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Symposium Sponsors

















Dear Colleagues,

The Peptide Therapeutics Foundation and its sponsors welcome you to the 10th Annual Peptide Therapeutics Symposium. This annual forum's goal is to present new advances and discoveries in the field of peptide-based research and development. This year's symposium represents another cutting-edge, thought provoking program designed to stimulate questions and conversations.

The symposium opens on Thursday with two Keynote addresses. Roger Perlmutter, Executive Vice President and President of Merck Research Laboratories, will discuss "Building Better Medicines: Translational Research in the 21st Century." Phil Scherer will then present "An Integrated View of the Adipocyte: From Feast to Famine." A series of state-of-the-art talks on alternative methods for peptide drug delivery and what no doubt will be a lively panel discussion will follow. We encourage you to join us for the Opening Reception and poster viewing session that will complete the program on Thursday evening.

Friday morning we commence the day with Plenary Lectures from Benjamin Cravatt and Randy Seeley highlighting "Activity-based Proteomics – Applications for Enzyme and Inhibitor Discovery" and "Molecular Targets for the Effects of Bariatric Surgery on Obesity and Diabetes" respectively. The remainder of the day consists of in-depth sessions describing the development programs for peptide therapeutics and exciting new peptide methodologies.

As in previous years the program, the venue, and social time have been designed to support networking with colleagues. We are delighted to host this meeting and look forward to meeting each of you.

Sincerely,

Richard DiMarchi Chairman of the Board

TRUSI

Peptide Therapeutics Foundation

film / ·

Soumitra Ghosh President

Peptide Therapeutics Foundation

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Ipsen Biosciences Inc.
MedImmune
The PolyPeptide Group
Roche
Zealand Pharma
Zydus Cadila



Ferring Research Institute Inc.

Headquartered in San Diego, California, Ferring Research Institute Inc. (FRI) is the global peptide therapeutics research center for Ferring Pharmaceuticals. Ferring first established a research group in San Diego in 1996, recognizing the vibrant opportunities in the region. Since establishing a presence in San Diego, Ferring has been able to assemble a world-class peptide research organization and establish collaborations with leading academic scientists. FRI's state-of-the art facility houses research laboratories for peptide medicinal chemistry, biochemistry, bioanalytical, and pharmacology. The small group of researchers working in San Diego has now grown to a staff of more than 70. In recent years the group developed five clinical compounds that have reached human clinical trials. FRI is committed to building a portfolio of novel, innovative peptide therapeutics to address areas of high unmet medical need.

Ferring Pharmaceuticals (Ferring) is a private, research-driven specialty biopharmaceutical company active in global markets. The company identifies, develops and markets innovative products in the fields of endocrinology, gastroenterology, infertility, obstetrics, urology and osteoarthritis. In recent years Ferring has expanded beyond its traditional European base: with over 4,500 employees worldwide, it operates subsidiaries in over 50 countries and makes its products available in more than 90 countries. The company has emerged as a world leader with one of the largest peptide therapeutics portfolios in the industry. As part of its commitment to developing innovative products to treat diseases with high unmet medical need, Ferring invests heavily in its research infrastructure both in terms of people and technology.



Ipsen Biosciences Inc.

Ipsen (Euronext: IPN; ADR: IPSEY) is a global specialty-driven pharmaceutical company with total sales exceeding €1.1 billion in 2011. Ipsen's ambition is to become a leader in specialty healthcare solutions for targeted debilitating diseases. Its development strategy is supported by four franchises: neurology/Dysport®, endocrinology/Somatuline®, uro-oncology/ Decapeptyl® and hemophilia. Moreover, the Group has an active policy of partnerships. R&D is focused on innovative and differentiated technology-driven platforms, peptides and toxins. In 2011, R&D expenditure totaled more than €250 million, above 21% of Group sales. The Group has total worldwide staff of close to 4,500 employees.



MedImmune

MedImmune is the worldwide biologics research and development arm of AstraZeneca with its headquarters in Gaithersburg, Maryland (MD, USA) and large R&D sites in Cambridge (UK) and Mountain View (CA, USA). The company is pioneering innovative research and exploring novel pathways across key therapeutic areas, including respiratory, inflammation and autoimmunity; cardiovascular and metabolic disease; oncology; neuroscience; and infection and vaccines.

The company's robust pipeline includes over 120 biologic compounds in R&D, more than 30 in clinical stage development and several marketed products, Synagis® (palivizumab) and Fluenz® (live attenuated influenza vaccine, LAIV) and others. Peptide drugs are a significant part of both MedImmune's and AstraZeneca's marketed (Zoladex®, Byetta®, Bydureon®) and (pre-)clinical portfolio.



The PolyPeptide Group

The PolyPeptide Group is a privately held group of six companies that employs 420 staff worldwide. The PolyPeptide Group focuses exclusively on the manufacture of peptides and related substances and is a leading provider of custom and generic GMP-grade peptides for a range of pharmaceutical and biotechnology applications. With corporate roots that began in the 1950s, the Group was formally launched in 1996. Today, it operates a growing international network of peptide manufacturing facilities. Its world-class chemists and support personnel offer an unparalleled range of services for clients of every size and at every stage of product development. The PolyPeptide Group has been pre-approval inspected by the FDA over fifteen times as well as by other Regulatory Authorities. Altogether, the Group 25 approved APIs.More information about PolyPeptide Group is available at www. PolyPeptide.com.

In addition to large-scale GMP manufacturing, the PolyPeptide Group offers a wide range of other peptide services including radiolabelling, organic synthesis, cosmetic peptides and small-scale custom synthesis. It also has an extensive catalog of peptides and building blocks. The Group's customers range from emerging pharmaceutical companies and biotech organizations through to Big Pharma. The remaining business is primarily linked to the sale of peptide generics, including Calcitonin, Deslorelin, Gonadorelin, Leuprolide, Octreotide, hPTH (1-34), Somatostatin, Triptorelin and Arg-Vasopressin.



Roche

Headquartered in Basel, Switzerland, Roche is a leader in research-focused healthcare with combined strengths in pharmaceuticals and diagnostics. Roche is the world's largest biotech company with truly differentiated medicines in oncology, virology, inflammation, metabolism and CNS. Roche is also the world leader in in-vitro diagnostics, tissue-based cancer diagnostics and a pioneer in diabetes management. Roche's personalized healthcare strategy aims at providing medicines and diagnostic tools that enable tangible improvements in the health, quality of life and survival of patients. In 2011, Roche had over 80,000 employees worldwide and invested over 8 billion Swiss francs in R&D. The Group posted sales of 42.5 billion Swiss francs. Genentech, United States, is a wholly owned member of the Roche Group. Roche has a majority stake in Chugai Pharmaceutical, Japan. For more information: www.roche.com.



Zealand Pharma

Zealand Pharma A/S (NASDAQ OMX Copenhagen: ZEAL) ("Zealand") is a biotechnology company based in Copenhagen, Denmark. Zealand has world-leading competences in peptide drug innovation, design and development with its main therapeutic expertise in the field of cardio-metabolic diseases — diabetes and obesity in particular. The company has built a broad and mature pipeline of novel drug candidates, which have all been invented based on internal discovery activities. The first Zealand invented drug, Lyxumia® (lixisenatide), a once-daily prandial GLP-1 agonist, is marketed for the treatment of Type 2 diabetes under a global license agreement with Sanofi. Lyxumia® is approved in Europe (March 2013) as well as in Japan, Australia and Mexico, and under regulatory review in a large number of other countries globally, including in the US (NDA submission accepted in Feb 2013).

Zealand has a partnering strategy for the development and commercialization of its products and in addition to the collaboration with Sanofi in Type 2 diabetes, the company has partnerships with Boehringer Ingelheim in diabetes/obesity, Helsinn Healthcare in chemotherapy induced diarrhea and AbbVie in acute kidney injury.



Zydus Cadila

Zydus Cadila is an innovative global pharmaceutical company that discovers, develops, manufactures and markets a broad range of healthcare products. The group's operations range from API to formulations, animal health and wellness products. Headquartered in the city of Ahmedabad in India, the group has global operations in four continents spread across USA, Europe, Japan, Brazil, South Africa and 25 other emerging markets.

In its mission to create healthier communities globally, Zydus Cadila delivers wide ranging healthcare solutions and value to its customers. With over 15,000 employees worldwide, a world-class research and development centre dedicated to discovery research and nine state-of-the-art manufacturing plants, the group is dedicated to improving people's lives.

From a turnover of Rs. 250 crores in 1995, the group posted revenues of Rs. 5200 crores in FY12. The group had posted a turnover of Rs. 4600 crores in FY 11, making it a billion dollar company. The group aims to be a leading global healthcare provider with a robust product pipeline; achieve sales of over \$3 bn by 2015 and be a research-based pharmaceutical company by 2020.



PEPTIDE THERAPEUTICS FOUNDATION

2015 Travel Grant Awardees

Emel Adaligil, Tufts University

Carmine Pasquale Cerrato, Stockholm University

Sing Yan Er, University of Queensland

Alfredo Erazo-Oliveras, Texas A&M University

Luis Daniel Ferreira Vasconcelos, Stockholm University

Aswini Giri, University of Arizona

Shruthi Kini, Nanyang Technological University

Geeta Kumari, Nanyang Technological University

Kristina Najjar, Texas A&M University

Nir Qvit, Stanford University

Jonathan Rittichier, Indiana University

Peter 't Hart, Utrecht University

Wei Liang Tan, Nanyang Technological University

Ting-Yi Wang, Texas A&M University

Thursday, October 22, 2015

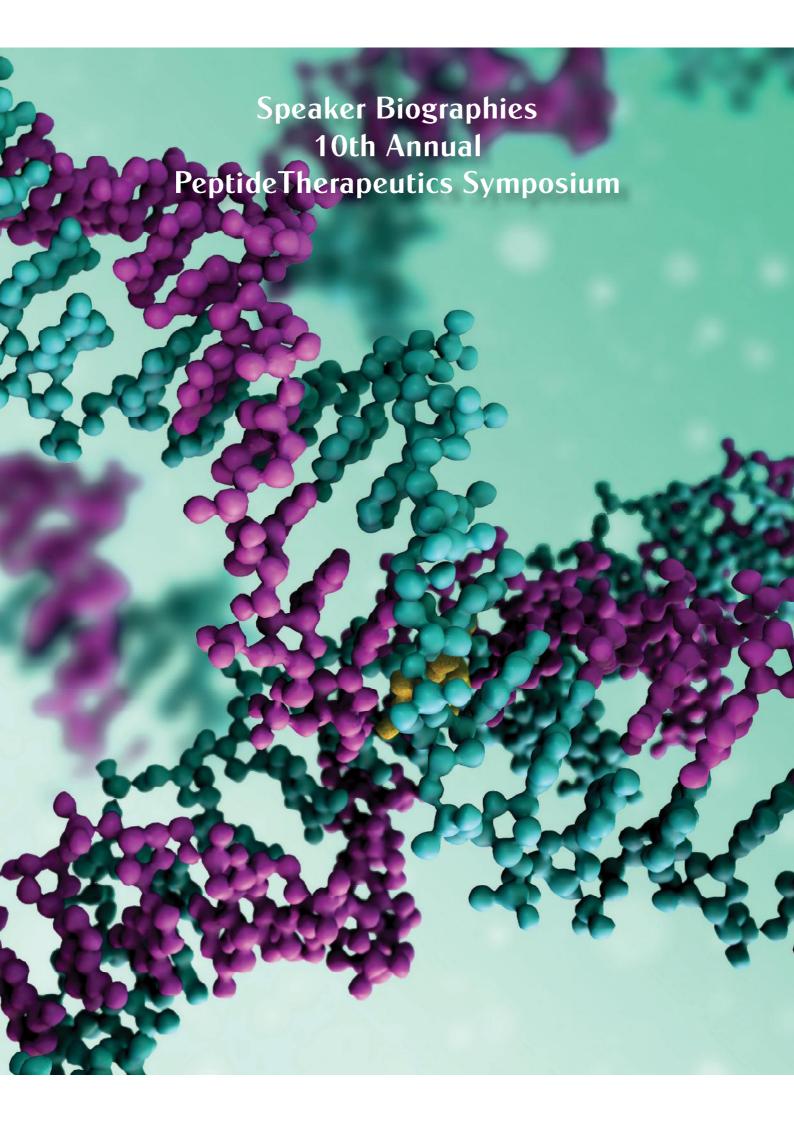
12:30 p.m. – 5:00 p.m.	Registration Check-in Fritz B. Burns Reception Center, Lower Level
1:30 p.m. – 6:00 p.m.	10th Annual Peptide Therapeutics Symposium Conrad T. Prebys Auditorium
1:30 p.m. – 1:40 p.m.	Opening Remarks Richard DiMarchi, Ph.D. Chairman of the Board, Peptide Therapeutics Foundation Standiford H. Cox Distinguished Professor of Chemistry, Jill & Jack Gill Chair in Biomolecular Sciences, Department of Chemistry, Indiana University
1:40 p.m. – 3:00 p.m.	Keynote Lectures Moderator Richard DiMarchi, Ph.D. Chairman of the Board, Peptide Therapeutics Foundation Standiford H. Cox Distinguished Professor of Chemistry, Jill & Jack Gill Chair in Biomolecular Sciences, Department of Chemistry, Indiana University
1:40 p.m. – 2:20 p.m.	Building Better Medicines: Translational Research in the 21st Century Roger M. Perlmutter, M.D., Ph.D. Executive Vice President and President, Merck Research Laboratories
2:20 p.m. – 3:00 p.m.	An Integrated View of the Adipocyte: From Feast to Famine Philipp E. Scherer, Ph.D. Professor, Department of Internal Medicine, Gifford O. Touchstone Jr. and Randolph G. Touchstone Distinguished Chair in Diabetes Research; Director, Touchstone Diabetes Center, The University of Texas Southwestern Medical Center
3:00 p.m. – 3:30 p.m.	Break Fritz B. Burns Reception Center, Lower Level
3:30 p.m. – 6:00 p.m.	Overview Drug Delivery Technologies and Panel Discussion Moderator Waleed Danho, Ph.D. Distinguished Research Leader (Retired), Hoffman-LaRoche, Inc.
3:30 p.m. – 3:55 p.m.	Oral Peptide Delivery: Deciphering the Reality from the Hype David J. Brayden, Ph.D. Professor of Advanced Drug Delivery, University College Dublin
3:55 p.m. – 4:20 p.m.	Oral Inhalation – A Simple Approach to Biologics Delivery John Freeman, M.S. Principal Scientist, MannKind Corporation
4:20 p.m. – 4:45 p.m.	ITCA 650 (Exenatide Delivered Continuously via a Matchstick-Size Subcutaneous Osmotic Mini-Pump) an Investigational Therapy for Type-II Diabetes Scott Peterson, Ph.D. Executive Director, Corporate Development, Intarcia Therapeutics, Inc.
4:45 p.m. – 5:10 p.m.	MICROCOR® Transdermal System: Technology Overview and Clinical Status Bobby Singh, Ph.D. Chief Technology Officer, Corium International, Inc.
5:10 p.m. – 6:00 p.m.	Panel Discussion Moderator Rodney Lax, Ph.D. Director, Peptide Therapeutics Foundation Business Development Consultant, The PolyPeptide Group
6:00 p.m. – 7:30 p.m.	Poster Session & Opening Reception Fritz B. Burns Reception Center, Lower Level

Friday, October 23, 2015

7:00 a.m. – 11:30 a.m. Registration Check-in Fritz B. Burns Reception Center, Lower Level 7:30 a.m. - 8:30 a.m. **Breakfast & Poster Viewing** Fritz B. Burns Reception Center, Lower Level 8:30 a.m. - 5:15 p.m. 10th Annual Peptide Therapeutics Symposium Conrad T. Prebys Auditorium 8:30 a.m. - 8:40 a.m. **Welcoming Remarks** Soumitra Ghosh, Ph.D. Director and President, Peptide Therapeutics Foundation President, Doon Associates LLC 8:40 a.m. - 10:00 a.m. **Plenary Lectures** Moderator Soumitra Ghosh, Ph.D. Director and President, Peptide Therapeutics Foundation President, Doon Associates LLC 8:40 a.m. - 9:20 a.m. Activity-based Proteomics - Applications for Enzyme and Inhibitor Discovery Benjamin F. Cravatt, Ph.D. Professor and Chair, Department of Chemical Physiology, The Scripps Research 9:20 a.m. - 10:00 a.m. Molecular Targets for the Effects of Bariatric Surgery on Obesity and Diabetes Randy J. Seeley, Ph.D. H.K. Ransom Professor of Surgery, University of Michigan 10:00 a.m. – 10:30 a.m. **Break & Poster Viewing** Fritz B. Burns Reception Center, Lower Level 10:30 a.m. - 12:00 p.m. Session I: Moderator Phil Dawson, Ph.D. Associate Professor, Associate Dean of Graduate Studies, Department of Chemistry, The Scripps Research Institute 10:30 a.m. - 11:00 a.m. Harnessing Chemistry and Glycoproteins to Discover Novel Targets for Cancer Therapy and Neurodegenerative Diseases Linda C. Hsieh-Wilson, Ph.D. Professor of Chemistry, Division of Chemistry and Chemical Engineering, California Institute of Technology 11:00 a.m. - 11:30 a.m. Engineered Cystine Knot Peptides: A New Class of Tumor Targeting Agents Jennifer R. Cochran, Ph.D. Associate Professor, Department of Bioengineering, and (by courtesy) Chemical Engineering, Stanford University 11:30 a.m. - 12:00 p.m. Phage Selection of Bicyclic Peptides for Application as Therapeutics Christian Heinis, Ph.D. Assistant Professor, École Polytechnique Fédérale de Lausanne (EPFL) 12:00 p.m. – 1:00 p.m. **Lunch & Poster Viewing**

Fritz B. Burns Reception Center, Lower Level

1:00 p.m. – 3:00 p.m.	Session II: Moderators Maria A. Bednarek, Ph.D. Director, Peptide Therapeutics Foundation Fellow and Head of the Peptide Platform, MedImmune/AstraZeneca
	James P. Tam, Ph.D. Professor, Herbalomics and Drug Discovery, School of Biological Sciences, Nanyang Technology University
1:00 p.m. – 1:30 p.m.	Kisspeptin and its Short-length Agonist Analogues: Discovery and Development Taiji Asami, Ph.D. Associate Director, Takeda Pharmaceutical Company Ltd.
1:30 p.m. – 2:00 p.m.	Phage-display Derived Peptidic Inhibitors of PCSK9 for LDL Cholesterol Lowering Daniel Kirchhofer, Ph.D. Principal Scientist, Department of Early Discovery Biochemistry, Genentech, Inc.
2:00 p.m. – 2:30 p.m.	Improved Chemical and Physical Stability in Liquid Formulations – How to Design Stable Glucagon Analogues Lise Giehm, Ph.D. Senior Scientist, Department of Pharmaceutical Development, Zealand Pharma A/S
2:30 p.m. – 3:00 p.m.	Selepressin, a Selective hV1a Agonist for the Treatment of Hypotension in Septic Shock Claudio D. Schteingart, Ph.D. Director, Peptide Therapeutics Foundation Vice President, Science & Technology – Research, Ferring Research Institute Inc.
3:00 p.m. – 3:30 p.m.	Break & Poster Viewing Fritz B. Burns Reception Center, Lower Level
3:30 p.m. – 5:00 p.m.	Session III: Moderator Abhijit Bhat, Ph.D. Director, Peptide Therapeutics Foundation Vice President, Chemistry, Ipsen Bioscience Inc.
3:30 p.m. – 4:00 p.m.	Addressing the Challenges with the Discovery and Development of Peptide Therapeutics Paul L. Feldman, Ph.D. Head of Discovery and Translational Medicine, Intarcia Therapeutics Inc. Former CEO & Co-founder, Phoundry Pharmaceuticalss
4:00 p.m. – 4:30 p.m.	NA-1: From Bench to Bedside – A Novel Peptide Therapeutic for the Treatment of Acute Stroke Dave Garman, Ph.D. Technology Officer, NoNO Inc.
4:30 p.m. – 5:00 p.m.	Relamorelin, a Ghrelin Agonist for the Treatment of Diverse Functional Gastrointestinal Disorders Lex H.T. Van der Ploeg, Ph.D. Chief Scientific Officer, Rhythm Pharmaceuticals
5:00 p.m. – 5:15 p.m.	Closing Remarks Adrienne Day, Ph.D. Secretary and Treasurer, Peptide Therapeutics Foundation Senior Director, Business Development, Ferring Research Institute Inc.
5:15 p.m. – 6:30 p.m.	Networking Reception Fritz B. Burns Reception Center, Lower Level





Taiji Asami, Ph.D. I Associate Director, Takeda Pharmaceutical Company Ltd.

Kisspeptin and its Short-length Agonist Analogues: Discovery and Development

Taiji Asami, Ph.D. is in responsible for all of the fundamental research and applications relating to peptide chemistry at Takeda Pharmaceutical Company Limited. He received his B.S., M.S. and Ph.D. degrees in medicinal chemistry from the University of Tokyo, Japan in 1988, 1990 and 2015, respectively. He joined Takeda in 1990 and has over 20 years' experience in the field of peptide science including basic research and peptide therapeutics. He has applied about 20 patents for peptide therapeutics, e.g., kisspeptin analogues. He is currently a Councilor of The Japanese Peptide Society and a Councilor of Division of Biofunctional Chemistry of The Chemical Society of Japan.



David J. Brayden, Ph.D. I Professor of Advanced Drug Delivery, University College Dublin

Oral Peptide Delivery: Deciphering the Reality from the Hype

David Brayden is Professor of Advanced Drug Delivery at the School of Veterinary Medicine, University College Dublin (UCD) and also a Fellow of the UCD Conway Institute. Following a Ph.D. in Pharmacology at the University of Cambridge, UK (1989), and a post-doctoral research fellowship at Stanford University, he set up Elan Corporation's pharmacology laboratory in Dublin (1991). At Elan, he became a senior scientist and project manager of several of Elan's Joint-Venture drug delivery research collaborations with US biotech companies. In 2001, he joined UCD as a lecturer in veterinary pharmacology and was appointed Associate Professor in 2006 and Full Professor in 2014. He was Director of the Science Foundation Ireland (SFI) Research Cluster (The Irish Drug Delivery Research Network) from 2007-2013, is Deputy Coordinator of an FP7 Consortium on oral peptides in nanoparticles ("TRANS-INT", 2012-2017), and is a Co-PI in "CURAM", SFI's new Centre for Medical Devices (2014-2020). He is the author or co-author of more than 200 research publications and patents. Professor Brayden serves on the Editorial Advisory Boards of Drug Discovery Today, European Journal of Pharmaceutical Sciences, Advanced Drug Delivery Reviews and the Journal of Veterinary Pharmacology and Therapeutics. In 2010 he became an Associate Editor of Therapeutic Delivery. Professor Brayden works as an independent consultant for drug delivery companies. He was made a Fellow of the Controlled Release Society in 2012.



Jennifer R. Cochran, Ph.D. | Associate Professor, Department of Bioengineering, and (by courtesy) Chemical Engineering, Stanford University

Engineered Cystine Knot Peptides: A New Class of Tumor Targeting Agents

Jennifer Cochran, Ph.D., is the Hitachi America Faculty Scholar Associate Professor of Bioengineering and (by courtesy) Chemical Engineering at Stanford University. She is also a member of the Stanford Biophysics and Cancer Biology graduate programs, and is currently the Director of Graduate Studies in Bioengineering. Dr. Cochran has over 14 years of experience in protein and peptide-based drug discovery and development for applications in regenerative medicine, ocular disease, and cancer imaging and therapy. Several of these designer proteins are at various stages of commercialization and clinical translation. Dr. Cochran obtained her Ph.D. in Biological Chemistry from the Massachusetts Institute of Technology, where she also completed a postdoctoral fellowship in Biological Engineering. She has received the National Cancer Institute Howard Temin Award, the Martin D. Abeloff Scholar Award from the V Foundation for Cancer Research, an American Cancer Society Research Scholar Award, and a Sidney Kimmel Scholar Award. She was also named the 47th Mallinckrodt Faculty Scholar from the Edward Mallinckrodt Jr. Foundation.



Benjamin F. Cravatt, Ph.D. I Professor and Chair, Department of Chemical Physiology, The Scripps Research Institute

Activity-based Proteomics - Applications for Enzyme and Inhibitor Discovery

Dr. Cravatt is a Professor in the Skaggs Institute for Chemical Biology and Chair of the Department of Chemical Physiology at The Scripps Research Institute. His research group is interested in understanding the roles that enzymes play in physiological and pathological processes, especially as pertains to the nervous system and cancer. Dr. Cravatt obtained his undergraduate education at Stanford University, receiving a B.S. in the Biological Sciences and a B.A. in History. He then trained with Drs. Dale Boger and Richard Lerner and received a Ph.D. in Macromolecular and Cellular Structure and Chemistry from The Scripps Research Institute (TSRI) in 1996. Professor Cravatt joined the faculty at TSRI in 1997 as a member of the Skaggs Institute for Chemical Biology and the departments of Cell Biology and Chemistry. Dr. Cravatt is a co-founder and scientific advisor of Activx Biosciences and Abide Therapeutics. His honors include a Searle Scholar Award (1998-2001), the Eli Lilly Award in Biological Chemistry (2004), a Cope Scholar Award (2005), the Irving Sigal Young Investigator Award (2007), the Tetrahedron Young Investigator Award in Bioorganic and Medicinal Chemistry (2008), a MERIT award from the National Cancer Institute (2009), and membership in the National Academy of Sciences (2014).



Adrienne Day, Ph.D. I Secretary and Treasurer, Peptide Therapeutics Foundation Senior Director, Business Development, Ferring Research Institute

Closing Remarks

Dr. Adrienne Day is the Senior Director of Business Development for Ferring Research Institute Inc. She has more than 20 years of experience in the biotechnology and biopharmaceutical industries, and has worked in the non-profit, for-profit and startup environments.

Prior to joining Ferring Dr. Day ran a successful consulting practice. She has previously served as Vice President of Business Development at what is now the Sanford Burnham Prebys Medical Discovery Institute, Vice President of Business Development Conforma Therapeutics, Senior Director of Business Development at Molecumetics Ltd., Associate Director of Corporate Development at Ligand Pharmaceuticals. She was Ligand Pharmaceuticals' first Project Manager, and began her biotechnology career at Invitrogen Corporation where she held various positions.

Dr. Day received her B.Sc., B.Sc. Honors, and Ph.D. degrees in Biochemistry from the University of Adelaide, Australia. She completed her postdoctoral training at the University of Southern California with Dr. Amy Lee and at the La Jolla Cancer Research Center in the laboratory of Dr. Eva Engvall.



Richard DiMarchi, Ph.D. I Chairman of the Board, Peptide Therapeutics Foundation Standiford H. Cox Distinguished Professor of Chemistry, Jill & Jack Gill Chair in Biomolecular Sciences, Department of Chemistry, Indiana University

Opening Remarks

Dr. DiMarchi's contributions in peptide & protein sciences consists of three decades of work in academia, the pharmaceutical industry and biotechnology companies. He is the Cox Distinguished Professor of Biochemistry and *Gill* Chair in Biomolecular Sciences at Indiana University. He is a co-founder of Ambrx, Inc., Marcadia Biotech, Assembly, Calibrium and MB2 Biotech. He has served as a scientific advisor to multiple pharmaceutical companies and three venture funds; 5AM, TMP, and Twilight. He is Chairman of the Peptide Therapeutics Foundation and external board member at Assembly Biosciences and On-Target Therapeutics.

Dr. DiMarchi is a retired Group Vice President at Eli Lilly & Company where for more than two decades he provided leadership in biotechnology, endocrine research and product development. He is readily recognized for discovery and development of rDNAderived Humalog® (LisPro-human insulin). As scientist and executive, Dr. DiMarchi also significantly contributed to the commercial development of Humulin®, Humatrope®, rGlucagon®, Evista®, and Forteo®. His current research is focused on developing macromolecules with enhanced therapeutic properties through biochemical and chemical optimization, an approach he has termed chemical-biotechnology.

Dr. DiMarchi is the recipient of numerous awards including the 2005 AAPS Career Research Achievement Award in Biotechnology, the 2006 ACS Barnes Award for Leadership in Chemical Research Management, the 2006 ACS Esselen Award for Chemistry in the Service of Public Interest, the 2007 Carothers Award for Excellence in Polymer Sciences, the 2009 Watanabe Award for Life Sciences Research, the 2011 Merrifield Award for Career Contributions in Peptide Sciences, the 2012 Phillip Nelson Innovation Award, the 2014 Erwin Schrödinger-Preis. He is a 2014 inductee to the National Inventors Hall of Fame, a 2015 awardee of the Meienhofer Award, and the Max Bergmann Medaille. He will receive the 2016 ACS Alfred Burger Award in Medicinal Chemistry.



Paul L. Feldman, Ph.D. I Head of Discovery and Translational Medicine, Intarcia Therapeutics Inc.; Former Chief Executive Officer, Co-founder, Phoundry Pharmaceuticals

Addressing the Challenges with the Discovery and Development of Peptide Therapeutics

Paul L. Feldman joined Glaxo Pharmaceuticals, Research Triangle Park, North Carolina in 1987 following receiving his Ph.D. from the University of California, Berkeley. From 1987-1995 Paul worked on a variety of drug discovery programs one of which led to the discovery of the marketed ultra short-acting analgesic opioid agonist remifentanil (Ultiva). In addition, he and academic collaborators worked on the biochemistry of nitric oxide production and first demonstrated that N-hydroxyarginine is an intermediate in the biosynthesis. From 1995-2000 Paul's department worked on the discovery of antiviral agents for treatment of HIV and HSV. One of the highlights during this period was the discovery of the marketed HIV protease inhibitor, fosamprenavir (Lexiva/Telzir). In 2000 Paul became Vice President of Chemistry for the Metabolic and Viral Diseases for GlaxoSmithKline (GSK) Pharmaceuticals. His group discovered two assets, the HIV integrase inhibitor dolutegravir (Tivicay) approved in 2013, and the phase 3 ultra short-acting benzodiazepine, remimazolam (outlicensed). In 2010 Paul was named Senior Vice President and his responsibilities included leading the Enteroendocrine Discovery Performance Unit, part of GSK's Metabolic Pathways Cardiovascular Unit, and leader of GSK's R&D Medicinal Chemistry Center of Excellence. The Enteroendocrine Unit focused on the discovery and early stage development of optimized combination peptide hormones, luminally restricted small molecules, and GRAS potentiators to treat diabetes and obesity. Several of these agents advanced into phase 2 clinical studies. In early 2015 Paul left GSK and co-founded Phoundry Pharmaceuticals, Inc., a biotechnology company focused on discovering peptide hormone therapeutics, where he is the CEO. Later in 2015 Phoundry was purchased by Intarcia Therapeutics, Inc. and Paul is currently Head of Discovery and Translational Medicine and a member of the Executive Leadership Team for Intarcia.

Paul has served as an adjunct Professor of Chemistry, Duke and North Carolina State Universities, chair of Heterocyclic Compounds Gordon Research Conference (2000), member at large (2000-2003) and alternate councilor (2012-2014) to the Executive Committee in the Division of Organic Chemistry of the American Chemical Society, and chair of the Division of Organic Chemistry Fellowship Evaluation Committee (2003). In 2014 Paul received the North Carolina Section of the American Chemical Society's Distinguished Lecturer award. Paul is currently on the editorial board for Organic Reactions. Paul has >50 journal publications and >40 invited lectures.



John Freeman, M.S. I Principal Scientist, MannKind Corporation
Oral Inhalation – A Simple Approach to Biologics Delivery

John Freeman earned his M.S. in chemistry from Polytechnic University. He has over 20 years of experience in the pharmaceutical industry which started at Emisphere Technologies, where he designed and synthesized small molecules for use as oral drug delivery carriers. He joined MannKind Corporation in 2003 and is currently a principal scientist in the R&D department at MannKind. In this role, he is responsible for conducting synthetic chemistry, manufacturing process development, and formulations development work that support MannKind's inhaled insulin, AFREZZA®, its product line extensions, and new products based on MannKind's Technosphere® oral inhalation platform.



Dave Garman, Ph.D. I Technology Officer, NoNO Inc.

NA-1: From Bench to Bedside - A Novel Peptide Therapeutic for the Treatment of Acute Stroke

Dave Garman is currently the Technical Officer for NoNO Inc, a biotechnology company directed at the development of novel peptide therapeutics to treat neurological disorders with unmet medical needs. His roles have encompassed most aspects of the development of a peptide therapeutic for the treatment of stroke from initial animal results in the laboratory through Phase 3 clinical trials. Dr. Garman's primary responsibilities include all aspects of manufacturing and regulatory interactions related to CMC, preclinical safety pharmacology and toxicity assessment of peptide therapeutics, intellectual property development and the development of both next generation and novel peptide therapeutics for the company pipeline.

Prior to joining NoNO Inc, Dr. Garman worked for 11 years at Arbor Vita Corporation, where he held roles from Scientist through Vice President, Business Development. His primary roles included working with the Arbor Vita team on the development of a medium throughput peptide:protein interaction platform to examine the selectively of peptide inhibitors. He also worked on the development of specific peptides for both clinical diagnostic and therapeutic uses.

Dr. Garman studied biochemistry and genetics at Carnegie Mellon before completing his Ph.D. in Developmental Biology from Stanford University. His work with peptides has spanned from early days of developing fluorescent peptide cleavage assays as a tool to study cellular enzymes through to the late stage clinical trials of peptide therapeutics. Dr. Garman is an inventor on over 30 issued patents and has more than 70 patents pending.



Soumitra Ghosh, Ph.D. I Director and President, Peptide Therapeutics Foundation President, Doon Associates LLC

Welcoming Remarks

Soumitra Ghosh is a biotechnology industry consultant and entrepreneur with extensive experience in drug discovery and drug development. His expertise is in formulating R&D strategy, building and directing research programs, technology licensing, developing partnering collaborations, and in managing intellectual property portfolios. His other current roles are acting CSO, Avexegen Therapeutics, Inc. and President of the Peptide Therapeutics Foundation. His biopharmaceutical experience includes R&D leadership positions at Amylin Pharmaceuticals and MitoKor. At these companies, he led multi-disciplinary research teams for the development of small molecule, peptide and protein based drug candidates for the treatment of metabolic diseases and CNS disorders. Several drug candidates were advanced to the clinic, or were partnered with companies for clinical development, and multiple patents and research publications resulted from the work. At Amylin, he also oversaw the research effort to support the programs for pramlintide (Symlin™), exenatide (Byetta™, Bydureon™) and metreleptin (MyaleptTM), first-in-class peptide therapeutics for the treatment of metabolic disorders. His work experience also includes development of DNA-based diagnostic tools and zinc peptidase inhibitors at Baxter Diagnostics, Inc. and at the Salk Institute Biotechnology/Industrials Associates (SIBIA). He received his MS and Ph.D. degrees in Chemistry from the Indian Institute of Technology and the University of Chicago, and conducted his post-doctoral research at the Rockefeller University in New York.



Lise Giehm, Ph.D. I Senior Scientist, Department of Pharmaceutical Development, Zealand Pharma A/S

Improved Chemical and Physical Stability in Liquid Formulations – How to Design Stable Glucagon Analogues

Lise Giehm holds a Ph.D. in protein biophysics from Aarhus University in Denmark with a strong focus on the molecular mechanisms underlying how protein and peptides form aggregates and amyloid-like structures.

For more than 10 years, it has been the major focus of Lise Giehm's academic research and industrial work to understand and prevent aggregation of proteins and peptides. Her academic work has focused on amyloid deposit diseases such as Alzheimer and Parkinson's. This has lead to discovering new structural features of the different species formed in the fibrillation process, and resolving the structure of an important oligomer believed to play a significant role in Parkinson's disease.

In her industrial work, Lise Giehm is a senior scientist in the department of pharmaceutical development at Zealand Pharma A/S, Denmark. Lise Giehm's primary focus has been to develop new innovative methods and technologies to evaluate liquid formulations and increase the screening throughput. This has included setting up external research collaborations as well as establishing state-of the art screening capabilities at Zealand Pharma A/S. In addition, design of peptides to increase their physicochemical properties in order to ease formulation development has likewise been a major focus.



Christian Heinis, Ph.D. I Assistant Professor, École Polytechnique Fédérale de Lausanne (EPFL)

Phage Selection of Bicyclic Peptides for Application as Therapeutics

Christian Heinis studied biochemistry at the Swiss Federal Institute of Technology in Zurich (ETHZ) where he also did a Ph.D. in the research group of Prof. Dr. Dario Neri. He was a postdoctoral fellow in the group of Prof. Dr. Kai Johnsson at the École Polytechnique Fédéral de Lausanne (EPFL) and in the group of Sir Gregory Winter at the Laboratory of Molecular Biology (LMB) in Cambridge, UK. In 2008 he was appointed assistant professor with tenure track for bioorganic chemistry at EPFL (SNSF professorship). The general research interest of Christian Heinis is the development of novel and innovative therapeutic strategies using chemical and biological methodologies. Christian is a scientific co-founder of the start-up company Bicycle Therapeutics.



Linda C. Hsieh-Wilson, Ph.D. I Professor of Chemistry, Division of Chemistry and Chemical Engineering, California Institute of Technology

Harnessing Chemistry and Glycoproteins to Discover Novel Targets for Cancer Therapy and Neurodegenerative Diseases

Dr. Linda Hsieh-Wilson is a Professor of Chemistry at the California Institute of Technology. She was born in New York City and was awarded her B.S. degree magna cum laude in chemistry from Yale University in 1990. In 1996, she received her Ph.D. in chemistry from the University of California at Berkeley, where she was a National Science Foundation predoctoral fellow in the laboratory of Professor Peter Schultz. In 1996, she moved to Rockefeller University to study neurobiology with Professor and Nobel Laureate Paul Greengard as a Damon Runyon-Walter Winchell postdoctoral fellow. Hsieh-Wilson joined the faculty at the California Institute of Technology in 2000, where she became an associate professor of chemistry in 2006 and full professor in 2010. In 2005, she was appointed an Investigator of the Howard Hughes Medical Institute.

Professor Hsieh-Wilson has pioneered the application of organic chemistry to probe the roles of carbohydrates and protein glycosylation in neurobiology and cancer. Her honors include the Beckman Young Investigator Award (2000), the Research Corporation Research Innovation Award (2000), an Alfred P. Sloan Fellowship (2003), the Eli Lilly Award in Biological Chemistry (2006), the Arthur C. Cope Scholar Award (2008), the Gill Young Investigator Award in Neuroscience (2009), and the Horace S. Isbell Award in Carbohydrate Chemistry (2014). In 2015, she was elected to the Academy of Arts and Sciences.



Daniel Kirchhofer, Ph.D. I Principal Scientist, Department of Early Discovery Biochemistry, Genentech, Inc.

Phage-display Derived Peptidic Inhibitors of PCSK9 for LDL Cholesterol Lowering

Currently, Dr. Kirchhofer is a project team leader and head of a research lab with emphasis on protein chemistry to develop new therapies for dyslipidemia and immune disorders. Genentech researches protein-based inhibitors (antibodies, Kunitz domains), peptides and small molecules to target specific disease pathways, including cancer. Previously, he was project leader in the cardiovascular research department at F.Hoffmann-La Roche, Switzerland, developing inhibitors of coagulation factors (Tissue Factor:Factor VIIa) for the prevention of myocardial infarction and thromboembolism.



Roger M. Perlmutter, M.D., Ph.D. I Executive Vice President and President, Merck Research Laboratories

Building Better Medicines: Translational Research in the 21st Century

Roger M. Perlmutter, M.D., Ph.D., is executive vice president and president of Merck Research Laboratories, Merck's global R&D organization. Before joining Merck, Dr. Perlmutter was executive vice president and head of R&D at Amgen, from January 2001 to February 2012. During his tenure, he oversaw the development and subsequent approval of a number of novel biologic and small molecule medicines in the areas of cancer, endocrinology, hematology, inflammation and osteoporosis.

Prior to joining Amgen, Dr. Perlmutter worked at Merck Research Laboratories and held roles of increasing responsibility from 1997 to 2001, culminating in his appointment to executive vice president of Worldwide Basic Research and Preclinical Development. He joined Merck as a senior vice president in February 1997. More recently, he served as a director of several biotechnology companies and was a science partner at The Column Group, a biotechnology-focused venture capital firm.

Before assuming leadership roles in industry, Dr. Perlmutter was a professor in the Departments of Immunology, Biochemistry and Medicine at the University of Washington, Seattle, and also served as chairman of its Department of Immunology, where he was a Howard Hughes Medical Institute investigator. His research focused on understanding the signaling pathways that control lymphocyte activation. Prior to his role at the University of Washington, he was a lecturer in the Division of Biology at the California Institute of Technology, Pasadena.

Dr. Perlmutter graduated from Reed College in Portland, Oregon, and received his M.D. and Ph.D. degrees from Washington University in St. Louis, Missouri. He pursued clinical training in internal medicine at Massachusetts General Hospital, Boston, and at the University of California, San Francisco.

Dr. Perlmutter is a Fellow of the American Academy of Arts and Sciences and the American Association for the Advancement of Science.



Scott Peterson, Ph.D. I Executive Director, Corporate Development, Intarcia Therapeutics, Inc.

ITCA 650 (Exenatide Delivered Continuously via a Matchstick-Size Subcutaneous Osmotic Mini-Pump) an Investigational Therapy for Type-II Diabetes

Scott is currently a member of Intarcia Therapeutics' Corporate Development Team. He is focused on identifying and evaluating opportunities for collaborations involving new molecules and Intarcia's proprietary, subcutaneous drug delivery system. The Intarcia osmotic minipump, combined with the company's unique formulations for stabilizing peptides and proteins at human body temperatures for extended periods of time, enables the continuous delivery of therapy for up to twelve months.

Scott received his Ph.D. in Synthetic Organic Chemistry from Harvard University under the mentorship of Prof. David A. Evans before joining Merck Sharp & Dohme's Research Laboratories. While at Merck, he contributed to the discovery and development of small molecule therapies for the treatment of human cancers, auto-immune and neurological diseases. Scott was also a member of Merck's Business Development and Licensing Innovation Hub in Boston, MA where he evaluated new opportunities for collaborations and partnerships, including those involving novel formulations and drug delivery platforms.



Philipp E. Scherer, Ph.D. I Professor, Department of Internal Medicine, Gifford O. Touchstone Jr. and Randolph G. Touchstone Distinguished Chair in Diabetes Research; Director, Touchstone Diabetes Center, The University of Texas Southwestern Medical Center

An Integrated View of the Adipocyte: From Feast to Famine

Philipp Scherer is Professor and Director of the Touchstone Diabetes Center at the University of Texas Southwestern Medical Center in Dallas. He received his Ph.D. degree from the University of Basel, Switzerland, followed by post-doctoral training the Whitehead Institute at MIT in Cambridge. In 1997, he joined the faculty of the Albert Einstein College of Medicine where he was a Professor for Cell Biology and Medicine. Throughout his career, he has maintained an interest in processes related to cellular and systemic energy homeostasis. During his Ph.D., he identified several components of the mitochondrial protein import machinery. While a post-doc, he identified adiponectin, one of the first secretory factors to be described that almost exclusively originate in adipose tissue and which is currently widely studied by many different research groups.

Current efforts in his laboratory are focused on the identification and physiological characterization of novel proteins that serve as potential links between the adipocyte, liver, the pancreatic beta cell and the processes of whole body energy homeostasis, inflammation, cancer and cardiovascular disease. His research team aims to identify novel targets for pharmacological intervention and to further define the role of adipose tissue as an endocrine organ.

Scherer has been on the faculty of UT Southwestern Medical Center since 2007 as a member of the Departments of Internal Medicine and Cell Biology. He holds the Gifford O. Touchstone Jr. and Randolph G. Touchstone Distinguished Chair in Diabetes Research and is a member of the Simmons Comprehensive Cancer Center. He won the 2012 O'Donnell Award in Medicine from the Academy of Medicine, Engineering & Science of Texas. In 2013, he received the Naomi Berrie Award for Outstanding Achievement in Diabetes Research from Columbia University, and he was awarded the 2015 Banting Medal for Scientific Achievement from the American Diabetes Association.



Claudio D. Schteingart, Ph.D. I Director, Peptide Therapeutics Foundation Vice President, Science & Technology – Research, Ferring Research Institute Inc. Selepressin, a Selective hV1a Agonist for the Treatment of Hypotension in Septic Shock

Dr. Schteingart is Vice President, Science & Technology – Research at Ferring Research Institute Inc. His current responsibilities are the evaluation of new technologies for the discovery and development of novel peptide therapeutics and to provide guidance to drug discovery programs at the Institute as well as supporting drug candidates in Development at Ferring Pharmaceuticals. He joined the Institute in 1996 as a research chemist and participated in the discovery of the peptidic GnRH antagonist degarelix, launched in 2009, and nine other peptidic drug candidates in various stages of clinical development for women's health, critical care medicine, and gastroenterology.

Dr. Schteingart received a Ph.D. in Chemistry at the University of Buenos Aires, Argentina. After postdoctoral studies in the Department of Chemistry at the University of California, San Diego, he moved to the Department of Medicine where he carried out research in the chemistry, physiology, metabolism, and physicochemical properties of biliary components and lipids.



Randy J. Seeley, Ph.D. I H.K. Ransom Professor of Surgery, University of Michigan Molecular Targets for the Effects of Bariatric Surgery on Obesity and Diabetes

Dr. Randy Seeley is the Henry K. Ransom Endowed Professor of Surgery at the University of Michigan School of Medicine. Prior to that, Dr. Seeley was Professor of Medicine and held the Donald C. Harrison Endowed Chair at the University of Cincinnati College of Medicine where he also served as the Director of the Cincinnati Diabetes and Obesity Center (CDOC). His scientific work has focused on the actions of various peripheral hormones in the CNS that serve to regulate food intake, body weight and the levels of circulating fuels. His work has also focused on new treatment strategies for obesity and diabetes. He has published over 280 peer-reviewed articles including articles in Science, Nature, Nature Medicine, Nature Neuroscience, Science Translational Medicine, Cell Metabolism, The Journal of Clinical Investigation and the New England Journal of Medicine. Collectively, this work has been cited more than 20,000 times and Dr. Seeley has an H-index of 66. Dr. Seeley has received numerous awards including the 2009 Outstanding Scientific Achievement Award from the American Diabetes Association. This award is presented to an individual medical researcher under age 45 who has made an outstanding contribution to diabetes research that demonstrates both originality and independence of thought. Dr. Seeley has also served on numerous review panels for the NIH and was Chair of the Integrative Physiology of Obesity and Diabetes review panel. He currently serves on the NIDDK Clinical Obesity Research Panel and on the Board of Reviewing Editors for Science.



Bobby Singh, Ph.D. I Chief Technology Officer, Corium International, Inc. *MICROCOR® Transdermal System: Technology Overview and Clinical Status*

Dr. Singh has over 19 years of experience in the pharmaceutical and drug delivery industry. He has led the research and development programs at Corium for over thirteen years. Prior to joining Corium, Dr. Singh held R&D and management positions at Ciba Geigy, Novartis and Vyteris. His work has resulted in the commercialization of a number of prescription transdermal and oral healthcare products. He has over 40 publications in peer reviewed journals and more than 25 issued and pending patents. Dr. Singh received his Ph.D. in pharmaceutics from the University of Queensland, Australia and completed a post-doctoral fellowship at the University of California at San Francisco. He is a member of the American Association of Pharmaceutical Scientists and the Controlled Release Society.



Lex Van der Ploeg, Ph.D. I Chief Scientific Officer, Rhythm Pharmaceuticals
Relamorelin, a Ghrelin Agonist for the Treatment of Diverse Functional Gastrointestinal Disorders

Lex H.T. Van der Ploeg is Managing Director for VDP, LLC, an independent consulting agency focused on guiding Research and Development for the Biotechnology and Pharmaceutical Industries and private investors. At VDP, LLC, Lex focuses on supporting R&D initiatives from a strategic and tactical perspective, as an integrated member of Management and R&D teams. Lex is CSO for Rhythm pharmaceuticals and functions as a member of the board of Directors for Retrotope Inc., he functions as an advisor to DeuteRx, LLC and is a member of the SAB for Excellentia Global partners.

Lex's expertise includes development of Diagnostics and Therapeutics including Cancer, Neurodegenerative disorders, Metabolic disorders and Infectious diseases. Prior to starting VDP, LLC Lex was the Sr. Vice President of Integrative Medicine and Translational Science at Abraxis/ Celgene. Previously, Dr. Van der Ploeg held the position of, Vice president, Basic Research, and site head at Merck Research Laboratories Boston focused on oncology and neurodegenerative disease. Prior to starting Merck Research Laboratories Boston in December 2003, Dr. Van der Ploeg held diverse functions at Merck Research Laboratories including site head MRL San Diego and Head Obesity research for Merck Rahway and Banyu, Japan. Lex held leadership roles in obesity and metabolism, oncology, neurodegenerative disease research and diagnostics.

Dr. Van der Ploeg received his M.S. degree (Summa Cum Laude) in Biochemistry in 1980 from the University of Amsterdam and his Ph.D. in Biochemistry/Enzymology/Genetics in 1984 from the University of Amsterdam/Netherlands Cancer Research Center. Following receipt of his Ph.D. Dr Van der Ploeg joined the faculty of the Dept of Genetics and Development of Columbia University, as an assistant Professor and received tenure at Columbia University in 1987. Dr Van der Ploeg held an adjunct faculty position at the Dept of Genetics and Development of Columbia University from 1992 through 2009. Dr. Van der Ploeg received numerous awards and grants for his research and has broadly published on his research in peer reviewed journals. He is an inventor on over 50 patents and patent applications.



Kisspeptin and its Short-length Agonist Analogues: Discovery and Development

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Kisspeptin/metastin (Kp), a 54-amino acid peptide, is the ligand of the orphan G-protein-coupled receptor hOT7T175, also known as AXOR12, and GPR54, currently renamed the KISS1 receptor, KISS1R. Recently, fundamental studies have suggested that Kp plays critical roles in the regulation of the hypothalamic–pituitary–gonadal (HPG) axis. Single administration of Kp markedly stimulated follicle stimulating hormone (FSH)/luteinizing hormone (LH) release in rat models, and continuous administration reduced testosterone levels. The molecular mechanisms for KISS1R signaling suggest that appropriate dosing of KISS1R agonists should activate or suppress the HPG axis, thereby potentially preventing or treating a number of sex-hormone-dependent diseases.

An N-terminally truncated decapeptide of Kp (Kp-10, Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Phe-NH₂) demonstrated biological activity *in vitro* and was 3–10 fold more potent than Kp, which indicated that the core residues for biological activity are located in C-terminal short peptides. However, Kp-10 was rapidly inactivated in serum.

Modifications of Kp-10 produced peptide analogues with higher metabolic stability than Kp-10. A nonapeptide **1** ([des-Tyr¹,D-Tyr²,D-Pya(4)³,azaGly²,Arg(Me)³]Kp-10) with both moderate agonist activity and metabolic stability showed more potent testosterone-suppressant activity than the endogenous ligand Kp in male rats. Compound **1** was the first short-length KISS1R agonist to exert comparable testosterone suppressive activity to that of a gonadotropin-releasing hormone (GnRH) analogue leuprolide *in vivo*.

Although compound **1** had more potent testosterone-suppressant activity than Kp-10, it possessed physicochemical instability at pH 7 and insufficient in vivo activity. Instability at pH 7 was dependent upon Asn⁴ and Ser⁵; substitution of Ser⁵ with Thr⁵ reduced this instability and maintained KISS1R agonist activity. Furthermore, compound **2** ([des-Tyr¹,D-Tyr²,D-Trp³,Thr⁵,azaGly⁷,Arg(Me)⁹,Trp¹⁰]Kp-10), showed 2-fold greater agonist activity than Kp-10 and an apparent increase in physicochemical stability. N-terminal acetylation of compound **2** resulted in a potent analogue **TAK-683** (Ac-[des-Tyr¹,D-Tyr²,D-Trp³,Thr⁵,azaGly⁷,Arg(Me)⁹,Trp¹⁰]Kp-10). With continuous administration, **TAK-683** possessed 10–50-fold more potent testosterone-suppressive activity in male rats than compound **1**. These results suggested that a controlled release of shortlength KISS1R agonist can suppress the HPG axis and reduce testosterone levels, and promoted us to select **TAK-683** as an investigational drug for sex hormone-dependent diseases.

In this presentation, the discovery and development of **TAK-683** including the related background research and the clinical results will be discussed.

Oral Peptide Delivery: Deciphering the Reality from the Hype

David J. Brayden, Ph.D. I Professor of Advanced Drug Delivery

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There is renewed interest in developing oral formulations for peptides and proteins. Part of this renaissance has arisen due to a combination of factors: a decrease in the cost of making synthetic peptides so that a large loss in the gastrointestinal tract and/or liver can be withstood commercially, relatively successful Phase III trials for oral salmon calcitonin (sCT) and octreotide, and a race between large Pharma to achieve oral versions of insulin and glucagon-like Peptide 1 analogues. Estimates of the absolute oral bioavailability of sCT and octreotide in the Phase III studies however are less than 1%. Because individual variation in oral bioavailability is exacerbated at low values, peptide selection is limited to highly potent molecules with a wide safety margin. Furthermore, the range of potential molecules for oral development is typically limited to ones already approved as parenteral injections, as Pharma will not take the additional risk of developing a new molecule by the most difficult route and therefore their physicochemical properties are sub-optimal for oral delivery before the project begins! Such peptides are hydrophilic in nature, have a high molecular weight, and are prone to metabolic attack by pancreatic secretions including serine proteases. The first two factors contribute to poor small intestinal epithelial permeability, while the latter leads to chemical instability. Both aspects have to be addressed by formulation in order to achieve oral bioavailability. While di- and tri-peptide products of digestion may potentially access the hPEPT1 intestinal carrier on small intestinal epithelia, this route has only been exploited for selected small molecule prodrugs to date, including valacyclovir and cephalexin. Attempts to target hPEPT1 by using peptide-loaded nanoparticles coated with ligands were unsuccessful, perhaps due to an overly-complex construct design, which could not be scaled up for subsequent manufacture. Peptide formulations therefore are currently attempting to take advantage of either accessing the paracellular route via temporarily opened tight junctions or to promote transcellular permeation as components attached to mixed micelles with bile salts. Intestinal permeation enhancers (PEs) including medium chain fatty acids, acyl carnitines, bile salts, EDTA, and alkyl maltosides have all been components of oral peptide formulations in clinical trials with varying degrees of success. Again, in order to reduce risk, the selection of PEs by Pharma is limited to substances with a history of use in man either as excipients or in food-grade products. Most of the promising PEs seem to open tight junctions and have a mild detergent effect on the epithelium in vivo due to their amphiphilic nature. These are incorporated in solid dosage forms or in water-in-oil emulsions where premature stomach release is impeded by surface coatings with pH-dependent polymers. Concerns over potential toxicity of such PEs include damage to the epithelium leading to absorption of bystander pathogens, but these have not been born out in clinical studies to date, although effects of repeat-dose administration are still being examined for most constructs. Academic groups have been developing formulations based on cell-penetrating peptide ad-mixtures to target the epithelium, or on motifs more specifically targeted to tight junction claudins to promote a sieve effect rather than to irreversibly open junctions. So far, they do not seem to be any more efficacious than the current crop of PEs in clinical trials.

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Engineered Cystine Knot Peptides: A New Class of Tumor Targeting Agents

Jennifer R. Cochran, Ph.D. I Associate Professor

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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, rational and combinatorial protein engineering methods are being used to generate tumor-targeting knottins with applications as cancer diagnostics and therapeutics. I will discuss some of our work in this area, where we took natural knottins found in plants, mammals, and insects, and used them as molecular scaffolds to engineer stable peptides that target tumor-associated cell adhesion receptors with high affinity. These engineered knottins were conjugated to molecular imaging probes and used as robust non-invasive tumor imaging agents in a variety of murine cancer models. We showed that one particular engineered knottin could localize to and illuminate intracranial brain tumors following intravenous injection. This knottin was able to traverse the blood-tumor-barrier and access brain tumor tissue, but did not accumulate in the surrounding healthy cerebellum. Engineered tumor-targeting knottins thus have great potential as clinical diagnostics, for applications including disease staging and management and image-guided surgical resection. More recently, we have been developing the engineered knottin peptides as vehicles for targeted drug delivery, expanding their application to tumordirected therapy.

Activity-based Proteomics - Applications for Enzyme and Inhibitor Discovery

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Genome sequencing projects have revealed that eukaryotic and prokaryotic organisms universally possess a huge number of uncharacterized enzymes. The functional annotation of enzymatic pathways thus represents a grand challenge for researchers in the genome era. To address this problem, we have introduced chemical proteomic and metabolomic technologies that globally profile enzyme activities in complex biological systems. These methods include activity-based protein profiling (ABPP), which utilizes active site-directed chemical probes to determine the functional state of large numbers of enzymes in native proteomes. In this lecture, I will describe the application of ABPP and complementary proteomic methods to discover and functionally annotate enzyme activities in mammalian physiology and disease, with a special emphasis on enzymes involved in metabolic disorders. I will also present competitive ABPP platforms for developing selective inhibitors for these enzymes and discuss ongoing challenges that face researchers interested in assigning protein function using chemoproteomic methods.

Addressing the Challenges with the Discovery and Development of Peptide Therapeutics

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There has been surgent interest and activity in the use of peptide therapeutics to treat a variety of human diseases. Three key challenges to address in the process of discovering and developing peptide therapies are: target selection, optimization, and delivery. In this lecture I will present our approaches to discover, optimize, and deliver peptides therapies for treatment of metabolic diseases. Our target selection utilizes observations resulting from Roux-en-Y gastric bypass surgery and rigorous pre-clinical assessment of peptide singleton and combinations. Peptide optimization is conducted to select for highly *in vivo* selective and potent peptides with physicochemical properties suitable for development. Additionally, various approaches to peptide delivery will be discussed and assessed for optimal patient benefit and adherence.

Oral Inhalation - A Simple Approach to Biologics Delivery

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Protein and peptide drugs are routinely delivered by injection. Oral inhalation, however, is a viable delivery option for these therapies. Some benefits of this delivery route include rapid onset of action, avoidance of first pass hepatic metabolism, and elimination of injections. While oral inhalation has long been the standard of care for delivering medicines directly to the lung for treating pulmonary diseases like asthma and COPD, only within the last decade has it been applied to therapies requiring systemic exposure. An example of such an application is Afrezza®, an orally inhaled insulin approved by FDA in 2014 for diabetes.

The technologies underlying Afrezza delivery comprises advanced, engineered dry powders and patient-friendly, breath-powered inhalers. These can be readily applied to a wide variety of peptide and protein drugs and clinical indications. The dry powders are based on an excipient (FDKP) that forms particles appropriately sized for inhalation. Protein and peptide drugs can be adsorbed onto the surfaces of pre-formed particles or incorporated into a composite particle matrix. The resultant dry powders are appropriately sized for inhalation into the deep lung. They are inhaled through a small, breath-powered inhaler, either reusable or single-use depending on the clinical indication. Upon inhalation, the powder dissolves immediately and the drug is absorbed into the systemic circulation. Oral inhalation drug delivery using this technology is characterized by rapid onset of action that is ideally suited for various clinical indications. The development of these non-invasive, patient-friendly combination products will be discussed and these technologies will be exemplified with non-clinical data in the therapeutic areas of diabetes (GLP-1), obesity (oxyntomodulin), and pain (novel tetrapeptides).

NA-1: From Bench to Bedside – A Novel Peptide Therapeutic for the Treatment of Acute Stroke

Dave Garman, Ph.D. I Technology Officer

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The development of a safe and effective treatment for acute ischemic stroke is one of the greatest challenges in all of biomedical sciences. Stroke afflicts about 1 million people in North America each year, and millions worldwide. It is a leading cause of mortality, and the most common cause of chronic disability. Despite over 200 attempted clinical trials with neuroprotective agents, the only effective therapeutic treatment for stroke remains the clot buster tPA, which is only given to 3-5% of stroke victims due to its restrictions. No neuroprotective therapeutic is approved in North America.

Dr. Michael Tymianski, founder of NoNO Inc., discovered of a novel class of neuroprotective therapeutic agents, termed PSD95 inhibitors, that show efficacy in stroke, traumatic brain injuries, and related CNS disorders. A single bolus dose of the lead inhibitor, NA-1, has been shown to reduce ischemia in rodent and primate models of stroke by more than 70%, while significantly improving functional outcomes. A Phase 2 clinical trial in 185 patients (US and Canada) further supports the safety and efficacy of NA-1 in reducing the number of strokes associated with endovascular repair of both unruptured and ruptured brain aneurysms.

NA-1 is currently being evaluated in a unique Phase 3 pivotal trial to determine whether a single dose of NA-1 administered by paramedics in the field within 3 hours of symptom onset will improve the outcome of patients with suspected acute stroke. The FRONTIER (Field Randomization Of NA-1 Therapy In Early Responders, NCT02315443) Trial is approved in Canada and the U.S. and enrolled its first patient in March 2015. NA-1 has undergone extensive preclinical safety pharmacology and toxicity studies, a phase 1 safety study with no drug related SAEs at any dose tested and a phase 2 efficacy trial in patients suffering from strokes in-hospital. NA-1's safety profile and efficacy in multiple models of stroke support the continued clinical development in both acute stroke and iatrogenic stroke indications such as those incurred as a result of surgical and endovascular procedures. NA-1 is being developed for use as either an adjunct to other reperfusion strategies or as an independent therapeutic.

Improved Chemical and Physical Stability in Liquid Formulations – How to Design Stable Glucagon Analogues

Lise Giehm, Ph.D. I Senior Scientist, Department of Pharmaceutical Development

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Glucagon is a 29 amino acid peptide hormone synthesized in the pancreas. In concert with insulin, this hormone is involved in the regulation of blood glucose levels by stimulating the liver to release glucose. It is used as a pharmaceutical for the treatment of severe episodes of hypoglycaemia in diabetic patients. The present pharmaceutical formulation is a lyophilized powder, which is reconstituted in water immediately prior to use. The underlying problem is that glucagon displays pH-dependent water-solubility with a low solubility at neutral pH, while being both physically and chemically unstable in aqueous solutions. It has a strong tendency to aggregate and form amyloid-like fibrils in addition to a rapid chemical degradation profile. Consequently, the stability of the aqueous formulation of glucagon after reconstitution is limited to a few hours, which makes convenient dosing in a ready-to-use rescue pen or the development of an artificial pancreas virtually impossible.

We have developed novel glucagon analogues through a series of iterative changes in the native sequence of glucagon with the goal of improving the physicochemical properties of the peptide whilst maintaining PK and PD profiles similar to those of native glucagon.

We present a glucagon analogue (ZP-GA-1) with optimized physicochemical properties that is suitable for long term storage as a liquid formulation. The solubility of ZP-GA-1 at physiological pH was shown to be >25 mg/mL, greatly superior to that of native glucagon (~0.2 mg/mL). In addition, no aggregates or fibrils were detected for ZP-GA-1 in optimized formulations, using methods such as turbidity analysis, Dynamic Light Scattering and Thioflavin T assays. Furthermore, the chemical stability has been dramatically improved compared to native glucagon.

The pharmacokinetic (PK) and pharmacodynamic (PD) properties of ZP-GA-1 and native human glucagon have been investigated in dogs. Animals were administered either subcutaneously (SC; 20 and 120 nmol/kg) or intravenously (75 nmol/kg). Our data demonstrate very similar PK as well as blood glucose (BG) profiles for ZP-GA-1 and glucagon. Furthermore, the effect of SC administered ZP-GA-1 on BG in a rat model of hypoglycemia was investigated, where both ZP-GA-1 and glucagon were shown to restore BG to baseline levels or above in a dose-dependent manner during insulin-induced hypoglycemia.

In conclusion, this data shows that ZP-GA-1 displays improved physicochemical properties while maintaining similar PK and PD profiles compared to native glucagon. This makes ZP-GA-1 an appealing alternative to native glucagon in situations requiring medium to long-term storage in a liquid format, as a ready-to-use rescue pen and a bionic pancreas.

Francesca Macchi, Ditte Riber, Anders Valeur, Mette Svendgaard, Keld Fosgerau, Pia Noerregaard and Lise Giehm.

Phage Selection of Bicyclic Peptides for Application as Therapeutics

Christian Heinis, Ph.D. I Assistant Professor

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My laboratory is engaged in the discovery and development of peptide macrocycles for therapeutic application. A major focus is the generation of antagonists based on bicyclic peptides by phage display. The bicyclic peptides combine key qualities of antibody therapeutics (high affinity and specificity) and advantages of small molecule drugs (access to chemical synthesis, diffusion into tissue, various administration options). We were able to generate bicyclic peptide antagonists with nanomolar or even picomolar binding constants for a range of human disease targets. Towards the therapeutic application of the peptides, we have extended their circulation time to several days in mice and we are now assessing the therapeutic effect of some of the peptides in vivo. An update on recently developed bicyclic peptides and their activities will be given.

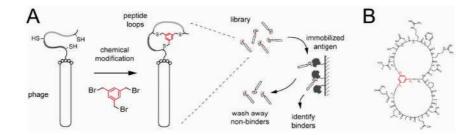


Figure: (A) Large libraries of random peptides (> 4 billion different peptides) are displayed on phage and cyclised in a chemical reaction (left). Binders to targets of interest are subsequently isolated in affinity selections (right). (B) Chemical structure of an isolated bicyclic peptide.

Harnessing Chemistry and Glycoproteins to Discover Novel Targets for Cancer Therapy and Neurodegenerative Diseases

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The dynamic post-translational modification of proteins by O-linked β -N-acetylglucosamine (O-GlcNAcylation) serves as a nutrient sensor to couple metabolic status to the regulation of cellular signaling pathways. O-GlcNAc transferase (OGT) attaches N-acetylglucosamine from uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) to serine or threonine residues of many intracellular proteins, including signaling proteins important for insulin resistance, oncogenes and tumor suppressors, and transcriptional co-activators that control gluconeogenesis. We will describe the synergistic application of organic chemistry and biology to understand the roles of O-GlcNAcylation in two contexts: cancer metabolism and neurodegeneration. Our studies reveal previously uncharacterized mechanisms for modulating the activities of metabolic enzymes and disease-associated proteins, as well as possible targets for therapeutic intervention.

Phage-display Derived Peptidic Inhibitors of PCSK9 for LDL Cholesterol Lowering

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) circulates in blood and is a negative regulator of liver LDL receptors. Therapeutic inhibition of PCSK9 by monoclonal antibodies shows remarkable reductions of LDL cholesterol levels in ongoing clinical trials. Development of small molecule inhibitors is hampered by the challenging nature of the target PCSK9. We used established phage display technologies to generate structured peptides that inhibit PCSK9 function, such as affinity-improved variants of the LDL-receptor EGF(A) domain, which neutralized PCSK9 in-vitro and in-vivo. In a different phage display approach, we were able to obtain a small 13 amino acid peptide, which completely inhibits LDL receptor binding and displays partial structural mimicry of the EGF(A) domain. The results demonstrate the feasibility of developing peptidic inhibitors to functionally relevant sites on PCSK9.

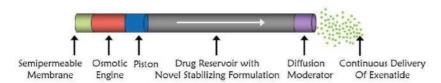
ITCA 650 (Exenatide Delivered Continuously via a Matchstick-Size Subcutaneous Osmotic Mini-Pump) an Investigational Therapy for Type-II Diabetes

Scott Peterson, Ph.D. I Executive Director, Corporate Development

Intarcia Therapeutics, Inc.

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Type 2 diabetes mellitus (T2DM) is a progressive disease that often requires combinations of oral antidiabetic drugs (OADs) and injectable medications to achieve adequate glycemic control. ADA and EASD treatment guidelines recommend that treatment be individualized for each patient based on prognosis, preferences, and co-morbidities. Nevertheless, a large segment of the T2DM population remains poorly controlled, even with three or more OADs (Hall, G.C., et al. *Diabet Med.* 2013; 30:681-6.) and only 50% of patients are adherent to chronic medications (including OADs; WHO, 2003). ITCA 650, an investigational therapy for T2DM currently in Phase 3 clinical trials, is an osmotic mini-pump that can subcutaneously deliver exenatide (a GLP-1 receptor agonist) for extended periods of time. This presentation will highlight 1) the technology behind the ITCA 650 delivery device; 2) recent data from an ongoing 2-year carcinogenicity study, which demonstrates one year of continuous exposure and tolerability in a nonclinical rodent model; and 3) the efficacy and tolerability of 39 weeks of ITCA 650 in patients with poorly controlled T2DM and high baseline HbA1c (>10%).



An Integrated View of the Adipocyte: From Feast to Famine

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The incidence of obesity and diabetes has assumed epidemic proportions. While increased fat mass is not necessarily associated with metabolic dysfunction, an increase in BMI is generally leading to an increased risk for diabetes, cardiovascular disease and cancer for the majority of individuals. A reduction in weight, even if modest, can lead to significant metabolic improvements. To achieve effective weight loss, it is critical to understand the concept of metabolic flexibility, which refers to the ability to adjust energy balance to different nutritional states. Obesity is associated with a decrease in energy expenditure. This reduction in energy expenditure may in fact be one of the driving forces for the development of obesity.

The adipocyte is center stage in terms of providing protective, anti-lipotoxic activities to the system on the basis of neutralizing excess lipids through storage and secreting anti-lipotoxic cytokines. During the progression from the lean to the obese state, adipose tissue undergoes **hyperplasia** as well as **hypertrophy** in an attempt to cope with the increased demand for triglyceride storage. This requires a high degree of plasticity at both the cellular and at the tissue level. The extracellular matrix of adipose tissue faces unique challenges with respect to adjusting to the need for remodeling and expansion. In parallel, the vasculature has to adapt to altered requirements for nutrient and oxygen exchange. **"Healthy" adipose tissue expansion** versus **"unhealthy" expansion** plays a key role in maintaining metabolic fitness system wide. We have defined a number of criteria that differentiate between these different states of adipose tissue. Chronic pharmacological intervention is challenging in light of the overlap between the physiological demands of an expanding fat pad and the expansion of a solid tumor mass at the level of vascularization, extracellular matrix remodeling and inflammatory processes. Yet, adipose tissue holds the key for systemic metabolic homeostasis and remains one of the prime targets for the manipulation of key biochemical pathways whose changes spill over into other tissues driving carbohydrate and lipid metabolism, such as the liver

Selepressin, a Selective hV1a Agonist for the Treatment of Hypotension in Septic Shock

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Sepsis is an excessive systemic inflammatory response to infection; the incidence of sepsis has increased in the past decade due to the aging population and the emergence of multiresistant bacteria, and although outcomes have improved, mortality remains significant. The standard of care is treatment with antibiotics, fluid resuscitation, and other supportive therapies. In spite of treatment, sepsis can progress to septic shock, characterized by systemic vasodilation and vascular leakage resulting in severe hypotension that is no longer responsive to fluids and requires treatment with vasopressor drugs. Norepinephrine (NE) is the most commonly used vasoconstrictor, but its use is limited by side effects such as increased tissue oxygen demand, reduced renal and mesenteric blood flow, pulmonary hypertension, and cardiac arrhythmias, and some patients may become catecholamine resistant. The hormone arginine vasopressin (AVP) can also produce vasoconstriction via V1a receptors on vascular smooth muscle cells and is currently recommended by the Surviving Sepsis Campaign as an adjunct vasopressor to NE. However, AVP is not selective for the V1a receptor versus the related V1b, V2, and oxytocin (OT) receptors. Activation of the V2 receptor results in water retention and antidiuresis by the kidney, and the release of von Willebrand factor (vWF) from the vascular endothelium, both of which may be counterproductive in the septic shock setting.

We will describe the structure-activity relationships leading to the discovery of selepressin, a peptide for the treatment of hypotension in septic shock patients by titratable intravenous infusion. Selepressin is a potent agonist at the hV1a receptor with excellent selectivity versus the related hV2, hV1b, and hOT receptors. In a rat model, selepressin reversed hypotension and vascular leakage induced by platelet activating factor, and reduced mortality to a greater extent than AVP. In normal sheep, an infusion of AVP at a rate equivalent to that required to treat septic shock caused significant release of vWF coagulation factor while selepressin did not. In preclinical studies in various sheep models of sepsis, selepressin maintained mean arterial blood pressure (MAP) and renal blood flow, and was superior to AVP in preventing fluid accumulation, plasma extravasation and tissue edema. Specifically, selepressin was more effective at reducing pulmonary edema and improving pulmonary function.

In a randomized double-blind placebo controlled Phase 2a clinical trial in septic shock patients requiring vasopressor support, selepressin dose dependently reduced or completely eliminated the requirements for NE while maintaining MAP within the target range (> 60 mmHg). Selepressin increased the proportion of days alive and free of mechanically assisted ventilation and the proportion of patients out of shock at 48 hours. There was a tendency to reduce the 7 day cumulative fluid balance at the highest infusion rate tested.

Selepressin appears a very promising agent for the treatment of hypotension in septic shock patients requiring a vasopressor. An adaptive Phase2b/3 clinical trial has been initiated to investigate the safety and efficacy of multiple dose regimens of selepressin in these patients.

Molecular Targets for the Effects of Bariatric Surgery on Obesity and Diabetes

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While various bariatric surgeries provide both the largest and most durable weight loss of any currently available therapy, there remain great uncertainties around the mechanisms that produce such weight loss. At least some surgical approaches also reduce obesity-related comorbidities including type 2 diabetes and hyperlipidemia. These weight and metabolic successes put a premium on understanding how these surgeries exert their effects. We have been using a variety of mouse models to test specific hypotheses about key molecular targets that mediate the benefits of bariatric surgery. Bariatric surgery produces changes in a number of brain-gut signaling systems that result in profound changes in food intake and food selection. In addition to typical gut hormones, bariatric surgery results in changes in bile acids and bile acid signaling that are crucial for many of the behavioral and metabolic effects of the surgery. These insights make it possible to devise new treatment strategies that take advantage of the potent effects of these procedures but are less invasive and more scalable to tackle the obesity and diabetes epidemics.

MICROCOR® Transdermal System: Technology Overview and Clinical Status

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Corium's MicroCor technology uses a fully integrated drug-device combination system for safe, effective, efficient and convenient delivery of drugs to the skin for local or systemic delivery. The microstructures can be fabricated from a variety of biocompatible materials, and in varying lengths, offering significant flexibility in the depth of drug delivery in the skin (stratum corneum to dermis) and duration of delivery (bolus to sustained administration). When placed against the skin and pressed with a slight force, the microstructures penetrate the skin and dissolve immediately to release their payload. The spring based mechanism provides consistent depth of penetration to achieve uniform and reproducible drug delivery. The delivery is painless and highly efficient. Since the microstructures dissolve inside the skin, there are no sharps left on the system eliminating any misuse and risk of accidental needle stick injury. The dry nature of the microstructures enables long term room temperature stability. Using this system we have demonstrated successful in vivo delivery of a number of macromolecules, including peptides, proteins, monoclonal antibodies and vaccines. Recently, we completed a single dose and 28-day multiple dose Phase 2a clinical safety, pharmacokinetic and pharmacodynamic study using a peptide drug, human parathyroid hormone (1-34), with positive results. The rate of absorption and the time to maximum concentration were faster with MicroCor PTH compared to the Forteo subcutaneous injection (brand product marketed by Eli Lilly), suggesting rapid delivery and uptake after a short patch wear time. Pulsatile delivery of PTH is known to be an important factor in stimulating bone formation. Increases in serum concentrations of bone-formation markers that are widely understood to be consistent with bone-building activity were comparable between MicroCor PTH and Forteo. Subjects treated with MicroCor PTH experienced excellent skin tolerability and no systemic adverse events beyond those observed in subjects treated with Forteo injections. In summary, MicroCor is a ground-breaking, clinically validated technology platform designed to overcome the limitations of injection-based delivery. MicroCor is designed to improve patient and healthcare outcomes, including ease of use, long term room temperature stability, short wear time, no remaining sharps and potentially enhanced quality and quantity of therapeutic response.

Relamorelin, a Ghrelin Agonist for the Treatment of Diverse Functional Gastrointestinal Disorders

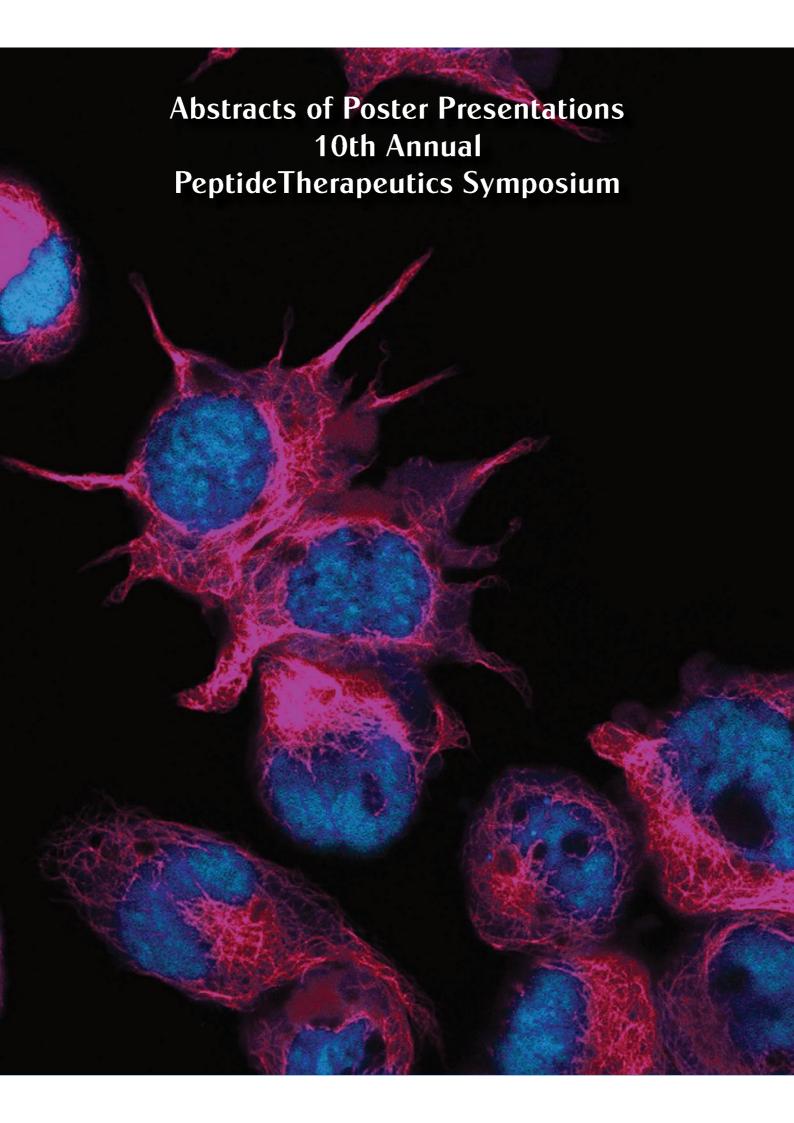
Lex H.T. Van der Ploeg, Ph.D. I Chief Scientific Officer

Rhythm Pharmaceuticals

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Ghrelin was identified in 1999 following the cloning of the growth hormone secretagogue receptor (GHSR) in 1996 (Muller et al., Molecular Metabolism. 2015; 4; 437-460). In addition to ghrelin's role in nutrient sensing and the control of energy expenditure, ghrelin may serve a critical role restoring gastrointestinal function in diverse functional gastrointestinal disorders. Several synthetic ghrelin agonists are being pursued for clinical development. Pralmorelin has been approved in Japan as a diagnostic test agent for growth hormone deficiency. Relamorelin a synthetic pentapeptide, is a potent agonist at the GHSR (Fisher et al., Front. Nutr., 12 January 2015 | doi: 10.3389/fnut.2014.00031) and has shown superior activity compared to numerous other ghrelin mimetics when tested in preclinical models of gastric emptying and gastrointestinal motility (Van der Ploeg et al., Life Sciences, 2014; 109(1):20-9). Preliminary data indicate that direct activation of GHSRs in the fundus of the stomach and GHSRs in the gastrointestinal tract, may contribute to relamorelin's mechanism of action (Sha et al., Gastroenterology, 2014, 146 (Suppl 1); S363). Relamorelin has been demonstrated to be safe and well tolerated in healthy volunteers and in patients with diabetic gastroparesis (DG) and chronic constipation. Relamorelin increases gastric emptying and decreases gastrointestinal transit times in healthy volunteers (Shin et al., 2013; Diabetes Care 36; 41-48) and in patients with DG. Relamorelin also reduced the incidence of vomiting in DG patients, and improved composite symptoms of nausea, abdominal pain, bloating and early satiety in a subgroup of patients with vomiting at baseline. In a clinical trial for the treatment of patients with chronic constipation, relamorelin demonstrated significant improvement in signs and symptoms of constipation and accelerated lower GI transit (Camilleri et al., Neurogastroenterology and Motility. 2014; 27; 324-332). Relamorelin is currently undergoing testing in a 12-week, Phase 2b dose-ranging trial for efficacy in patients with diabetic

Lex Van der Ploeg, Bart Henderson, Sharon Spence, Fred Fiedorek, Elizabeth Stoner and Keith Gottesdiener

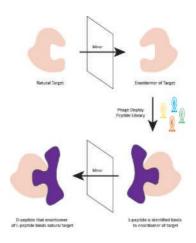


PO1 Discovery of D-Peptide Antibiotics Using Mirror Image Phage Display

Emel Adaligil, Kalyani Patil, Krishna Kumar

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Extensive and improper use of antibiotics has triggered a "bacterial resistance" problem. Vancomycin is one of the last resort antibiotics used in the treatment of life threatening hospital infections caused by Gram-positive bacteria. VREs (vancomycin-resistant Enterococci) have emerged a serious threat to the dwindling arsenal of effective antibiotics. We are developing D-peptide antibiotics capable of binding both vancomycin-sensitive and vancomycin-resistant strains. Such peptides have advantages over their enantiomeric L-forms, such as resistance to hydrolytic enzymes, being less immunogenic, and the possibility of being absorbed after oral administration. We use mirror image phage display to select D-peptide ligands for the designed targets.³ Briefly, the mirror image forms of the targets are synthesized and screened against bacteriophage libraries displaying short peptides (7 and 12-mers, both linear and cyclic). Several peptides have been identified from our screening experiments using enantiomers of the targets, and some of them have showed antibacterial activity against gram-positive bacterial strain. Biophysical and microbiological experiments will establish the potency of these identified compounds. These studies will pave the way for identification of a new class of peptide antibiotics with properties considered important for use as therapeutic.



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P02 Etchable Peptide Probes to Image and Quantify Cellular Internalization In Vivo

Gary B. Braun, Hong-Bo Pang, Alessia Pallaoro², Tatiana Hurtado de Mendoza, Anne-Mari A. Willmore³, Venkata Ramana Kotamraju, Aman P. Mann, Zhi-Gang She, Kazuki N. Sugahara, Tambet Teesalu³, Erkki Ruoslahti ¹Cancer Research Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA ²Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, USA ³Laboratory of Cancer Biology, Institute of Biomedicine, Centre of Excellence for Translational Medicine, University of Tartu, Tartu, 50411, Estonia

There is considerable interest in using peptides to target cell receptors, as peptides can increase the uptake of a cargo into cells and thereby improve specificity of drug delivery in the body. A key issue for tissue and cell targeting is to improve binding and endocytosis (cell internalization). Tools for monitoring endocytosis separately from the binding step include confocal microscopy and acid washing of probes from the cell surface, but are currently lacking for *in vivo* use. We developed a rapid, non-toxic etching process that selectively removes the extracellular nanoparticles but leaves the intracellular nanoparticles unharmed. We have shown that the technique works in living mice — the mild etchant can be injected by intravenous or intraperitoneal routes, or during terminal perfusion. Etching leaves the internalized pool for imaging and quantification. Etchant dissolves the nanoparticle core, removing those signals coming from nanoparticles that are still circulating or in the tissue but not yet internalized. Using this technique we track the tumor homing kinetics of peptide-silver nanoparticle and quantum dots in a tumor model in mice. In one example, we observed that accumulation in the tumor (unetched condition) occurred progressively over ~24 hours, but that endocytosis was not significant until after 4 h and indicates a potential rate limiting step. Efficiency of peptide based drug delivery may be optimized based on rate of internalization combined with information on total accumulation at the target tissue. Furthermore, microscopy can be used to correlate peptide internalization to receptor expression levels in the tissue microenvironment.

PO3 ADEP Antibiotics: Optimization and Insights into Mechanism of Action

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Cyclic peptide natural products can often times circumvent the typical liabilities that undermine the use of peptides as therapeutics and thus have had tremendous success in clinical medicine. The cyclic acyldepsipeptides (ADEPs) are an exciting new class of nonribosomal peptide antibiotics that exhibit potent broad spectrum activity against gram+ bacteria including drug resistant strains of deadly pathogens such as methicilin resistant *S. aureus* (MRSA) and Vancomycin resistant *enterococci* (VRE). This poster presentation will summarize the recent collaborative developments in the Sello (Brown University) and Sauer (MIT) labs towards the medicinal chemistry optimization of ADEPs as well as efforts to further our understanding of their mechanism of action. The antibacterial potency of the ADEPs is vastly improved by conformational restriction and strengthening of intramolecular hydrogen bonding networks. The ADEPs also highlight an interesting case wherein peptides and peptidomimicry are judiciously merged in order to optimize biological activity. These strategies should be generally applicable and highlight important lessons from nature about the design of potent and efficacious peptide therapeutics.

PO4 Recombinant Human Albumin: A Versatile Tool in Formulation and Delivery of Peptide Therapeutics

<u>Mikael Bjerg Caspersen</u>¹, Neil Dodsworth², Karen Bunting², Nanna Ny Kristensen¹, Jason Cameron², Lizzie Allen², Filipa Antunes², Francesca Macchi¹, Morten Jonas Maltesen¹

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The expanding field of peptide therapeutics gives promise for improvement of treatments against many diseases. However, many of the peptides found to be efficacious face challenges in relation to formulation due to their hydrophobicity or they have fast systemic clearance due to their small size.

Here we present the use of recombinant human albumin (rAlb) as a means to counter such events. First, we find rAlb to be superior to human sourced albumin in terms of purity and homogeneity, making rAlb the preferred option when developing formulations and half-life extension based on human albumin.

Human albumin is an obvious candidate for serum half-life extension; it is non-immunogenic and has a natural serum half-life of 19 days. We have designed albumin variants that have increased binding affinity for the neonatal Fc receptor (FcRn). This improves the albumin's recycling by the FcRn increasing its serum half-life further. Peptides, in need of increased serum half-life, can either be chemical conjugated to the free thiol of rAlb, or genetically fused to the rAlb. Examples of both strategies are shown, illustrating the robustness of the FcRn interaction with albumin-drug conjugates and retention of efficacy of conjugated peptide.

Human albumin has many biophysical properties: It binds a wide range of molecules typically hydrophobic, negatively charged ones. Albumin has also been shown to cover, both hydrophobic and hydrophilic, surfaces, in a near to one protein layer. These properties are illustrated in stabilizing the fibrillating Teduglutide and in adsorption prevention during handling of Glucagon.

P05 Novel Cell-Penetrating Peptide Targeting Mitochondria

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Cell-penetrating peptides (CPPs) are short, nontoxic peptides with cationic and/or amphipathic properties able to cross the cellular membrane. CPPs are used for the delivery of a wide variety of cargoes, such as proteins, oligonucleotides, and therapeutic molecules. The aim of the present study was to synthesize unusually small novel CPPs targeting mitochondria based on the Szeto-Schiller peptide (SS-31) to influence intra mitochondrial processes and to improve the biologic effects. All the peptides used were synthesized manually using 9-fluorenylmethyloxycarbonyl chemistry. In the first part of the study, HeLa 705, U87, and bEnd.3 cells were used as in vitro delivery model. Cells were incubated for 24 h at 37° C and 5% CO2 with different concentrations of our peptides. Cell proliferation assay was performed to evaluate cell viability. Biologic effects such as mitochondrial membrane potential and antioxidant activity were evaluated. H₂O₂ was used as positive control. Uptake studies were performed using peptides conjugated with 5(6)-carboxyfluorescein (FAM). Fluorescent microscopy was used to determine presence and localization of peptides into the cells. Isolated mitochondria from pre-treated cells and mitochondria treated after isolation were used to confirm the targeting ability of the peptide. Uptake of FAM alone was used as negative control. Microscopy studies confirmed the ability of peptides to penetrate cell. Localization analysis showed increase in uptake by35%compared with SS-31. Mitochondrial CPP 1 (mtCPP-1) had no effect on mitochondrial membrane potential and prevented reactive oxygen species formation in bEnd.3 cells by 2-fold compared with SS-31. No cytotoxicity was observed even at high concentration (100 mM). These data suggest that mtCPP-1 is a mitochondrial CPP and protect mitochondria from oxidative damage due to its own antioxidant activities. — Cerrato, C. P., Pirisinu M., Vlachos E. N., Langel, Ü. Novel cell penetrating peptide targeting mitochondria. FASEBJ. 2015 Jul 20. pii: fj.14-269225 www.fasebj.org

Key Words: mitochondrial targeting • ROS reduction • mitochondrial membrane potential • antioxidant activity

P06 Formulation Development of Novel Peptides for the Treatment of Metabolic Diseases

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Peptide therapeutics present a fast growing share of new drug pipelines, with projections for a global market value of \$25.4 Billion by 2018. As therapeutic products, peptides occupy a niche between typical small molecule drugs and larger proteins. Just as the latter, they feature a modular structure with amino acids linked by peptide bonds as base units, while their size is generally limited, with FDA guidance identifying a boundary of up to 40 residues. Within these limits, peptides nonetheless exhibit assorted chemical and physical stability liabilities related to their amino acid sequences. Proper formulation development is thus required to ensure stability of the drug product, which is critical for preserving the peptide during shelf-life storage and in-use period, as well as for preventing the formation of aggregates which can induce undesirable immune reactions *in vivo*. At Merck, Compound A is currently being investigated in clinical trials for diabetes. Small molecule analytical approaches are leveraged to identify chemical degradation mechanisms, while various large-molecule biophysical characterization techniques are adapted to probe physical and structural stability. The comprehensive characterization toolkit and generated experimental datasets have enabled the rational selection of excipients and composition for definition of a robust formulation. Our learnings are further leveraged to support the development and scale-up of manufacturing processes and to similarly facilitate the preparation of strong Chemistry, Manufacturing and Controls (CMC) regulatory filings.

P07 Isolation and Pharmacological Characterisation of π -TRTX-Hm1a, a Potent Modulator of Acid-Sensing Ion Channel 1a (ASIC1a) from Venom of the Spider *Heteroscodra Maculata*

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Acid-sensing ion channels (ASICs) are chordate-specific members of the degenerin/epithelial sodium channel family that are widely distributed in both the central and peripheral nervous system. The ASIC1a subtype has been implicated in a variety of neuropathological conditions, including pain, psychiatric illness, multiple sclerosis, epilepsy and stroke, and is therefore considered a promising therapeutic target. Currently, the only potent and selective inhibitor of ASIC1a known is PcTx1, a peptide isolated from the venom of the Trinidad chevron tarantula, *Psalmopoeus cambridgei*.

In response to the increasing interest in ASIC1a as a therapeutic target, we used two-electrode voltage clamp (TEVC) electrophysiology on *Xenopus laevis* oocytes expressing homomeric ASIC1a to screen tarantula venoms for novel ASIC inhibitors. Here we describe the isolation and recombinant expression of π -theraphotoxin-Hm1a (Hm1a), a potent ASIC1a modulator from venom of the Togo starburst tarantula *Heteroscodra maculata*. Hm1a rapidly, selectively, and reversibly inhibits ASIC1a with an IC $_{50}$ value of 1.3 nM. Hm1a increases the apparent affinity of ASIC1a for protons, inducing steady-state desensitisation of ASIC1a at physiological pH. Despite sequence similarity with PcTx1, Hm1a is less potent on hASIC1a but more potent on ASIC1b. The shorter C-terminal region of Hm1a may result in improved structural rigidity as compared to PcTx1. Structure-activity relationship studies comparing PcTx1 and Hm1a may be helpful in elucidating important residues for engineering better inhibitors of ASIC1a.

P08 Late Endosomes and its Unique Anionic Lipid Bis(monoacylglycero)Phosphate Act as Doorways for the Endosomal Escape and Cytosolic Entree of the Fluorescently-Labeled Dimeric Cell-Penetrating Peptide dfTAT Alfredo Erazo-Oliveras, Kristina Najjar, Dat Truong, Ting-Yi Wang, Dakota J. Brock, Austin R. Prater & Jean-Philippe Pellois* Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA

Endosomal entrapment is one of the key challenges and a multibillion dollar issue in the field of delivery of biologics/ therapeutics inside live cells. Currently, there is an urgent need to define cell penetration and endosomal escape mechanisms in order to improve existing delivery strategies. We have shown that dfTAT, a recently identified delivery tool, can circumvent this hurdle. In this work we describe the use of dfTAT, a dimeric peptide found to promote endosomal leakage extremely efficiently and without deleterious effects to cells, as a model cell-penetrating peptide that can reveal the cellular organelles and factors involved in endosomal escape. By modulating the trafficking of dfTAT within the endocytic pathway, we identify late endosomes as the unique organelles rendered leaky by dfTAT and as the exit point for dfTAT endosomal escape. dfTAT binds bis(monoacylglycero)phosphate (BMP), a negatively-charged phospholipid found in late endosomes and, consequently, causes the leakage of bilayers containing BMP but not bilayers containing two other negatively-charged lipids, phosphatidic acid (PA) and phosphatydilglycerol (PG), *in vitro* (PG being a structural isomer of BMP). Finally, we establish that an anti-BMP antibody is capable of blocking leakage *in vitro* and endosomal escape in cellulo. Together, these data not only identify late endosomes as the cellular gateways utilizable for efficient delivery protocols, but also sheds light on a mechanism that may provide a fundamental basis for the rational design of future cell-permeable molecules.

P09 Characterization of Cell-Penetrating Peptide Complexes with Oligonucleotides by Fluorescence Correlation Spectroscopy

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Stearylated Transportan 10 (TP10) derivatives, named PepFects (PFs), are a class of peptide vectors known to form nanometer-sized, non-covalent complexes with oligonucleotide cargo (siRNA, pDNA) and transduce into cells *in vitro* and *in vivo*. The structural details and mechanism of transduction are not known for these potential drug delivery systems. The physicochemical nature of the nanocomplexes, namely their size and amphipathicity, limit the biophysical techniques that can be used to address these questions. Fluorescence correlation spectroscopy (FCS) was used to determine the diffusion time (τ D) and concentration of PF14 and siRNA complexes. PF14, the most efficient peptide from the PF family, was labeled with rhodamine 6G, while the cargo was labeled with Cy5. The dynamics of complex formation was studied in aqueous solution at different molar ratios and pHs. It was found that the τ D for PF14K-Rh6G is 60 ± 5 μ s and for Cy5-siRNA is $160 \pm 5 \mu$ s. For the [PF14-Rh6G: Cy5-siRNA] complexes, preliminary data suggest a τ D in the order of 500 μ s and an average molecular weight of 3 MDa. Characterization of complexes in solution by FCS has been successful. This opens the perspective for further cellular uptake and cytoplasmic localization studies.

- Andaloussi, S.E. *et al.* Design of a peptide-based vector, PepFect6, for efficient delivery of siRNA incell culture and systemically *in vivo*. *Nucleic acids research* 39, 3972-3987 (2011).
- 2 Vasconcelos, L. *et al.* Effects of cargo molecules on membrane perturbation caused by transportan10 based cell-penetrating peptides. *Biochimica et biophysica acta* 1838, 3118-3129 (2014).

P10 Single Amino Acid Derivatives and Small Peptides Based Nano-Structures: Examining Their Potential in Drug Delivery

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Design and synthesis of single amino acid derivatives and small peptides with the aim of converting them to desired nano-structures to examine their potential in drug delivery will be discussed. These molecules have been transformed to nano-structures simply by warming ($\approx 60-65\,^{\circ}\text{C}$) them in water. The factors (e.g. time, concentration of the parent molecule, nature of amino acid and its derivative), which influence the formation of these structures having different shapes and sizes, will be highlighted with examples. TEM images have been taken to analyze them. UV-Vis spectra have been recorded before and after the formation of nano-structures to see the change in absorbance. Plausible logics behind the construction of various kinds of nano-structures including nano-tubes (NTs), nano-vesicles and nano-rods will be presented. Preliminary theoretical calculations in support of forming nano-structures will be discussed. Successful internalization of Phe-Phe dipeptide NT in CHO cell with the help of TEM image analysis will be presented.

Acknowledgement: We thank the US Public Health Service, National Institute of Health (NIH) for supporting this project. BK is thankful to UGC-India for awarding him a Raman Postdoctoral Fellowship to take part in this research at the University of Arizona.

- 1 Giri, A. K.; Karthikeyan, B.; Hruby, V. J. The single amino acid derivatives produce bio inspired nano tubes and nano vesicles (Manuscript is in progress).
- 2 Karthikeyan, B.; Giri, A. K.; Kulkarni, V. V.; Hruby, V. J. Simple size-selective separation and microscopic characterization of Phe-Phe nanotubes and study of its internalization inside CHO cells. *Journal of Microscopy and Ultrastructure* 2014, 2, 224-229.

P11 Synthetic Membrane Anchored Ligands: An Innovative Approach to Drug Discovery

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Novel strategies are needed to expedite the generation and optimization of peptide probes targeting G protein-coupled receptors (GPCRs). We have previously shown that membrane tethered ligands (MTLs), constructs comprised of a membrane anchor, an extracellular linker, and a peptide ligand, can be used to develop targeted receptor modulators. Although MTLs provide a useful tool to identify and/or modify active peptides, a major limitation of this strategy is the reliance on recombinant protein expression. We now report the generation and pharmacological characterization of prototype peptide-linker-lipid conjugates, synthetic membrane anchored ligands (SMALs), which are designed as mimics of corresponding recombinant MTLs. Using a range of GPCRs (NK1R, CCK2R, CMKLR1, MrgprX1, GLP-1R), we illustrate the feasibility of the approach and highlight selected pharmacological properties of MTLs/ SMALs both *in vitro* and *in vivo*. Our results suggest that MTLs offer an expedited method to validate peptides that will have enhanced function as SMALs. Once identified and optimized as an active MTL, SMAL mimics can be generated and characterized. These synthetic constructs offer the possibility of direct administration rather than recombinant expression. Our work illustrates a two-step approach of optimizing MTLs and then synthesizing corresponding SMALs. These membrane anchored probes provide powerful tools to investigate GPCR physiology and provides a basis for the development of novel therapeutics. Efforts are in progress to apply the MTL/ SMAL approach to the study of other cell surface proteins.

P12 Chitin-Binding Cysteine-Rich Peptides from Alternanthera Sessilis

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Fungal outbreaks in rice, wheat and maize cost global agriculture \$60 billion per year.¹ Currently, many commercially available anti-fungal agents are toxic to animals and humans. Plants produce a myriad of anti-fungal cysteine-rich peptides as an innate host-defense mechanism with the family of hevein-like peptides being a prime example shared by plant antifungal peptides in their ability to bind to the chitin-containing cell wall to elicit fungicidal effects. Chitin, a polysaccharide containing repeating N-acetylglucosamine (GlcNAc) units, is a primary constituent of fungal cell walls. To gain an insight into this interaction, we used proteomic and genomic methods to isolate and characterize six novel hevein-like peptides, aSR1-R3 and aSG1-G3, collectively called altides from red and green varieties of *Alternanthera sessilis*, a perennial herb belonging to the Amaranthaceae family. ¹H-NMR titration experiments showed that altides bind to (GlcNAc)_n oligomers with dissociation constants in the micromolar range. Changes in chemical shift of aromatic residues occupying the conserved chitin-binding domain indicated their involvement in binding. To our knowledge, aSR1 is the smallest hevein-like peptide with dissociation constant towards (GlcNAc)_n oligomers can help to develop strategies to combat fungal attacks in plants eliminating the use of toxic chemical pesticides.

Fisher, M. C., Henk, D. a., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, 484(7393), 186–194. http://doi.org/10.1038/nature10947

P13 Targeted Pro-apoptotic Peptides Depleting Adipose Stromal Cells Inhibit Tumor Growth

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Progression of many cancers is associated with tumor infiltration by mesenchymal stromal cells (MSC). Adipose stromal cells (ASC) are MSC that serve as adipocyte progenitors and endothelium-supporting cells in white adipose tissue (WAT). Clinical and animal model studies indicate that ASC mobilized from WAT are recruited by tumors. Direct evidence for ASC function in tumor microenvironment has been lacking due to unavailability of approaches to specifically inactivate these cells. We recently designed a proteolysis-resistant targeted hunter-killer peptide D-WAT composed of a cyclic domain CSWKYWFGEC homing to ASC and of a pro-apoptotic domain KLAKLAK2. We reported that ASC depletion with D-WAT limits WAT growth and suppress obesity development in mice (Daquinag et al., Cell Death Differ. 2015 22(2):351-63). Here, we investigate the effects of D-WAT in cancer models. Using mouse bone marrow transplantation models, we show that D-WAT treatment specifically depletes tumor stromal and perivascular cells without directly killing malignant cells or tumor-infiltrating leukocytes. In several mouse carcinoma models, targeted ASC cytoablation reduced tumor vascularity and cell proliferation resulting in hemorrhaging, necrosis, and suppressed tumor growth. We also validated a D-WAT derivative with a pro-apoptotic domain KFAKFAK2 that was found to have an improved cytoablative activity. Our results for the first time demonstrate that ASC, recruited as a component of tumor microenvironment, support cancer progression. We propose that peptide-based drugs targeting ASC can be developed as a combination therapy complementing conventional cancer treatments.

P14 Discovery of New Scaffold for Drug Design from Jasminum Sambac

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Naturally occurring cysteine-rich peptides (CRPs) are known for their diverse range of biological activity and privileged structures. These structures are highly compact and have been successfully used as scaffolds for designing peptide drugs. Here we report the discovery of a new scaffold and its structure of a novel family of CRP, jasmintides isolated from Jasminum sambac of the Oleaceae family. Jasmintide jS1 contains 27 amino acids. It also contains an N-terminal pyro-glutamine which provides resistance to degradation by aminopeptidases. Structure characterization of jS1, elucidated by mass spectrometry and further validated by NMR, revealed a new disulfide connectivity of Cys I-V, Cys II-IV and Cys III-VI and framed by three short antiparallel β -sheets, a structure which is not reported previously in plant CRPs. The compact cysteine-rich structure together with the protected N-terminal site confers jasmintide with high resistance to heat, enzymatic degradation, including exopeptidase treatment and serum. Taken together, these results reveal a new scaffold from plants with unique cystine connectivity for designing of peptide drugs.

P15 The GPCR PAR2 Regulates Regeneration, Transdifferentiation, and Death: Modulation by a PAR2 Agonist Peptide

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Cell and tissue damage, particularly to pancreatic β -cells, is a fundamental aspect of diabetes, being caused by β -cell autoimmunity in type 1 and by obesity-associated factors in type 2. However, the mechanisms by which cells sense and respond to injury remain poorly understood. Recently, we found that pancreatitis induced by duct ligation or the peptide caerulein in the setting of profound β -cell deficiency led to islet cell transdifferentiation by a pathway involving conversion of β -cells to β -cells and then to β -cells. Relevance to diabetes was demonstrated by finding evidence for this pathway in murine and human type I diabetes.

One feature common to all forms of pancreatitis is the aberrant release of pancreatic exocrine enzymes, including proteases. Thus, we identified protease-activated receptors (PARs) as candidates for playing a role in the stimulation of islet cell transdifferentiation. Of the four members of the PAR family, the best candidate for playing a role in α - to β -cell transdifferentiation was PAR2, as it is activated in models of pancreatitis by trypsin. PAR2 is under active investigation as a therapeutic target, with pain and inflammation as the primary indications.

We used a peptide agonist of PAR2 and mice carrying a mutated, inactive form of PAR2, to show that it was necessary and sufficient to induce transdifferentiation in the context of severe β -cell deficiency. Surprisingly, we also found that PAR2 was required for β -cell survival in pancreatitis. Furthermore, it was a key factor in the response to injury in other organs, including the liver and extremities, being required for regeneration of the distal phalange. The ability of a pharmacologically relevant single target, PAR2, to control regeneration and survival in multiple tissues identifies PAR2 as a therapeutic target in those and possibly other disorders involving tissue damage and regeneration, including type 1 diabetes.

P16 Design and Synthesis of Novel Piperine-Peptide Hybrids for Activity Against Trypanosoma Cruzi

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Chagas disease (American Trypanosomiasis) is a zoonosis caused by the protozoan Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae). This parasitic infection is recognized by WHO as one of the thirteen most neglected diseases in the World (WHO, 2012). There are only two drugs for the treatment of chagasic patients: the nitroheterocyclic derivatives benznidazole and nifurtimox. However, none of them meets the criteria (i.e. parasitical cure both in acute as well as in chronic phase of the infection and reduced side effects) required to be an optimal drug to treat this illness. Recently, we reported the trypanocidal effects of the natural amide piperine and its derivatives (FRANKLIM, 2013). It is known that the presence of a fibronectin receptor on the parasite's cell membrane can recognize the peptidic sequence RGDS (OUAISSI, 1986). We have taken a novel approach

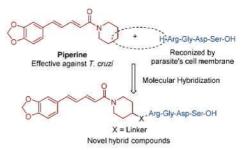


Figure 1. Design of novel piperine-peptide hybrids

of combining these chemical structures to achieve our goal. A known trypanocidal natural product i.e. piperine and the tetrapeptide (Arg-Gly-Asp-Ser) have been taken as reference to our molecular planning (Figure 1). Herein, we describe design and synthesis and the results obtained with these new hybrids for their high affinity to the parasite's cell surface.

References:

FRANKLIM T. N. et al. (2013) Molecules, 18: 6366-6382, and references cited therein.

OUAISSI, M.A. et al. (1986) Science, 234, pp. 603-697.

WHO-World Health Organization (2012). Research priorities for Chagas disease, human African trypanosomiasis and leishmaniasis. Technical report series, nr. 975.

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P17 Protein Kinase C (PKC) Delta Activation Negatively Regulates Phorbol 12-Myristate 13-Acetate (PMA) Induced Superoxide (SO) Release in Polymorphonuclear Leukocytes (PMNs)

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It's known that PMA is a broad-spectrum PKC activator that augments SO release in PMNs via NADPH oxidase activation. It was shown that PKC delta (δ) negatively regulates PMN elastase release which suggests that PKC δ may also negatively regulate PMN NADPH oxidase SO release. To test the hypothesis, we altered PKC δ activity using selective cell permeable PKC δ activator, inhibitor peptides, and rottlerin, another type of PKC δ inhibitor. We predict that PKC δ activation would attenuate PMA-induced PMN SO release, whereas PKC δ inhibition would augment the response. Isolated PMNs (5x106) from male Sprague-Dawley rats were incubated in the presence/absence of PKC δ activator (Myr-MRAAEDPM, MW=1130 g/mol, 2.5-20 μ M, n=6-8), PKC δ inhibitor (Myr-SFNSYELGSL, MW=1326 g/mol, 1-20 μ M, n=6-10) or rottlerin (MW=516 g/mol, 2.5-20 μ M, n=8-9) prior to PMA (MW=616 g/mol, 15 or 30 nM, n=11-16) stimulation. SO release was measured spectrophotometrically by the reduction of ferricytochrome C. At the end of the assay, cell viability was determined by 0.3% trypan blue exclusion. As expected, PKC δ peptide activator attenuated PMN SO release up to 56%, whereas, rottlerin augmented the PMA response up to 90% compared to untreated controls (p<0.05). However, the PKC δ peptide inhibitor only enhanced PMN SO release up to 26% and was not significantly different from controls. Moreover, cell viability was >92±5% in all study groups. The data suggest that PKC δ negatively regulates PMN NADPH oxidase SO release. PKC δ peptide inhibitor is relatively ineffective regulating PMN SO release compared to rottlerin.

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

P18 Structure and Activity Relationship Studies of a Highly Endosomolytic Fluorescently Labeled Dimer of the Cell Penetrating Peptide TAT (dfTAT): Effects on Cytosolic Cellular Penetration

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The plasma membrane acts as a barrier to hydrophilic molecules and hinders delivery of cell-impermeable macromolecules into the cytosolic space of live cells. Cell-penetrating peptides (CPPs) have been used to aid in the internalization of macromolecules inside cells utilizing the endocytic pathway. However, endosomal entrapment severely limits the efficiency of macromolecular delivery into the cytosolic space of cells. Achieving efficient release of macromolecules from endosomes could increase the potential of this technology in medicine as well as in biomedical research. In our laboratory we have generated a peptide dfTAT, derived from the prototypical CPP TAT, which penetrates live cells by escaping from endosomes with a particularly high efficiency and no noticeable impact on cell physiology. Understanding the molecular features that confer the high endosomolytic activity of dfTAT is crucial and has not yet been identified. Therefore this project focuses on establishing the structure activity relationships that will help in identifying the molecular properties required for this efficient endosomal escape. In this study, a focused library of peptide analogues based on the prototype dfTAT was synthesized and characterized for cellular penetration and endosomolytic activity. This knowledge is critical since it will aid in the understanding of molecular features that are essential to achieve efficient endosomolytic activity. This project also lays a foundation for the design of cellular delivery agents with improved therapeutic value.

P19 Decoding the Cellular Transport Mechanisms for Peptide-Mediated Drug Delivery

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Cationic cell-penetrating peptides (CPPs) have proven their usefulness in overcoming barriers (such as cell membranes) for drug delivery. We recently discovered a novel class of CPPs characterized by a basic sequence R/KXXR/K motif. With this motif at the carboxy(C)-terminus, peptides bind to neuropilin-1 (NRP1), and initiate active endocytosis into cells and through tissues (C-end Rule or CendR motif). Tumor-penetrating CendR peptides contain a cryptic CendR motif, which is proteolytically exposed after they have specifically homed to a tumor. The activation of the CendR process allows the peptides to extravasate and penetrate deep into extravascular tumor tissue, and can take along coupled or co-administered cargo. Here, by comparatively studying CendR and other cationic CPPs, we have characterized the cellular machinery of the cell and tissue penetration, and unveiled an underlying regulatory mechanism.

Through a genome-wide RNAi screening and subsequent validation studies, we found that CendR peptides initiate a receptor-dependent macropinocytosis-like process, which uses genetic machinery distinct from all known endocytic pathways. Furthermore, the activity of this pathway is stimulated by mTOR inhibition and nutrient deprivation. We also observed cell-to-cell transport of CendR cargo, which, like the endocytosis, is stimulated by nutrient deprivation. By comparing CendR and TAT peptides, we established CendR pathway as a cell entry route also used by certain TAT variants, and determined the subcellular transport route by ultrastructural imaging. We found that the CendR-NRP1 interaction determines the ability of CPPs to induce vascular permeability. These results highlight the importance of cell biology studies to the drug delivery field.

P20 Inhibition of One Substrate Phosphorylation of a Protein Kinase out of Many Substrates by a Selective Peptide Inhibitor of Kinase-Substrate Interaction

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Many kinases phosphorylate multiple substrates. To assess the role of an individual phosphorylation event without using laborious mutagenesis studies, selective pharmacological tools that inhibit phosphorylation of specific one substrate are needed. We focused on delta protein kinase C (delta-PKC) because delta-PKC activation after heart attack leads to cardiac damage. We reasoned that since pyruvate dehydrogenase kinase (PDK) phosphorylation by delta-PKC leads to inhibition of ATP production, it might be critical in the injurious effect delta-PKC after heart attack. To determine if of the many substrates of delta-PKC, PDK is the substrate that mediates this injurious effect in the heart, we designed a short protein-protein interaction inhibitory peptide (PPIIP) to selectively inhibit delta-PKC phosphorylation of PDK. PDK/delta-PKC interaction was inhibited by PPIIP *in vitro* and *in vivo*. PDK/ delta-PKC PPIIP selectively inhibited phosphorylation of PDK without affecting phosphorylation of several other delta-PKC substrates. Treatment with PDK/ delta-PKC PPIIP inhibited myocardial infarction-induced PDK phosphorylation without inhibiting the phosphorylation of other delta-PKC substrates. Further, PDK/ delta-PKC PPIIP treatment led to a 50% reduction in infarct size, in release of cardiac enzyme and in JNK phosphorylation, all markers of cardiac injury (Figure 1). PDK/delta-PKC PPIIP is a selective inhibitor of PDK phosphorylation by delta-PKC. Of the several delta-PKC-mediated phosphorylation events following heart attack, delta-PKC-induced PDK phosphorylation is sufficient to cause cardiac injury as a selective inhibitor of this phosphorylation event alone was sufficient to provide great reduction in delta-PKC-mediated cardiac injury after heart attack.

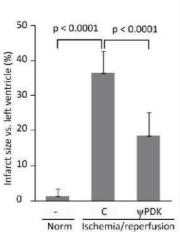


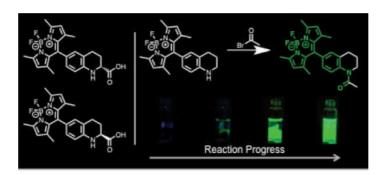


Figure 1: PPIIP protect from cardiac injury in animal model.

Rats subjected to 30 minutes ischemia and 24 hours reperfusion in vivo were treated with PPIIP or control, and infarct size was determined using TTC staining. Infarct size/left ventricle (%) is provided on the left and examples of TTC staining on the right. PPIIP treatment led to a 50% reduction in infarct size.

P21 Design, Synthesis and Proof of Concept for the First Fluorogenic Amino Acid That 'Turns-on' Its Fluorescence Upon Amide Bond Formation

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Fluorescent probes and probes with clickable functional handles for fluorescence tagging are an invaluable tool to the life sciences and are widely applied across many biological and chemical disciplines. This is especially true for amino acid based probes and biomarkers within the context of peptide and drug research. A common downfall to these methods is the researchers inability to observe realtimelabeling due to the experimental subject being flooded with fluorescent probes. Thus additional fixing and washing steps are often required for labeling protocols. With this understanding we have recently designed and synthesized three bodipy based fluorogenic probes possessing a tetrahydro-quinoline amine. Two of these are L and D amino acids. Our main intent was to design amino acids that become fluorescent upon forming an amide (peptide) bond. A proof of principle experiment was designed and carried out using the decarboxylated version of this probe. The experiment chemically transformed the amine of the probe to an amide bond. The remarkable enhancement of fluorescence upon amide bond formation was quantified to be over 100 times brighter at an emission of 512 nm. The amino acid version also showed an enhanced fluorescence upon amide bond formation. To the best of our knowledge, this marks the first fluorogenic amino acid that turns on its fluorescence due to forming an amide bond.

P22 Plaque-Penetrating Peptide Inhibits Atherogenesis

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The cardinal feature of atherosclerosis is the formation of plaques in arterial wall, which gradually obstruct blood flow. Rupture of a plaque can cause sudden closure of the vessel by a blood clot, leading to catastrophic ischemic event such as heart infarct or stroke. Atherosclerosis treatments are generally aimed at altering systemic lipid metabolism such that atherogenesis, the formation of plaque, is curtailed. The plaques themselves offer some potential therapeutic targets. For example, selective depletion of macrophages, which play a key role in atherogenesis, inhibits plaque formation, as does reducing the macrophage lipid content. However, it has not been possible to take advantage of these targets because the drugs that have been tested have not been sufficiently selective. We have developed as a peptide, LyP-1, which specifically targets atherosclerotic plaques, penetrates into plaque interior, and accumulates in plaque macrophages. We have also shown that LyP-1 can cause apoptosis in cells that take it up effectively. These observations prompted us to test the possibility that characteristics might render LyP-1 capable of inhibit plaque formation. Here we show using three different atherosclerosis models in ApoE null mice that prolonged treatment with systemic injections of LyP-1 indeed reduces plaque, and that it does so without causing detectable side effects. We also show that LyP-1 recognizes human plaque. These findings suggest that LyP-1 could serve as a prototype for the development of a new class of anti-atherosclerosis drugs.

P23 Rapid SAR of Peptide Therapeutics for Diabetes and Beyond

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We have leveraged a hybrid method of peptide manufacturing for the rapid discovery of novel thermostable insulin (TSI) and solution stable glucagon peptides. The peptide production method combines recombinant production with chemical cleavage to achieve orthogonal purification with high efficiency. Leveraging this technology we have produced a large number of target peptides and created a structure activity tree that has allowed us to discover both short and long acting thermostable insulins as well as glucagon that is solution stable. We will provide an overview of the rapid SAR method and its application to peptide drug discovery of novel TSI and glucagon, and will present stability data, as well as in-vitro and in-vivo activity data from a select number of candidate TSIs demonstrating that we can modulate activity via both conformational constraint and standard mutations within both the A and B chains. We will also present solution stability data and *in-vitro* activity for a series of novel glucagon analogs that have 40oC solution stability ranging from 7 days to 3 months in phosphate buffered saline. We will conclude with initial results applying the method to peptides in new therapeutic and commercial areas including bowman-birk inhibitors, toxins and the natural sweetener brazzein.

P24 Novel Lipid II-Binding Antibiotic Peptides Identified via Bicyclic Phage Display

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With the emerging threat of the "post-antibiotic era" novel antibiotics are in dire need. The majority of known antibiotics derive from natural sources and as such natural resistance mechanisms have also evolved with them.¹ We here describe the *de novo* discovery of novel Lipid II-binding antibiotic peptides from non-natural sources via bicyclic phage display.² Lipid II (Figure 1) is the monomeric building block used in bacterial cell wall biosynthesis and is a validated antibacterial target. Sequestration of Lipid II can obstruct cell wall synthesis leading to bacterial cell death.³ We employed a pair of enantiomeric Lipid II analogues (Figure 1, target 1 and 2) for use in (mirror image) peptide phage display. The Lipid II inspired targets 1 and 2 were synthesized in a 30-step convergent synthetic route and used in an optimized phage display selection protocol. The output of the phage display selections was analyzed by high-throughput DNA sequencing technology and resulted in the identification of three novel antibiotic peptide sequences. The peptides were synthesized and tested against various bacterial strains. Most noteworthy are the results obtained with vancomycin-resistant enterococci against which the peptides have potent antibacterial effects. Further experiments showed that these peptides indeed have the capacity to bind to lipid II. With these peptides serving as lead compounds, strategies for enhancing their activities (i.e. alanine scans and lipidation) were also explored. We here demonstrate that bicyclic peptide phage display is a useful approach for identifying novel antibiotic peptides that do not come from natural sources.

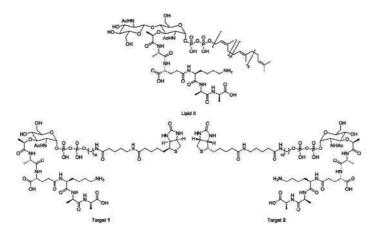


Figure 1. Structures of Lipid II and Lipid II inspired targets used in bicyclic peptide phage display

References

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P25 Discovery of a Novel Carboxypeptidase Inhibitor from the Medicinal Herb Wolfberry, *Lycium Barbarum* Wei Liang Tan, Giang K. T. Nguyen, Ka H. Wong, James P. Tam*

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Metallocarboxypeptidaes (MCPs) are zinc-dependent exoproteases that are involved in a wide range of physiological processes. Unregulated expression of MCPs in mammalian extra-pancreatic tissues has been implicated in the cause and progression of human diseases and their inhibitors could be potentially useful as therapeutics. Carboxypeptidase inhibitors (CPIs) have been used in the treatment of human blood coagulation disorders. Since the discovery of the prototypic proteinaous CPIs four decades ago from the potato and tomato plants, only nine naturally occurring CPIs have been isolated and characterized thus far, and none from plants. Here, we report the isolation and characterization of a novel CPI from a highly popular medicinal herb wolfberry, wolfberry carboxypeptidase inhibitor (WCI). Transcriptome mining of protein databases revealed the existence of 30 plant CPIs, expanding their number to 32 members from plants in Solanaceae and Asteraceae families. Sequence analysis revealed amino acid residues that are highly conserved throughout the CPI family that could play a role in their inhibitory function. The identification of CPI sequences provide a basis for studying diversity within the CPIs family, and which could provide leads for developing therapeutics for human diseases.

P26 Membrane Oxidation and Oxidized Lipids Mediate Efficient Delivery of Cell-Penetrating Peptides into Live Cells

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Cell-penetrating peptides (CPPs) are promising approaches to deliver biologically active molecules or probes into live cells. However, the underlying mechanism by which CPPs translocate across cellular membrane is not thoroughly unveiled. This impedes their applications for therapeutic treatments and for the study of important biological processes. Here we describe a novel and unexpected mechanism for the cell penetration by polyarginine CPPs. For the first time, we reveal that the cell penetration of CPPs is dependent upon cellular oxidative stress and on the presence of oxidized lipids at the plasma membrane of human cells. Oxygen tension and antioxidants can dramatically impact cell penetration of the peptide, which involves direct membrane translocation. We also show that naturally occurring oxidized lipids within the plasma membrane directly mediate the rapid and efficient transport of the peptide across this biological barrier by forming inverted micelles with CPPs. Our findings would thereby provide new fundamental insights on the process of membrane translocation. This novel mechanism also leads to new opportunities for rationale design of highly efficient cell-permeable compounds and drug delivery strategies.

P27 In Vitro Evaluation of New, Potent and Selective V2 Receptor Agonists

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The vasopressin analogue desmopressin (dDAVP) is a potent V_2 receptor agonist that also activates the related V_{1b} receptor. dDAVP is approved in many countries for the treatment of diabetes insipidus, primary nocturnal enuresis, nocturia, and coagulation disorders including hemophilia A and von Willebrand's disease.

In search of novel potent and selective peptidic V_2R agonists, we synthesized a series of C-terminally truncated [Val⁴]dDAVP¹ analogs modified in positions 2 (Ar¹), 3 (Ar²), 7 (Z) and/or at the disulfide bridge (X, Y). The peptides were evaluated for *in vitro* potency (hV₂R) and selectivity (hV_{1a}R, hV_{1b}R, hOTR). Analogs in which the C-terminal glycine amide was replaced by various R¹ groups (H, CH₂OH, C(O)NR³R⁴) displayed high potencies *in vitro* as hV₂R agonists and superior selectivity profiles as compared to dDAVP. Synthetic details and comprehensive *in vitro* data for the new compounds will be presented.

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P28 Magnetic Nano-Particles Assisted Enzymatic Synthesis of Glycopeptides and Glycoamino Acids Hailiang Zhu, Zhigang Wu, Mohui Wei, Christopher Gibbons and Peng George Wang Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA

Glycosylation of peptides is one of the most significant of peptide modifications. Solid phase peptide synthesis(SPPS) provides a very useful synthetic tool for the synthesis of glycopeptides. However, it is still laborious work to synthesize glycopeptides either use a chemical or enzymatic method, especially when glycans are complex. Laborious work is mainly caused by the purification steps. In our work, we want to develop a solid phase synthesis method to chemo-enzymatically synthesize complex glycopeptides to reduce laborious purification steps. Solid phase supports play a very important role in chemical and enzymatic reactions. The regular resins for SPPS have a limited application in enzymatic glycosylation reactions of glycopeptides. Herein, we are focusing on using magnetic nano-particles as our solid support to run enzymatic glycosylation reactions. For the enzymatic reactions on nano particles, linker and spacer are another two critical roles to finish the compounds without significant side reactions, therefore a photo cleavable linker is incorporated onto a PEGlated poly glycine spacer. Any glycopeptides with a free amine either on its N terminal or side chains can be connected onto NHS activated photo cleavable linker and then enzymatic extension can be run on the glycopeptides. When enzymatic extension is finished, the extended glycopeptide can cleaved with a UV lamp under 366 nm wave length.

















