as carrier molecules for this purpose[1]. Interaction of a modified ligand with its receptor leads to cellular internalization of the peptide-receptor complex and thus enables a tumor-selective uptake of the peptide-attached cargo.

A promising target receptor for cancer therapy is the human Y_1 receptor (hY_1R), which is part of the four-membered Y receptor family in humans and is activated by the neuropeptide Y (NPY)[2]. The hY1R was found to be overexpressed in breast cancer cells, while in non-neoplastic breast tissue expression of the human Y_2 receptor (hY_2R) was observed[3]. Using a hY_1R-selective NPY analog[4] as carrier system is therefore suitable for breast cancer targeting[5,6].

Tumor-targeted drug delivery can be used in a binary radiation therapy such as boron neutron capture therapy (BNCT) to achieve a double-selective therapeutic effect. The principle of BNCT is the accumulation of a non-toxic boron compound in tumor cells, followed by local irradiation with slow (epi)thermal neutrons. Neutron capture of intracellular boron-10 generates excited boron-11, which decays into high energy particles that destroy the cancer cell from inside. For the routine use of BNCT, however, selective boron delivery agents with high accumulation in tumor cells, but a low uptake in healthy tissue, are urgently needed[7].

Here we describe the solid phase peptide synthesis of highly carbaborane-loaded, hY_1R-selective NPY conjugates as potential new boron compounds for BNCT. The suitability of the carbaborane-NPY conjugates as receptor-targeted boron carrier was evaluated in receptor activation and internalization studies.

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YI-P249 Precisely-Regulated and One-Pot Locking of Linear Peptides into Stable Multicyclic Topologies

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Structurally-constrained peptides have been considered as promising scaffolds for developing protein ligands and inhibitors. Compared to their linear counterparts, constrained peptides are usually much more resistant to enzymatic hydrolysis and exhibit enhanced binding affinity and specificity to target proteins. Here, we introduce the discovery of a small phenyl molecule with four isosteric thiolate-reactive groups of sequentially-varied reactivity (i.e., 2,3,5,6-tetrafluoroterephthalonitrile), and how this molecule was exploited in combination with cysteine/penicillamine thiolates of different nucleophilic reactivity for precisely-regulated and one-pot locking (**PROP**-Locking) of linear peptides into multicyclic topologies. The **PROP**-Locking relies on multistep and sequential thiolate/fluorine nucleophilic substitutions, which is not only rapid but highly specific, thus enabling rapid locking of peptides with high amino acid diversities without protecting groups. Several different tricyclic peptide templates were designed and synthesized via the **PROP**-Locking strategy. Moreover, tricyclic peptide structures constrained through stable thioether bonds, unlike their disulfide-bridged analogues, are tolerant to the highly reducing conditions of the cytoplasm; thus they should be more suitable to be exploited as structurally-constrained scaffolds for developing potential therapeutics and ligands for intracellular targets.

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YI-P250 A 31-residue Fragment of Tau Shows Prion-like Behavior in Cells

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The self-propagation of misfolded conformations of tau underlies neurodegenerative diseases, including Alzheimer's disease. There is considerable interest in discovering the minimal sequence and active conformational nucleus that defines this self-propagating event. The microtubule-binding region, spanning residues 244-372, reproduces much of the aggregation behavior of tau in cells and animal models. Further dissection of the amyloid-forming region to a hexapeptide from the third microtubule-binding repeat resulted in a peptide that rapidly forms fibrils *in vitro*. We show here that this peptide lacks the ability to seed aggregation of $tau_{244\text{-}372}$ in cells. However, as the hexapeptide is gradually extended to 31 residues, the peptides aggregate more slowly and gain potent activity to induce aggregation of $tau_{244\text{-}372}$ in cells. X-ray fiber diffraction, hydrogen-deuterium exchange and solids NMR studies map the beta-forming region to a 25-residue sequence. Thus, the nucleus for self-propagating aggregation of $tau2_{44\text{-}372}$ in cells is packaged in a remarkably small peptide.

YI-P251 Helix Nucleation by Constrained Capping Motifs

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Protein-protein interactions (PPIs) are involved in virtually all kinds of biological processes. The modulation of these interactions by synthetic molecules or biologics presents a strategy to study these interactions, as well as a way to treat a variety of diseases. One feature of PPIs is the large interface, which makes modulation by small molecules challenging. Peptides derived from fragments of the protein interface can be used for inhibiting these interactions. However, short peptides are less likely to adopt well-defined secondary structures, and they generally have poor pharmacological properties, such as *in-vivo* stability and cell permeability. These drawbacks have significantly limited the use of peptides as therapeutic agents. To overcome this issue, macrocyclization, such as peptide stapling, is used. However, this method leaves the two termini of the helices exposed, which decreases the stability and permeability. To address this issue, we designed head-to-tail cyclized "cap-strapped peptides" using optimized linkers that can couple both helix N- and C-capping motifs. This led to well-defined tertiary folding in a 14-residue peptide as characterized by CD spectroscopy, 2D NMR, hydrogen/deuterium (H/D) exchange, and X-ray crystallography. In addition, the T-jump IR study revealed unique folding/unfolding property of this type of constrained peptides.

YI-P252 Design of Self-Assembling Peptide Gels for 3D Cell Culture and Cell Delivery

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Extracellular matrices (ECMs) mainly interact with cells via integrin receptors and regulate cellular functions. Controlling integrin-matrix interactions is an essential design element in constructing novel biomaterials. Previously, we demonstrated that hydrogels prepared from positively charged self-assembling peptides can be used to encapsulate and deliver cells by syringe injection.[1,2] The positively charged peptides that comprise these gels interact with cell surface negatively charged glycoproteins, but not with integrins. In this study, we newly developed an integrin-binding peptide gel. We utilized negatively charged β-hairpin peptides to eliminate binding to cell surface glycoproteins. The designed process began with the peptide, AcVES3 (Ac-VEVSVSVEV^{D}PPTEVSVEVEV-NH_2), which has an overall charge state of -5 at neutral pH and forms rigid gels. The tetrapeptide (RGDV), which binds to the integrin αvβ3, was incorporated at the C-terminus to afford, AcVES3-RGDV. In 3D cell culture experiments, encapsulated fibroblasts developed extended pseudopodia and proliferated in the AcVES3-RGDV gels. In vivo experiments of cell delivery show that fibroblasts delivered with the peptide gel via syringe are retained in mouse subcutaneous tissue over 40 days and eventually engraft to the tissue. These results demonstrate that the combination of integrin-binding motifs and negatively charged β-hairpin peptide gels afford an injectable medium for 3D cell culture and cell delivery.

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P253 Operationally Convenient and Scalable Asymmetric Synthesis of (2*S*)- and (2*R*)-α-(Methyl) Dysteine Derivatives via Alkylation of Chiral Alanine Schiff Base Ni(II) Complexes

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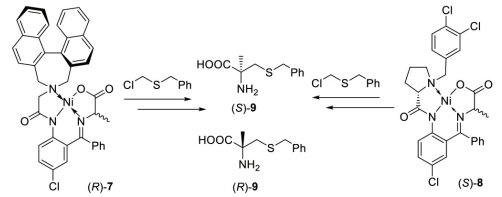
This research demonstrates that the methylation of N-benzyl cysteine Schiff bases derived Ni(II) complexes leads to the formation of the corresponding dehydroalanine containing products and cannot be used for preparation of the target α-(methyl)cysteine. In sharp contrast, the alternative strategy involving the thiomethylation of the Ni(II) complexes of alanine

Schiff bases, is viable and practically attractive approach affording the desired α-(methyl)cysteine containing derivatives. This work also reveals a significant, and rather unexpected, difference in the stereochemical performance of proline and 3,5-dihydro-4*H*-dinaphth[2,1-*c*:1',2'-*e*]azepine derived chiral ligands, showing a clear superiority of the former in terms of chemical yields and diastereoselectivity of the α-(methyl) cysteine products formation.



P254 Truncation of the Peptide Sequence in Designed Bifunctional Ligands for Next Generation Analgesics

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Current treatments of neuropathic pain generally only modulate the pain, tolerance occurs, severe toxicities appear, and treatment often leads to more pain. The mechanisms for these effects are still not clear. However, it is clear that prolonged pain states lead to neuroplastic changes. To address this problem we have taken a new approach to drug design for a novel type of analgesic. A designed bifunctional ligand, possessing both the NK1 antagonist pharmacophore and the opioid agonist pharmacophore could counteract these system changes to have significant analgesic efficacy without undesirable adverse effects. Our lead compound TY027 (Tyr-*D*Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-3',5'-Bzl(CF_3)$_2$) produced significant anti-nociception in naïve animals, and anti-hyperalgesia and anti-allodynia in nerve-injured animals, without showing anti-nociceptive tolerance when administered spinally and systemically.[1]

The short peptides have several advantages over long peptides for easier synthesis, lower preparative cost, and being the better template to be orally-available small molecule peptide mimetics. To this end the peptide sequence of TY027 was truncated based on our overlapping-pharmacophores concept. As a result, NP66 possessing only three amino acid residues showed balanced and potent opioid agonist as well as substance P antagonist activities in cell-based binding, cell-based functional and isolated tissue-based assays.[2, 3]

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YI-P255 Peptide Ligation on DNA Scaffold

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Peptide ligation reaction exemplified by native chemical ligation is an indispensable step in chemical protein synthesis, which enables site-specific incorporation of multiple unnatural amino-acids into proteins of interest. To date, various chemoselective and regioselective reactions that proceed in mild aqueous condition, have been developed. However, most of the reactions need high concentration of peptide substrates to compete hydrolysis of activated carboxlic acid. In order to improve the reaction efficiency of peptide ligation, we devised the use of adjuvant DNA as a scaffold in peptide ligation. Two complementary oligonucleotides were conjugated to amino-acids near the ligation site on two different peptide sequences via cleavable linker to generate two peptide-DNA conjugates. When these conjugates are mixed together, reaction sites should locate in close proximity by the formation of DNA duplex, which would improve the reaction rate and the selectivity. After ligating the peptide moiety, DNA was removed to obtain the ligated peptide product. We found that peptide ligation reaction was accelerated about 1000-fold on DNA scaffold. Furthermore, we investigated the effects of the peptide length between DNA duplex scaffold and the scope of the ligation for various amino acids. It is notable that our developing method has potential to extend available peptide substrates with low solubility by not only reducing peptide concentration in ligation reaction but also by accelerating peptide solubility by attaching hydrophilic DNA moiety.

P256 Ribosomal Synthesis of L-boronophenylalnine and L-carboranylalanine Containing Peptides

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Boron neutron capture therapy (BNCT) is a promising therapeutic technique for cancers by using the highly energetic $^4He^{2+}$ and $^7Li^{3+}$ arising from the thermal neutron capture by boron-10 (^{10}B). It can selectively treat cancers without damaging normal cells nearby, and has focused primarily on the treatment of high grade gliomas, recurrent cancers of the head and neck region, etc. The primary and most important factor for successful BNCT is the development of nontoxic boron delivery agents which can selectively target tumor cells and accumulate in sufficient amounts. Although considerable efforts have been made, only disodium mercaptododecaborate (BSH) and L-boronophenylalanine (L-BPA) are being used in clinical trials. However, they are not ideal ^{10}B carriers due to insufficient tumor selectivity and much faster clearance rate after injection. Therefore, novel and powerful ^{10}B delivery agents are in high demand. As L-BPA has a similar structure to tyrosine, we found that L-BPA could be charged onto tRNAs by flexizyme, and expressed by means of translation machinery under the reprogrammed genetic code. L-Carboranylalanine (L-CBA) has been reported as a bioisostere of L-phenylalanine, because the dimension of carborane in L-CBA is only slightly larger than the space occupied by a benzene ring rotating about its C(I)-C(4) axis. In this paper, we also synthesized L-CBA, and successfully incorporated it into peptides by flexible in vitro translation (FIT) system. This provide novel strategies for screening of boronated peptides as BNCT agents.

YI-P257 A Spiro-Piperidine Scaffold: New α-Helix Mimetics for Targeting Protein-Protein Interactions

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Protein-protein interactions (PPIs) have been widely recognized as a master regulator of many biological processes. Targeting PPIs using small molecules is a challenging approach to control such biological processes[1]. To the end, we developed new mimetics of α-helix containing spiro carbon atom. Spiro compounds are molecules containing two rings with just one shared carbon atom, and they have been widely used to stabilize ligand conformations due to structural rigidity. Spiro-rings fusion represents one of the potential tools to reduce the conformational entropy. In this work, we designed and synthesized α-helix mimetics bearing a spiro-piperidine moiety. Owing to its 3D properties, structural novelty, and rigidity, the spiro-piperidine scaffold appears to be promising for mimicking α-helices. Molecular modeling showed outstanding helix mimicry as the conformation of a spiro-piperidine superimposes well on an α-helix (RMSD = 0.9 Å) for the the side chains of amino acid residues at *i, i+4*, and *i+7* positions. The construction of these mimetics was split into two parts, and the spiro-rings fusion was carried out by a condensation reaction of the two monomers with a catalytic amount of iodine in ionic liquids[2]. Spiro-piperidine compounds were designed to mimic helical BH3 domains of pro-apoptotic Bcl-2 proteins and examined on their biological activities. Based on the results, the spiro-piperidine scaffold appears to be an attractive template for the development of small molecules to mimic α-helices.

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YI-P258 Triple-Tagged Peptides Targeting Intracellular Proteins

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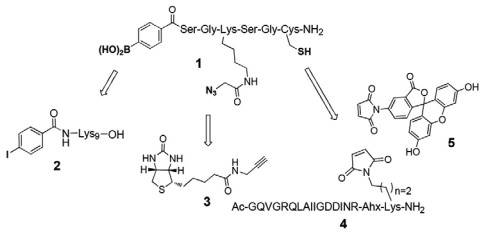
Tagging peptides with chemical labels have been widely used in biomedical applications. Peptides have been labeled, for example, with fluorescent dyes for imaging, biotinylation for affinity-based protein, and cell permeable peptides to access the intracellular targets. Numerous coupling methods have been reported, such as alkyne-azide cycloaddition (CuAAC), thiolmaleimide coupling, Staudinger ligation, and oxime/hydrazone formation[1]. However, the combination for orthogonal coupling reactions has not been well investigated, while each of these coupling reactions has been effectively used alone in peptide labeling. Herein, we report a triple combination of orthogonal coupling reactions: thiol-maleimide coupling, CuAAC, and Suzuki-Miyaura cross coupling. Suzuki-Miyaura coupling has been rarely performed in peptide labeling and we have optimized it for peptide ligation previously[2]. We designed and synthesized peptide **1** bearing three functional groups such as phenyl boronic acid, a sulphydryl group, and an azide. Labeling of peptide **1** was carried out with poly-lysine **2**, biotin **3** and BAK BH3 peptide **4** or fluorescein **5** via Suzuki-Miyaura cross-coupling, CuAAC, and thiol-maleimide methods, respectively. In order to evaluate the utility of this strategy, the multiply labeled peptide was incubated with cells and used to pull down Bcl-2 proteins that bind to the BAK peptide. These results suggest that peptides bearing such multiple labels may serve as powerful research tools for diverse biomedical studies.



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YI-P259 Discovery of Required Element on Human β-Defensins as Melanocortin Receptor Antagonists

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Melanocortin receptors are a family of G protein-coupled receptors that regulate many key physiological processes such as skin pigmentation, energy homeostasis, feeding behavior and sex function. The endogenous agonists (MSHs) and inverse agonists (AGRP, ASIP) have been extensively studied and used as templates for developing melanocortin receptor ligands. However, little is known about how the endogenous antagonists human β-Defensins interacts with melanocortin receptors. We have identified the key element on Human β-Defensin 1 that can selectively bind to melanocortin receptors. This element was determined as antagonist, with the ability to displace the agonist binding while not triggering cAMP production. Moreover, our docking results suggest that the binding happens on less conserved residues of the melanocortin receptors, which suggests that selectivity could be more easily achieved. Our discovery provides novel template for developing selective antagonists targeting melanocortin receptors.

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Bearfoot Icebar

SLCC Dinner

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