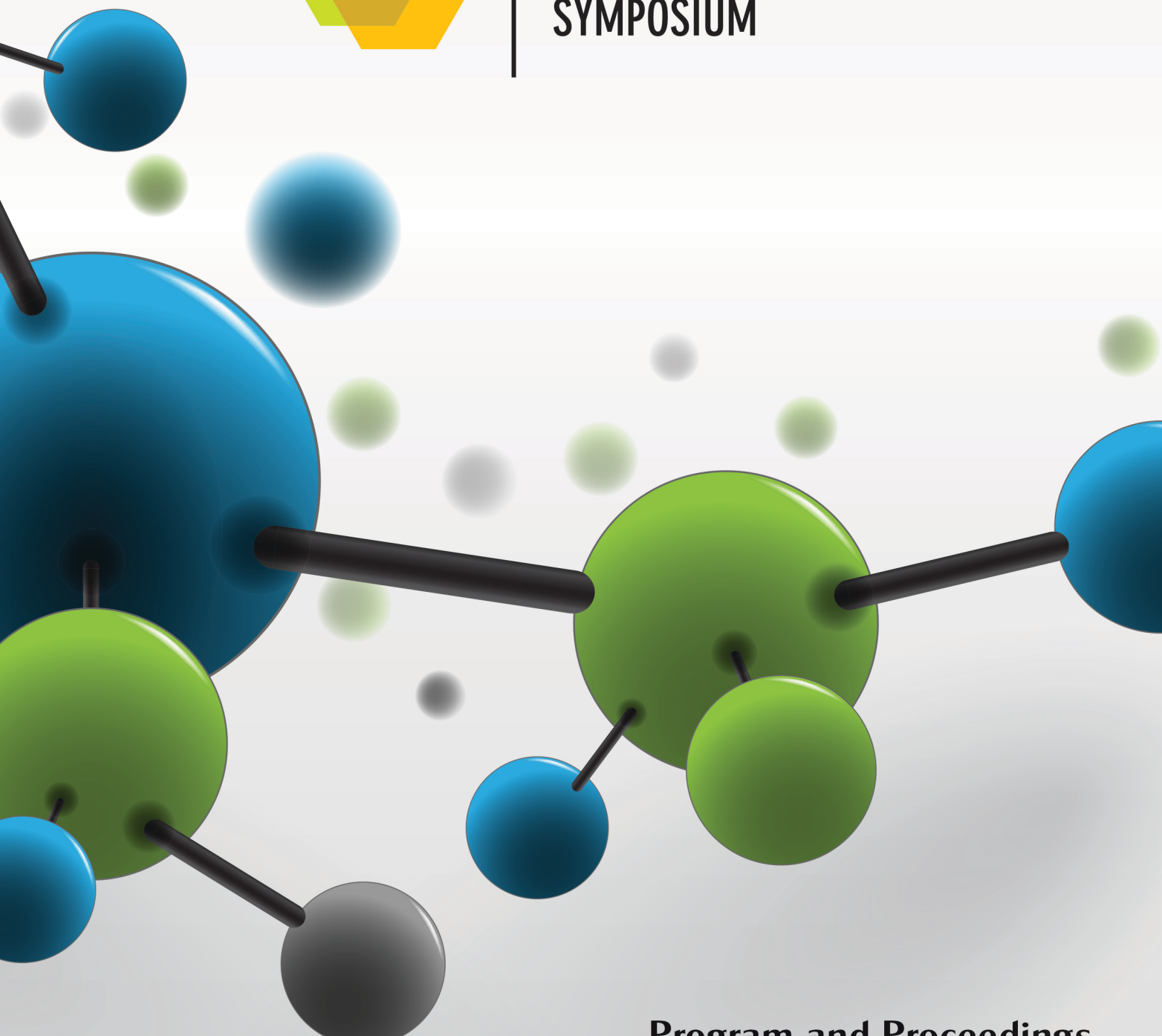




**PEPTIDE  
THERAPEUTICS  
SYMPOSIUM**



**Program and Proceedings  
11th Annual Peptide Therapeutics Symposium**

**October 27 – 28, 2016  
Salk Institute for Biological Studies, La Jolla, CA**

**[www.peptidetherapeutics.org](http://www.peptidetherapeutics.org)**

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### Symposium Sponsors



# 11th Annual Peptide Therapeutics Symposium

## Dear Colleagues,

The Peptide Therapeutics Foundation and its sponsors welcome you to the 11th Annual Peptide Therapeutics Symposium. The goal of this annual conference 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 a lecture from Atul Chopra, Professor Baylor College of Medicine, describing pioneering work that led to the recent discovery of a new hormone, named Asprosin. A series of state-of-the-art talks, largely directed at the quest for Class B GPCR-directed therapy that is orally administered, will follow. The Opening Reception and poster viewing session will complete Thursday's program.

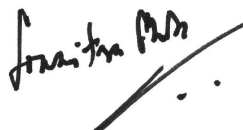
Friday morning we commence the day with Plenary Lectures from Professors Frances Arnold and Chi-Huey Wong highlighting "Innovation by Evolution: Expanding the Enzyme Universe" and "Homogeneous Glycoproteins for Structural and Functional Study," respectively. The remainder of the day consists of in-depth 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  
Peptide Therapeutics Foundation



Soumitra Ghosh  
President  
Peptide Therapeutics Foundation

## Sponsors, Peptide Therapeutics Foundation

Ferring Research Institute, Inc.  
Ipsen Biosciences Inc.  
MedImmune  
The PolyPeptide Group  
Zealand Pharma  
Zydus Cadila  
Peptide Therapeutic Foundation



## 11th Annual Peptide Therapeutics Symposium



### Ferring Research Institute, Inc.

Headquartered in San Diego, California, Ferring Research Institute, Inc., (FRI) is the global peptide/protein therapeutics research center for Ferring Pharmaceuticals. Established in 1996, FRI is located in the heart of the Southern California biopharmaceutical community. The center has attracted a diverse group of highly skilled professionals representing over twenty four countries of origin. FRI is focused on the following key therapeutic areas: endocrinology, reproductive health, urology, and gastroenterology. Our state-of-the art facility includes peptide and protein drug design, chemistry, pharmacology, biology, and preclinical ADME capabilities. FRI is committed to building a portfolio of novel, innovative peptide and protein 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 5,000 employees worldwide, it operates subsidiaries in 56 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

Ipsen is a global specialty care-driven biopharmaceutical group with total sales exceeding €1.4 billion in 2015. Ipsen sells more than 20 drugs primarily across three franchises: oncology, endocrinology, and neurology. Ipsen's internal R&D focuses on its innovative and differentiated technological platforms: peptides and toxins. Ipsen's R&D sites are located in the hearts of leading life sciences hubs (Cambridge, USA; Oxford, UK; Les Ulis, France) and are supported by an active policy of partnerships.

#### Peptides: long-standing expertise

Ipsen has long-standing expertise in peptides, ranging from discovery to delivery, that is being leveraged to create highly differentiated drugs addressing targets that are not readily druggable by small molecules or antibodies. Ipsen Bioscience, Ipsen's new R&D center in Cambridge, develops peptide-based drugs to fulfill major unmet medical needs in endocrinology and oncology.

# 11th Annual Peptide Therapeutics Symposium



## MedImmune/AstraZeneca

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; 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 manufacturing sites which focus on proprietary and generic GMP-grade peptides for the pharmaceutical and biotechnological market. With more than 60 years of experience, the Group is committed to the highest quality of peptide manufacturing for commercial peptide drug substances, GMP peptides in clinical trials, or small-scale non-GMP custom syntheses.

The PolyPeptide Group has grown by selective acquisition of existing expertise, culminating in its position today as a leader in peptide manufacturing. The Group has manufacturing facilities in Sweden (Malmo), France (Strasbourg), India (Ambernath) and two sites in the USA (San Diego CA & Torrance CA). As a multinational company with about 520 employees worldwide, its diversity brings breadth and depth of knowledge and experience to the Group.

The Group's long-established core strength in GMP manufacturing and broad range of services supports peptide & peptide-like projects, including conjugation to non-peptide moieties, from the bench through to commercialization. With continually increasing capacity for GMP manufacturing, the PolyPeptide Group is stronger and better equipped to serve the needs of its customers at all stages of pharmaceutical peptide development. With its multinational organization, strict focus on peptides and solid financial base, the Group offers an almost unique security of supply to its customers.

## 11th Annual Peptide Therapeutics Symposium



### Zealand Pharma

Zealand Pharma A/S (NASDAQ OMX Copenhagen: ZEAL) (“Zealand”) is a biotechnology company specialized in the discovery, design and development of peptide-based therapeutics. The company has a portfolio of medicines and product candidates under license collaborations with Sanofi, Boehringer Ingelheim and Helsinn and a proprietary pipeline of product candidates, which primarily target specialty diseases with significant unmet needs. Zealand builds on internal innovation and has a partnering strategy to leverage its competencies and grow the pipeline through to commercialization.

Zealand is the inventor of lixisenatide, a once-daily prandial GLP-1 analog for the treatment of type 2 diabetes, which is licensed to Sanofi and marketed globally outside the U.S. as Lyxumia®. In July 2016, lixisenatide received U.S. FDA approval as Adlyxin. Sanofi has also developed iGlarLixi, a fixed-ratio combination of lixisenatide and Lantus® (insulin glargine) which product is under regulatory review in the U.S. and in Europe.

Zealand’s proprietary pipeline includes glepaglutide\*, a GLP-2 analog in Phase II for the treatment of short bowel syndrome, and dasiglucagon\*, a glucagon analog in Phase II as a single-dose rescue therapy for severe hypoglycemia and in Phase II preparation as a multiple-dose component in a dual-hormone artificial pancreas system; and other earlier stage clinical and preclinical peptide therapeutics. The company has approximately 120 employees and is based in Copenhagen, Denmark.

\* Glepaglutide and dasiglucagon are proposed International Nonproprietary Names (pINNs)



### 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.

# 11th Annual Peptide Therapeutics Symposium



## PEPTIDE THERAPEUTICS FOUNDATION

### Peptide Therapeutics Foundation

Peptide Therapeutics Foundation is a not-for-profit 501C (3), established in 2008 to promote research and development of peptide therapeutics. The Foundation is currently supported by six corporate sponsors; Ferring Research Institute, Inc., Ipsen Biosciences Inc., MedImmune, the PolyPeptide Group, Zealand Pharma, and Zydus Cadila.

The Foundation sponsors an annual meeting, Peptide Therapeutics Symposium, which brings together world leaders from academia, the biopharmaceutical industry, CMOs, CROs, and investors interested in all aspects of peptide R&D, including drug discovery, safety and toxicology, clinical development, manufacturing, pharmaceutical development, formulation, drug delivery and regulatory affairs.

### 2016 Travel Grant Awardees

**Kimberly Barnash**, University of North Carolina

**Christina Brock**, Texas A&M, College of Veterinary Medicine

**Dakota Brock**, Texas A&M

**Sasha Fraser**, Tufts University

**Antony Kam**, Nanyang Technological University

**Shining Loo**, Nanyang Technological University

**Dominic McBrayer**, University of Nevada, Reno

**Julia Piccoli**, Universidade Estadual Paulista

**Christina Schroeder**, The University of Queensland

**Luis Daniel Vasconcelos**, Stockholm University

**Fa Zhang**, Indiana University

**Dan Zhou**, California Institute of Technology

## 11th Annual Peptide Therapeutics Symposium

### Thursday, October 27, 2016

- 12:30 p.m. – 5:00 p.m.      **Registration Check-in**  
Fritz B. Burns Reception Center, Lower Level
- 1:30 p.m. – 5:30 p.m.      **11th Annual Peptide Therapeutics Symposium**  
Conrad T. Prebys Auditorium
- 1:30 p.m. – 1:45 p.m.      **Opening Remarks**  
Richard DiMarchi, Ph.D.  
*Chairman of the Board, Peptide Therapeutics Foundation*  
*Distinguished Professor of Chemistry, Gill Chair in Biomolecular Sciences,*  
*Department of Chemistry, Indiana University;*  
*Site Director, Novo Nordisk Research Center, Indianapolis*
- 1:45 p.m. – 2:30 p.m.      **Plenary Lecture**  
**Moderator**  
Richard DiMarchi, Ph.D.
- Asprosin, a Fasting-Induced Glucogenic and Orexigenic Protein Hormone**  
Atul Chopra, M.D., Ph.D.  
*Caroline Wiess Law Scholar, Assistant Professor, Department of Molecular and Cellular*  
*Biology, Department of Molecular and Human Genetics, Baylor College of Medicine*
- 2:30 p.m. – 3:30 p.m.      **Session I:**  
**Moderator**  
Andrew Parker, Ph.D., MBA  
*Chief Science Officer and Senior Vice President, Head of Research & External*  
*Innovation, Zealand Pharma*
- 2:30 p.m. – 3:00 p.m.      **Tailoring Peptides for Less Frequent Dosing and Non-invasive Delivery**  
Jesper Lau, Ph.D.  
*Vice President, Diabetes Protein and Peptide Chemistry, Diabetes Research Unit,*  
*Novo Nordisk A/S*
- 3:00 p.m. – 3:30 p.m.      **Non-Peptide GLP-1 Receptor Agonists; From Idea to Medicine**  
Carmen Valcarce, Ph.D.  
*Senior Vice President and Chief Scientific Officer, vTv Therapeutics*
- 3:30 p.m. – 4:00 p.m.      **Break**  
Fritz B. Burns Reception Center, Lower Level
- 4:00 p.m. – 5:30 p.m.      **Session II:**  
**Moderator**  
Yvonne Angell, Ph.D.  
*Director of Peptide Chemistry, ChemPartner – San Francisco*
- 4:00 p.m. – 4:30 p.m.      **Structural Elucidation of Ligand Binding Sites in Class B GPCRs and Their Application in Drug Discovery**  
Ali Jazayeri, Ph.D.  
*Chief Technology Officer, Heptares Therapeutics*
- 4:30 p.m. – 5:00 p.m.      **Selective Inhibition of Cytokines with Peptide Therapeutics: A Unique Alternative to Conventional MAB and JAK Inhibitor Approaches for Cytokine Modulation**  
Nazli Azimi, Ph.D.  
*Founder, President and CEO, Bioniz Therapeutics, Inc.*
- 5:00 p.m. – 5:30 p.m.      **Peptide-based Strategies to Treat Inflammatory and Autoimmune Diseases: From the Peptide P140 to Lupuzor™**  
Sylviane Muller, Ph.D.  
*Professor and CNRS Director, Molecular and Cellular Biology Institute, University of Strasbourg*

# 11th Annual Peptide Therapeutics Symposium

5:30 p.m. – 7:30 p.m.

## Poster Session & Opening Reception

Fritz B. Burns Reception Center, Lower Level

## Friday, October 28, 2016

7:00 a.m. – 11:30 a.m.

## Registration Check-in

Fritz B. Burns Reception Center, Lower Level

7:00 a.m. – 8:15 a.m.

## Breakfast & Poster Viewing

Fritz B. Burns Reception Center, Lower Level

8:15 a.m. – 5:15 p.m.

## 11th Annual Peptide Therapeutics Symposium

Conrad T. Prebys Auditorium

8:15 a.m. – 8:30 a.m.

## Welcoming Remarks

Soumitra Ghosh, Ph.D.

*Director and President, Peptide Therapeutics Foundation*

*President, Doon Associates LLC*

8:30 a.m. – 9:50 a.m.

## Plenary Lectures

### Moderator

Phil Dawson, Ph.D.

*Associate Professor, Associate Dean of Graduate Studies, Department of Chemistry,*

*The Scripps Research Institute*

8:30 a.m. – 9:10 a.m.

## Innovation by Evolution: Expanding the Enzyme Universe

Frances H. Arnold, Ph.D.

*Dickinson Professor of Chemical Engineering Bioengineering and Biochemistry,*

*Division of Chemistry and Chemical Engineering, California Institute of Technology*

9:10 a.m. – 9:50 a.m.

## Homogeneous Glycoproteins for Structural and Functional Study

Chi-Huey Wong, Ph.D.

*Professor of Chemistry, Department of Chemistry, The Scripps Research Institute*

9:50 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

Abhijit Bhat, Ph.D.

*Director, Peptide Therapeutics Foundation*

*Vice President Chemistry, Ipsen Bioscience Inc.*

10:30 a.m. – 11:00 a.m.

## AB103: Host-Oriented Therapeutics to Control Life Threatening Infections

Anat Shirvan, Ph.D.

*Executive Vice President, Research & Development, Atox Bio, Ltd.*

11:00 a.m. – 11:30 a.m.

## Engineering Potent and Selective Inhibitors of Nav1.7 Sodium Channel

Les Miranda, Ph.D.

*Director Research, Therapeutic Discovery, Amgen Inc.*

11:30 a.m. – 12:00 p.m.

## Targeting Novel Polysaccharide-binding Proteins to Block Tissue Fibrosis

Eva Ann Turley, Ph.D.

*Co-Founder, Novare Pharmaceuticals; Distinguished Oncology Scientist, London*

*Regional Cancer Program, Lawson Research Institute; Professor, Departments of*

*Oncology, Biochemistry and Surgery, University of Western Ontario*

12:00 p.m. – 1:00 p.m.

## Lunch & Poster Viewing

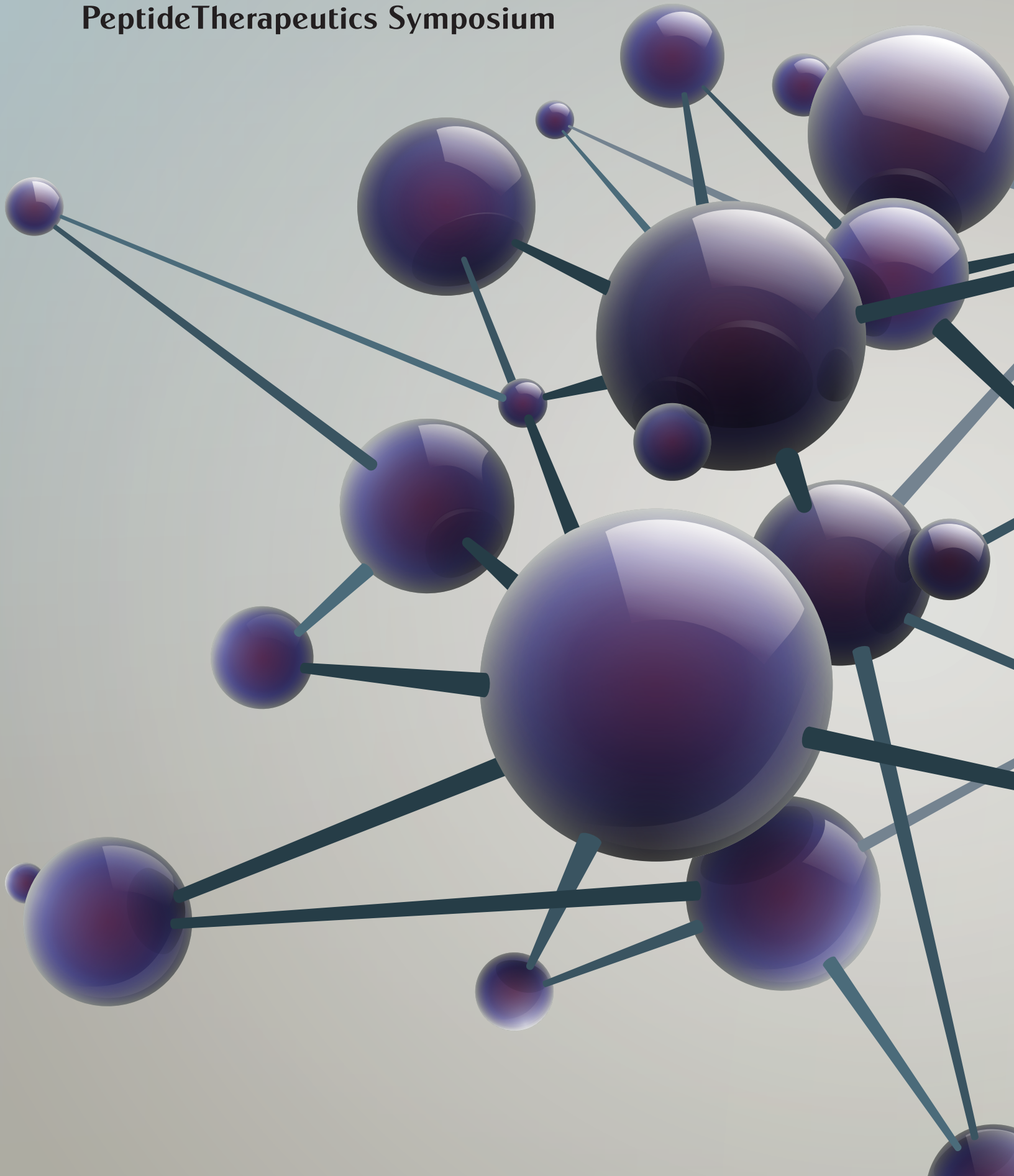
Fritz B. Burns Reception Center, Lower Level

## 11th Annual Peptide Therapeutics Symposium

1:00 p.m. – 3:00 p.m.	<p><b>Session II:</b>  <b>Moderators</b>            Rodney Lax, Ph.D.  <i>Director, Peptide Therapeutics Foundation</i>  <i>Senior Director of Business Development, The PolyPeptide Group</i></p> <p>Waleed Danho, Ph.D.  <i>Distinguished Research Leader (Retired), Hoffman-LaRoche, Inc.</i></p>
1:00 p.m. – 1:30 p.m.	<p><b>TLQP-21 and C3aR1, a Novel Ligand/Receptor Target for Lipolysis and Obesity</b>            Alessandro Bartolomucci, Ph.D.  <i>Associate Professor of Physiology, Department of Integrative Biology and Physiology, University of Minnesota</i></p>
1:30 p.m. – 2:00 p.m.	<p><b>Discovery and Clinical Development of Elamipretide</b>            Hazel H. Szeto, M.D., Ph.D.  <i>Adjunct Professor, Weill Cornell Medicine</i></p>
2:00 p.m. – 2:30 p.m.	<p><b>Unlocking the Mysteries of Amyloid Diseases with Macrocyclic <math>\beta</math>-Sheet Peptides</b>            James S. Nowick, Ph.D.  <i>Professor of Chemistry and Department Chair, University of California, Irvine</i></p>
2:30 p.m. – 3:00 p.m.	<p><b>Endocrine FGFs and Structure-Activity-Relationship</b>            Alexei Kharitononkov, Ph.D.  <i>Professor, Department of Chemistry, Indiana University</i></p>
3:00 p.m. – 3:30 p.m.	<p><b>Break &amp; Poster Viewing</b>            Fritz B. Burns Reception Center, Lower Level</p>
3:30 p.m. – 5:00 p.m.	<p><b>Session III:</b>  <b>Moderator</b>            Claudio D. Scheingart, Ph.D.  <i>Director, Peptide Therapeutics Foundation</i>  <i>Vice President, Science &amp; Technology, Ferring Research Institute, Inc.</i></p>
3:30 p.m. – 4:00 p.m.	<p><b>New Therapeutics from the Human Microbiome: Discovery of Novel Proteins and Peptides for the Treatment of Gastrointestinal Barrier Related Disorders</b>            Karim Dabbagh, Ph.D.  <i>Chief Scientific Officer, Second Genome, Inc.</i></p>
4:00 p.m. – 4:30 p.m.	<p><b>Chemo-enzymatic Peptide Synthesis (CEPS): A Generally Applicable, Traceless Ligation Technology for the Synthesis of Peptide-to-peptide and Peptide-to-protein Conjugates</b>            Timo Nuijens, Ph.D.  <i>Lead Scientist, EnzyPep</i></p>
4:30 p.m. – 5:00 p.m.	<p><b>InGell® – Injectable Drug Depots for Short and Mid-term Release of Soluble and Non-soluble API's</b>            Mike G.W. de Leeuw, MSc, BBA  <i>Founder and Chief Business Officer, InGell Labs BV</i></p>
5:00 p.m. – 5:15 p.m.	<p><b>Closing Remarks</b>            Adrienne Day, Ph.D.  <i>Secretary and Treasurer, Peptide Therapeutics Foundation</i>  <i>Senior Director, Business Development, Ferring Research Institute, Inc.</i></p>
5:15 p.m. – 6:30 p.m.	<p><b>Networking Reception</b>            Fritz B. Burns Reception Center, Lower Level</p>



**Speaker Biographies**  
**11th Annual**  
**Peptide Therapeutics Symposium**



## 11th Annual Peptide Therapeutics Symposium



**Frances H. Arnold, Ph.D. | Dickinson Professor of Chemical Engineering, Bioengineering and Biochemistry, Division of Chemistry and Chemical Engineering, California Institute of Technology**

*Innovation by Evolution: Expanding the Enzyme Universe*

Frances Arnold's research focuses on protein engineering by directed evolution, with applications in alternative energy, chemicals, and medicine. Dr. Arnold pioneered the 'directed evolution' of proteins, mimicking Darwinian evolution in the laboratory to create new biological molecules. Her laboratory has developed methods of laboratory evolution and structure-guided recombination that are used widely in industry and basic science to engineer proteins with new and interesting properties.

Dr. Arnold chairs the Advisory Panel of the David and Lucile Packard Foundation Fellowships in Science and Engineering program and serves as a judge for the Queen Elizabeth Prize in Engineering. Dr. Arnold's honors include the Millennium Technology Prize (2016), the ENI Prize in Renewable and Nonconventional Energy (2013), the US National Medal of Technology and Innovation (2011), and the Charles Stark Draper Prize of the US National Academy of Engineering (2011). She was inducted into the US National Inventors Hall of Fame in 2014 and has been elected to membership in all three US National Academies, of Science, Medicine, and Engineering and the American Academy of Arts and Sciences. Dr. Arnold has received honorary doctorates from the University of Stockholm, the ETH Zurich, and the University of Chicago.

Dr. Arnold is inventor on more than 50 US patents and is active in technology transfer to the private sector. She is a Director of Illumina and Provivi and has served on the science advisory boards of numerous companies. She co-founded Gevo, Inc. in 2005 to make fuels and chemicals from renewable resources and Provivi, Inc. in 2013 to develop non-toxic modes of agricultural pest control.



**Nazli Azimi, Ph.D. | Founder, President and CEO, Bioniz Therapeutics, Inc.**

*Selective Inhibition of Cytokines with Peptide Therapeutics: A Unique Alternative to Conventional MAB and JAK Inhibitor Approaches for Cytokine Modulation*

Dr. Azimi is the founder of Bioniz, co-inventor of its core technology. She has served as the Company's Chief Executive Officer since its inception. After graduating from the University of Tehran with a doctorate in pharmacy, Dr. Azimi completed her post-doctoral immunology program at one of the premier research groups at the National Institutes of Health (NIH), directed by Dr. Thomas Waldmann, a pioneer in the field of immune-therapy. During a decade of research at the NIH as a post-doctoral fellow and an independent scientist, Dr. Azimi made seminal contributions to the field of immunology, which later became the foundation for Bioniz's platform technology. In 2004, Dr. Azimi joined the faculty at the Fred Hutchinson Cancer Research Center, where she studied immune cytokine responses to herpes virus. Prior to founding Bioniz, Dr. Azimi served as founder and CEO of Dermaheal USA, a privately held dermatology company. Dr. Azimi has authored over 30 peer-reviewed, high impact scientific publications and is the inventor of more than a dozen patents, including the Bioniz intellectual property patent estate.

# 11th Annual Peptide Therapeutics Symposium

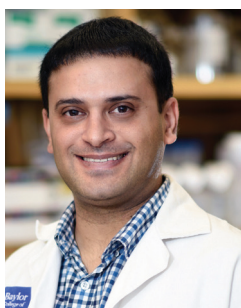


**Alessandro Bartolomucci, Ph.D. | Associate Professor of Physiology, Department of Integrative Biology and Physiology, University of Minnesota**

*TLQP-21 and C3aR1, a Novel Ligand/Receptor Target for Lipolysis and Obesity*

Dr. Alessandro Bartolomucci is an Associate Professor and the 2016-17 Fesler-Lampert Chair in Aging Studies, in the Department of Integrative Biology and Physiology, University of Minnesota who specializes in obesity and stress physiology. Dr. Bartolomucci graduated at the University of Parma, Italy, and completed his training in behavioral neuroscience and psychoneuroendocrinology in Italy, Germany and France. After starting an independent laboratory at the University of Parma in 2006 he was recruited in 2010 to the University of Minnesota as Assistant Professor and funding Associate Director of the Stress Physiology Center. He also serves as the Director of the Phenotyping Core Facility of the Medical School.

Dr. Bartolomucci's laboratory is interested in the autonomic and neuroendocrine regulation of metabolic functions and their imbalance in pathological conditions relevant for obesity and stress physiology. Two are the main current research area: 1) the functional role of *Vgf*-derived peptides in obesity and metabolism and their development as innovative drug targets for obesity-related disease; 2) the mechanism(s) of stress-induced obesity and metabolic disease and the impact of stress on aging. Dr. Bartolomucci published over 70 research papers in leading biomedical journals that collectively received more than 3500 citations and received financial support by NIH, Foundations grants and private industry.



**Atul Chopra, M.D., Ph.D. | Caroline Wiess Law Scholar, Assistant Professor, Department of Molecular and Cellular Biology, Department of Molecular and Human Genetics, Baylor College of Medicine**

*Asprosin, a Fasting-Induced Glucogenic and Orexigenic Protein Hormone*

Dr. Atul Chopra is the Caroline Wiess Law Scholar and physician-scientist at Baylor College of Medicine in Houston, Texas with clinical expertise in Medical Genetics and research expertise in the field of energy homeostasis and metabolic disease. Research in Dr. Chopra's laboratory is guided by clinical observations from patients with rare diseases manifesting with defects in energy balance. These observations are then translated into a specific genotype using state-of-the-art human exome sequencing, followed by cutting edge molecular biology, cell biology, biochemistry and mouse genetics to fill-in the gap from the genotype to the observed phenotype. This approach has resulted in the recent discovery of Asprosin, a novel 30-kDa protein hormone. Patients deficient in circulating asprosin manifest with a very low body weight and adiposity, in conjunction with insulin sensitivity. Harnessing this information, Dr. Chopra's laboratory demonstrated that monoclonal antibodies that deplete circulating asprosin can reduce adiposity and body weight in obese mice and can increase the insulin sensitivity of diabetic mice.

## 11th Annual Peptide Therapeutics Symposium



### **Karim Dabbagh, Ph.D. | Chief Scientific Officer, Second Genome, Inc.**

*New Therapeutics from the Human Microbiome: Discovery of Novel Proteins and Peptides for the Treatment of Gastrointestinal Barrier Related Disorders*

Karim Dabbagh leads the R&D organization at Second Genome. Prior to that, he led the immunoregulation department at Pfizer, an R&D group focused on innovative approaches to elicit homeostatic immune responses, including microbiome research, for the treatment of immune related disorders. At Pfizer, he also led external R&D innovation for autoimmune and inflammatory diseases. Past responsibilities include founding Modus BioMedicine, a start-up biotechnology company focused on treatments for transplantation and autoimmune disease, as well as spending nine years at Roche Pharmaceuticals in Inflammation Discovery Research. Dr. Dabbagh received his Ph.D. in biochemistry from University College, London and his BS in biotechnology from the Imperial College of Science, Technology, and Medicine in London. He completed postdoctoral fellowships at the Cardiovascular Research Institute at the University of California, San Francisco and at Stanford University where he worked on elucidating the role played by the microbiome in the hygiene hypothesis.



### **Adrienne Day, Ph.D. | Secretary and Treasurer, Peptide Therapeutics Foundation Senior Director, Business Development, Ferring Research Institute, Inc.**

*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 Conformia 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.



### **Mike G.W. de Leeuw, MSc, BBA | Founder and Chief Business Officer, InGell Labs BV**

*InGell® – Injectable Drug Depots for Short and Mid-term Release of Soluble and Non-soluble API's*

Mike de Leeuw (1960, the Netherlands) has a 30-year experience in setting-up and turning around innovative technology companies and business units in Life Sciences, Pharma and Biomedical Materials in Europe, China and India. After an international career with Unilever, Shell and DSM, Mike has started up three companies between 2008-2013 in polymer-enabled drug delivery (Branching Tree, InGell Labs, OrthoGell), all based on the InGell® biodegradable drug delivery depots. In 2006 he initiated the Dutch public-private collaboration program BMM, with €90 million in funding for innovative drug-delivery and cell-therapy projects.

During the formation of the above companies, he has also held interim CEO positions for Beta Cell (cell therapy for Type-1 Diabetes), ACS Biomarker (microRNA's for early detection of heart failure) and consulted with various companies in Regenerative Medicine. He has lead or co-invented on 7 patents and patent applications in the past 7 years, and has co-authored various articles covering the innovations his teams and his clinical and academic collaborators have developed.



# 11th Annual Peptide Therapeutics Symposium



**Richard DiMarchi, Ph.D. | Chairman of the Board, Peptide Therapeutics Foundation  
Distinguished Professor of Chemistry, Gill Chair in Biomolecular Sciences,  
Department of Chemistry, Indiana University;  
Site Director, Novo Nordisk Research Center, Indianapolis**

*Opening Remarks*

Dr. DiMarchi contributions in peptide and protein sciences consists of three decades of work in academia, the pharmaceutical industry and biotechnology companies. He is co-founder of Ambrx, Marcadia, Assembly, Calibrum and MB2 biotechnology companies. He has served as a scientific advisor to multiple pharmaceutical companies and three venture funds; 5AM, TMP, and Twilight.

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). 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 AAPS Career Achievement Award in Biotechnology, the Carothers Award for Excellence in Polymer Sciences, the Merrifield Award for Career Contributions in Peptide Sciences, the Meienhofer Award, the Max Bergmann Medaille, Erwin Schrödinger-Preis, and the Alfred Burger Career Award in Medicinal Chemistry. He is a member of the National Inventors Hall of Fame and the National Academy of Medicine.



**Soumitra Ghosh, Ph.D. | 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 (Myalept™), 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.

## 11th Annual Peptide Therapeutics Symposium



**Ali Jazayeri, Ph.D. | Chief Technology Officer, Heptares Therapeutics**

*Structural Elucidation of Ligand Binding Sites in Class B GPCRs and Their Application in Drug Discovery*

Ali joined Heptares in 2007 and as one of the first scientists he was involved in the transfer of the receptor stabilisation (StaR®) technology from MRC Laboratory of Molecular Biology to Heptares. Following successful implementation and industrialisation of the original technology, he led the development of novel methodologies that significantly increased the efficiency and reach of the StaR® technology. Prior to Heptares, Ali worked as a post-doctoral scientist in Clare Hall Laboratories (a Cancer Research UK institute) and Kudos Pharmaceuticals. In both cases he carried out research on the role of cell cycle checkpoint kinases in DNA damage response pathways. He has a BSc in Genetics from University of Manchester and obtained his Ph.D. in molecular biology with Prof Steve Jackson at the Gurdon Institute from University of Cambridge.



**Alexei Kharitonov, Ph.D. | Professor, Department of Chemistry, Indiana University**

*Endocrine FGFs and Structure-Activity-Relationship*

Alexei Kharitonov obtained Ph.D. in Biochemistry at the Moscow State University in Moscow, Russia, in 1990. In 1992-1997 Dr. Kharitonov was a postdoctoral fellow and then a staff scientist at the Max-Planck-Institute for Biochemistry, Martinsried, Germany, in the Molecular Biology Department led by Dr. Axel Ullrich. In 1998 Dr. Kharitonov joined Lilly Research Laboratories where he headed the Metabolism group in Diabetes Research. During this tenure Dr. Kharitonov discovered FGF21 and developed this novel metabolic target from the initial hit in the in vitro assay to the evaluation of its therapeutic utility in clinic. In 2014 Dr. Kharitonov partnered with the team of Richard DiMarchi at Indiana University, Bloomington. Dr. Kharitonov's current research interests lie within the translational aspects of metabolism with the intent to expand the boundaries of metabolic drug discovery.



**Jesper Lau, Ph.D. | Vice President, Diabetes Protein and Peptide Chemistry, Diabetes Research Unit, Novo Nordisk A/S**

*Tailoring Peptides for Less Frequent Dosing and Non-invasive Delivery*

After his Ph.D. in organic chemistry in 1990 at University of Southern Denmark and stay in the group of Professor Barry Trost at Stanford University in California, he joined Health Care Discovery at Novo Nordisk.

Dr. Lau has 24 years experience in pharmaceutical discovery and holds a broad experience in small molecule based therapeutics as well as a comprehensive experience within protein engineering especially within diabetes care with particular interest in glucagon like peptide 1 (GLP-1). He was project manager for once weekly GLP-1 and is first inventor of semaglutide.

In addition to project management Dr. Lau has been line manager since 1995 and was in 2008 appointed Vice President of Protein and Peptide Chemistry with a staff of about 80 researchers.

# 11th Annual Peptide Therapeutics Symposium



**Les Miranda, Ph.D. | Director Research, Therapeutic Discovery, Amgen Inc.**

*Engineering Potent and Selective Inhibitors of Nav1.7 Sodium Channel*

Les Miranda is Director of Research in Therapeutic Discovery at Amgen Inc. A graduate of the University of Western Sydney (Australia), he obtained his Ph.D. in synthetic peptide and protein chemistry from the University of Queensland (Australia). Les held a research position at the Carlsberg Laboratory (Denmark) working on the development of one-dimensional orthogonal protection strategies, and then served as an Associate Director at Gryphon Therapeutics (South San Francisco, USA) with Professor Stephen B.H. Kent, where he contributed to the development of methods for the total chemical synthesis and medicinal optimization of protein therapeutics. At Amgen, he is responsible for hybrid modality discovery, structural biology, and molecular modeling; and has supported numerous preclinical and early development programs. Les has led several cross-functional teams through various stages of drug discovery and development. During his career, Les has authored more than 40+ original peer reviewed publications and contributed to more than 20 published/issued patents.



**Sylviane Muller, Ph.D. | Professor and CNRS Director, Molecular and Cellular Biology Institute, University of Strasbourg**

*Peptide-based Strategies to Treat Inflammatory and Autoimmune Diseases: From the Peptide P140 to Lupuzor™*

Sylviane Muller received her doctoral degrees in Molecular Biology (1978) and Science (1984) from the University of Strasbourg (France). She worked as a post-doctoral fellow at the Max-Planck Institute for Immunobiology in Freiburg (Germany). She is currently Distinguished class Research Director at the CNRS and Professor at the Institute of Advanced Studies of the Strasbourg University (chair Therapeutic immunology). She is the director of the Molecular and Cellular Biology Institute, Director of the CNRS Unit Immunopathology and therapeutic chemistry, and Head of the Drug discovery center for cancer and inflammation Medalis awarded 'Laboratory of Excellence'. Her research interests focus on molecular and cellular events involved in autoimmunity, especially in the lupus disease. She discovered the P140/Lupuzor peptide that is currently evaluated in a phase III clinical trial for lupus. She is the co-author of over 350 publications, co-inventor of ~30 patents and co-founder of NeoMPS (1986) and ImmuPharma (2002) companies. She received the CNRS Silver Medal (2009), the Paul-Ehrlich Award from the French Society of therapeutic chemistry and Janssen research & development (Johnson & Johnson) (2015), and the CNRS Innovation Award (2015).



## 11th Annual Peptide Therapeutics Symposium



**James S. Nowick, Ph.D. | Professor of Chemistry and Department Chair, University of California, Irvine**

*Unlocking the Mysteries of Amyloid Diseases with Macrocyclic  $\beta$ -Sheet Peptides*

James Nowick is a Professor of Chemistry at the University of California, Irvine. He received his A.B. (Bachelors) degree in Chemistry in 1985 from Columbia University and his Ph.D. degree in Organic Chemistry in 1990 from MIT, where he was both an NSF Graduate Fellow and an ACS Division of Organic Chemistry Graduate Fellow. After an NSF postdoctoral fellowship in supramolecular chemistry at MIT, he began his independent career as an Assistant Professor at UCI in 1991. He was promoted to Associate Professor in 1996 and Professor in 1998. He is currently Chair of the Department of Chemistry.

Dr. Nowick's research interests include peptidomimetic chemistry, molecular recognition, and supramolecular chemistry, with a central focus on understanding how peptides fold and interact. In recognition of his scientific contributions, he has received a Camille and Henry Dreyfus Foundation New Faculty Award, an American Cancer Society Junior Faculty Research Award, an NSF Young Investigator Award, an Arnold and Mabel Beckman Foundation Young Investigator Award, a Presidential Faculty Fellow Award, a Camille Dreyfus Teacher-Scholar Award, an Alfred P. Sloan Research Fellowship, and an American Chemical Society Arthur C. Cope Scholar Award. He is a Fellow of the American Association for the Advancement of Science and a Fellow of the American Chemical Society. For his contributions to research and education at UCI, he has received the Award for Outstanding Faculty Contribution to Undergraduate Research, the Chancellor's Award for Excellence in Undergraduate Research, and the School of Physical Sciences Award for Outstanding Contributions to Undergraduate Education.



**Timo Nuijens, Ph.D. | Lead Scientist, EnzyPep**

*Chemo-enzymatic Peptide Synthesis (CEPS): A Generally Applicable, Traceless Ligation Technology for the Synthesis of Peptide-to-peptide and Peptide-to-protein Conjugates*

Timo Nuijens graduated in Drug Innovation at the University of Utrecht. He performed his Ph.D. project on enzymatic peptide synthesis within DSM Innovative Synthesis B.V. (Geleen) under the supervision of Dr. Peter Quaedflieg (DSM Innovative Synthesis B.V.) and Prof. Rob Liskamp (Univ. Utrecht). His Ph.D. was obtained in May 2012. Since mid 2012 he is working as a lead scientist at EnzyPep and has the responsibility of the scientific development and co-ordinating the R&D team in Geleen.

# 11th Annual Peptide Therapeutics Symposium



**Anat Shirvan, Ph.D. | Executive Vice President, Research & Development, Atox Bio, Ltd.**

*AB103: Host-Oriented Therapeutics to Control Life Threatening Infections*

Dr. Shirvan joined Atox Bio in 2010 and is serving as an executive VP for R&D, responsible for all R&D, academic activities, regulatory strategy and affairs and its execution, and is managing the contract with the US government (BARDA). Dr. Shirvan has a research experience in a broad range of scientific disciplines, and an overall 20 years of experience in leadership positions in the Biotech industry in Israel. She has extensive experience in drug development, bringing a small early stage discovery biotech companies from inception to a phase III clinical stage, with global studies performed at EU and US sites. Dr. Shirvan published more than 55 peer reviewed manuscripts; presented in numerous scientific meetings which were recognized with distinguished prizes, and is an author on 14 granted independent patents, and numerous additional pending patent applications.

Before joining Atox Bio she was the co-founder, EVP for R&D and director at Aposense Ltd, in which she served for 13 years. Dr. Shirvan led the drug development of Aposense technology from its conception, advancing it from discovery stage to pre-clinical and clinical development while overseeing all related regulatory aspects. Dr. Shirvan earned her Ph.D. in molecular biology from The Hebrew University of Jerusalem, and did post-doctoral research for 4 years at NIH studying the annexin family of calcium-binding proteins.



**Hazel H. Szeto, M.D., Ph.D. | Adjunct Professor, Weill Cornell Medicine**

*Discovery and Clinical Development of Elamipretide*

Dr. Szeto recently left Weill Cornell Medical College where she was Professor of Pharmacology and Director of the Research Program in Mitochondrial Therapeutics. Dr. Szeto pioneered the development of mitochondria-targeted peptides that preserve mitochondrial structure and restore cellular bioenergetics by targeting cardiolipin, a unique phospholipid on the inner mitochondrial membrane. Mitochondrial dysfunction and bioenergetics failure is a hallmark of rare genetic diseases and common age-associated chronic diseases. Preclinical studies have demonstrated remarkable efficacy of these compounds in cardiorenal and metabolic diseases, neurodegenerative diseases, skeletal muscle weakness, and neuropathic pain. These compounds are now in clinical development sponsored by Stealth Biotherapeutics, a company founded by Dr. Szeto in 2006. The first drug candidate (Elamipretide, Bendavia™) is currently undergoing multiple Phase 2 clinical trials for mitochondrial diseases, heart failure, and age-related macular degeneration and muscle weakness. The US Food & Drug Administration granted Fast Track designation for elamipretide for primary mitochondrial myopathy in patients with genetic mitochondrial diseases. Dr. Szeto received her M.D. and Ph.D. (Pharmacology) from Cornell University Medical College.

## 11th Annual Peptide Therapeutics Symposium



**Eva Ann Turley, Ph.D. | Co-Founder, Novare Pharmaceuticals; Distinguished Oncology Scientist, London Regional Cancer Program, Lawson Research Institute; Professor, Departments of Oncology, Biochemistry and Surgery, University of Western Ontario**

*Targeting Novel Polysaccharide-binding Proteins to Block Tissue Fibrosis*

Dr. Turley is a Distinguished Oncology Scientist at the London Regional Cancer Program and Professor at Western University in Dept. Oncology, Biochemistry and Surgery. She has had extensive experience in translating her basic research through interactions with the Pharmaceutical and Biotechnology sectors. She has co-founded 4 Biotech start-up companies and has performed consulting and advisory board functions for many other companies. Her research focuses upon mechanisms of cell motility that contribute to tumor invasion and metastasis. Her research particularly focuses upon the signalling mechanisms by which pro-fibrotic and pro-inflammatory extracellular matrix molecules such as hyaluronan contribute to tumor progression and selection of aggressive metastatic variants. Her laboratory discovered and characterized the first cellular hyaluronan receptor, RHAMM.



**Carmen Valcarce, Ph.D. | Senior Vice President and Chief Scientific Officer, vTv Therapeutics**

*Non-Peptide GLP-1 Receptor Agonists; From Idea to Medicine*

Carmen Valcarce is the Senior Vice President and Chief Scientific Officer at vTv Therapeutics. In her role, Dr. Valcarce oversees Preclinical Research, Bioanalytical and Quality Assurance. As the company's Diabetes portfolio subject matter expert, she is also responsible for all development activities of the company's diabetes programs and acts as the scientific liaison when in discussions with national and international regulatory agencies.

Dr. Valcarce joined vTv Therapeutics in 2007 to lead the Diabetes and Metabolic Disorders Research. She has since held a series of leadership roles of increasing responsibility, including, Senior Vice President of Preclinical Research (Pharmacology, DMPK, Toxicology and Pharmaceutical Development), where she was responsible for the preparation of 11 INDs which were successfully approved by FDA.

Prior to joining vTv Therapeutics, Dr. Valcarce spent 7 years at Novo Nordisk (Denmark) as the Project Leader and Scientific Coordinator for the Glucokinase Activator project advancing this program from an idea to clinical development.

Dr. Valcarce holds a Ph.D. in Biochemistry and Molecular Biology from the Universidad Autonoma de Madrid (Spain) and the title of Docent in Experimental Clinical Chemistry from Lund University (Sweden). Dr. Valcarce is the author of numerous papers and presentations, and is an inventor on several patents and patent applications.

# 11th Annual Peptide Therapeutics Symposium



**Chi-Huey Wong, Ph.D. | Professor of Chemistry, Department of Chemistry,  
The Scripps Research Institute**

*Homogeneous Glycoproteins for Structural and Functional Study*

Professor Wong received his B.S. (1970) and M.S. (1977) degrees from National Taiwan University, and Ph.D. (1982) in Chemistry (with George M. Whitesides) from MIT. He then worked at Harvard University as a postdoctoral fellow (with George M. Whitesides) for another year, and became a faculty member at Texas A&M University (1983) where he was promoted to full professor in 1987. He then moved to The Scripps Research Institute in 1989 as Professor and Ernest W. Hahn Chair in Chemistry. From October 2006-May 2016, he was President of Academia Sinica with a joint appointment as Professor of Chemistry at The Scripps Research Institute.

Professor Wong is a recipient of many awards and honors, notably, the Presidential Young Investigator Award in Chemistry, USA (1986), the Roy Whistler Award of the International Carbohydrate Organization (1994), the American Chemical Society Claude S. Hudson Award in Carbohydrate Chemistry (1999), the International Enzyme Engineering Award (1999), the US Presidential Green Chemistry Challenge Award (2000), The American Chemical Society Award for Creative Work in Synthetic Organic Chemistry (2005), Humboldt Research Award for Senior Scientists (2006), the FA Cotton Medal (2008), the Nikkei Asia Prize for Science, Technology and Innovation (2012), the American Chemical Society Arthur C. Cope Award (2012), the Wolf Prize in Chemistry (2014), and the Royal Society of Chemistry Robert Robinson Award (2015).

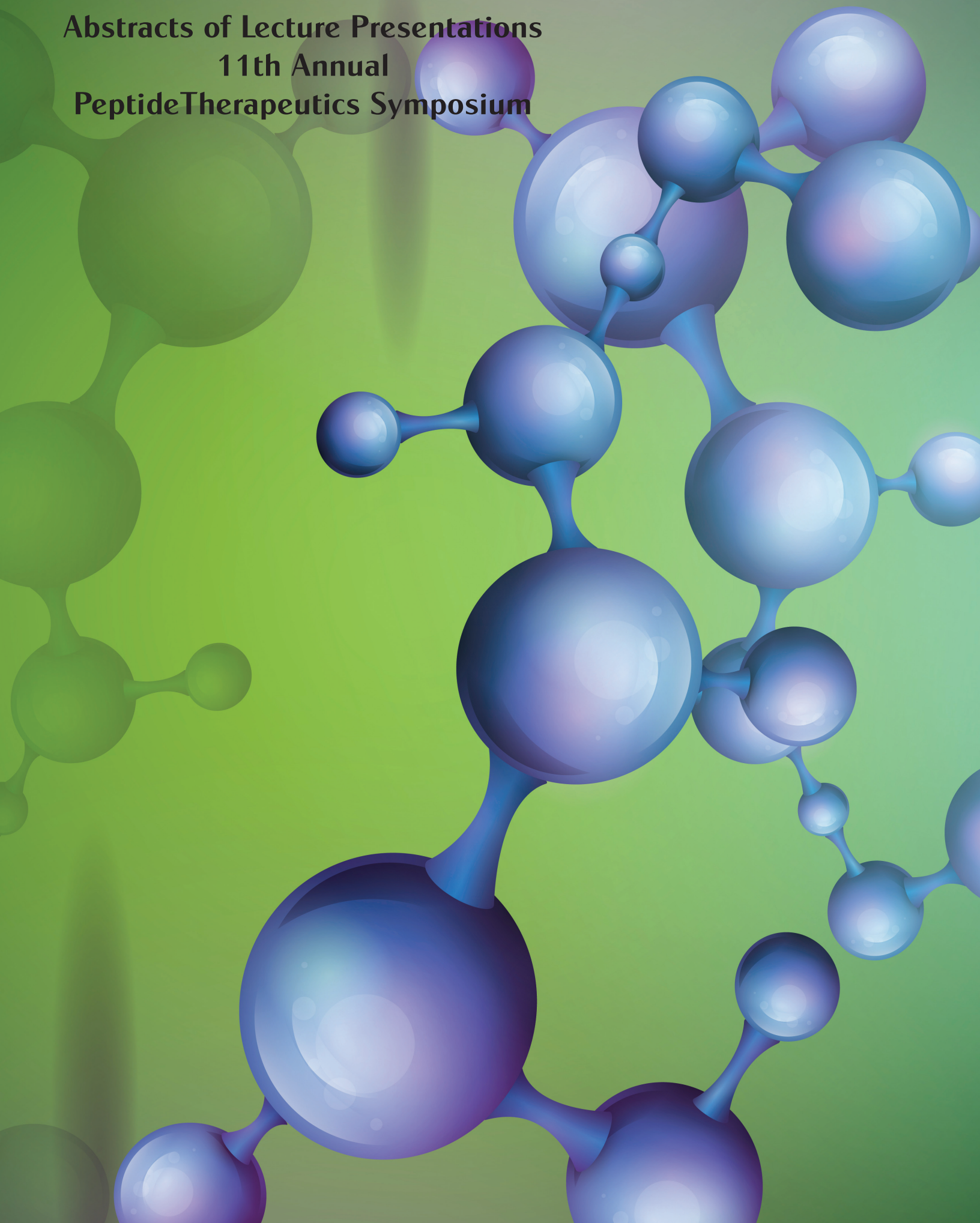
He is a member of the American Academy of Arts and Sciences (1996) and the US National Academy of Sciences (2002). He served as an Editorial Advisory Board member for the Journal of American Chemical Society and *Angewandte Chemie*, Chairman of the Executive Board of Editors of the Tetrahedron Publications (2006-2008), Head of the Frontier Research Program on Glycotechnology at RIKEN in Japan (1991-1999), and a board member of the US National Research Council on Chemical Sciences and Technology (2000-2003) and a committee member of the US National Academy of Sciences (2011) for assessing the importance of glycoscience and glycomics. In addition, he has received many honorary doctor degrees and served as science advisor to many organizations, including the Max-Planck Institute (2000-2008), RIKEN Advisory Council (2010- ), and the Board of Scientific Governors of the Scripps Research Institute (2009-2015) and as the Chief Science Advisor of the Taiwan Government (2008-2015).

His research interests are in the field of chemical biology, with particular focus on the development of new methods for the synthesis of complex carbohydrates and glycoproteins, elucidation of carbohydrate-mediated biological recognition associated with protein folding, cancer progression and microbial infection, and development of carbohydrate-based medicines and devices. He has published over 700 papers and 100 patents, and is a highly cited scientist with H-index of 107.





**Abstracts of Lecture Presentations  
11th Annual  
Peptide Therapeutics Symposium**



# 11th Annual Peptide Therapeutics Symposium

## Innovation by Evolution: Expanding the Enzyme Universe

**Frances H. Arnold, Ph.D. | Dickinson Professor of Chemical Engineering Bioengineering and Biochemistry, Division of Chemistry and Chemical Engineering**

California Institute of Technology

228B Spalding, Mail Code 210-41, Pasadena, CA 91125 | (626) 395-4162

Not satisfied with nature's vast catalytic repertoire, we want to create new enzymes and expand the range of chemical reactions that can be genetically encoded. I will describe how we can use the most powerful algorithm for biological design and evolution, to optimize existing enzymes and invent new ones. Mimicking nature's evolutionary tricks and using a little chemical intuition, we can generate whole new enzyme families that catalyze important reactions not (yet) known in nature, thereby adding new capabilities to the chemistry of the biological world and increasing the scope of molecules and materials we can build. I will show that heme proteins can catalyze an array of increasingly challenging carbene- and nitrene-transfer reactions and that these new activities can be enhanced by directed evolution. These experiments illustrate the mechanisms by which new catalysts have been and will continue to be generated by nature's innovation machine, evolution.

Prier, C. K. and Arnold, F. H., Chemomimetic Biocatalysis: Exploiting the Synthetic Potential of Cofactor-Dependent Enzymes to Create New Catalysts, *Journal of the American Chemical Society* 137, 13992-14006 (2016)

Renata, H., Wang, Z. J., Arnold, F. H. Expanding the Enzyme Universe: Accessing Non-Natural Reactions by Mechanism-Guided Directed Evolution, *Angewandte Chemie* 54, 3351-3367 (2015).

## Selective Inhibition of Cytokines with Peptide Therapeutics: A Unique Alternative to Conventional MAB and JAK Inhibitor Approaches for Cytokine Modulation

**Nazli Azimi, Ph.D. | Founder, President and CEO**

Bioniz Therapeutics, Inc.

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**Introduction:** Cytokines are valid therapeutic targets. However, single MAB therapy targeting a cytokine has shown limited success in the clinic due to presence of other cytokines that exhibit overlapping functions. Furthermore, JAK inhibitors (JAKi) that disrupt the signaling of all cytokines have a broader effect inhibiting the cytokines but are associated with life threatening side effects. Bioniz Therapeutics is developing peptide therapeutic agents that exhibit a selective cytokine inhibitory profile. They display an optimal balance between efficacy and toxicity *in vivo*, and fill a critical therapeutic void that exists between single MAB therapy, which lacks optimal efficacy when multiple cytokines are disease modulators, and small molecule JAK inhibitors, which display pan-cytokine inhibition and unwanted side-effects.

Bioniz initial focus is on a group of cytokine family called gamma-c ( $\gamma$ c) family that consists of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. While each cytokine has distinct biological effects, they have many overlapping functions, which make single MAB therapy against one cytokine inefficient where multiple  $\gamma$ c cytokines are disease drivers. The  $\gamma$ c cytokines are linked to a variety of inflammatory and autoimmune diseases. Bioniz has developed BNZ132-1, an antagonistic peptide specific for blocking the signaling of the  $\gamma$ c cytokines IL-2 and IL-15, without displaying significant inhibitory activity against the remaining cytokines in the  $\gamma$ c family. A PEGylated version of BNZ132-1 (BNZ132-1-40) showed favorable *in vivo* half-life and potent *in vivo* activities in a variety of disease models.

In a 13-week repeat dose GLP toxicology studies, the pharmacodynamics and toxicokinetics effect of BNZ132-1-40 was evaluated at multiple concentrations C57BL/6 mice and cynomolgus monkeys. BNZ132-1-40 displays highly targeted effects against lymphoid/immune cell lineages expected following an effective blockade of both IL-2 and IL-15 in both mouse and monkey. The compound did not exhibit any adverse effects thus highlighting the agent's selective cytokine inhibitory profile without the strong off-target activity often observed following the administration of small molecule JAK inhibitors.

**Conclusion:** BNZ132-1-40 is effective at targeting IL-2 and IL-15 *in vivo*, and is well-tolerated following long-term administration. The compound displays a high selectivity for IL-2 and IL-15, without significant impact on the remaining  $\gamma$ -c cytokines or other cytokine families. The lack of off-target effects of BNZ132-1-40 is in stark contrast to small molecule JAK inhibitors, which act to block signaling of the entire  $\gamma$ -c cytokine family and thereby elicit cytotoxic effects following *in vivo* administration. BNZ132-1-40 represents a safe and effective alternative for therapeutic strategies that employ single MAB and small molecule JAK inhibitors. BNZ132-1-40 is in phase I trial in collaboration with the NIH for the treatment of HTLV-Associated Myelopathy (HAM) and T-cell leukemia.



# 11th Annual Peptide Therapeutics Symposium

## TLQP-21 and C3aR1, a Novel Ligand/Receptor Target for Lipolysis and Obesity

**Alessandro Bartolomucci, Ph.D.** | Associate Professor of Physiology, Department of Integrative Biology and Physiology  
University of Minnesota  
Cancer & Cardiovascular Research Building, 2231 6th Street SE, Minneapolis, MN 55455 | (612) 624-7006

Obesity is characterized by excessive fat mass and is associated with serious diseases such as type 2 diabetes and hypertension. Reducing fat mass by sustained lipolysis has the potential to exert a major beneficial effect but has been a major challenge for anti-obesity therapies due to unwanted cardiometabolic side effects. In this context, TLQP-21, a 21 amino acid peptide encoded by the pro-peptide VGF (non-acronymic), is emerging as a novel and safe neuromodulator of lipolysis and a target for obesity-associated diseases. TLQP-21 is expressed in the CNS and peripheral nerves and acts as an endogenous ligand for the complement 3a receptor 1 (C3aR1). A recent structure-function relationship study demonstrated that the C-terminus of TLQP-21 is critical for C3aR1 activation and that the peptide adopts an  $\alpha$ -helical conformation upon targeting cells expressing the receptor. Furthermore, TLQP-21 acts as an enhancer of beta-Adrenergic Receptor-induced lipolysis in murine adipocytes, despite not being pro-lipolytic *per se*. Finally, TLQP-21/activation exerts an anti-obesity effect, normalizes molecular markers of obesity-associated lipolytic catecholamine resistance and hypertension in mice, without causing adverse cardiometabolic side effects. Overall, our work identifies an alternative pathway to safely enhance adipocyte lipolysis and a novel druggable target for obesity and obesity-associated diseases.

Supported by NIH/DK102496 and the Minnesota Partnership for Biotechnology and Medical Genomic, Decade of Discovery in Diabetes Grant.

## Asprosin, a Fasting-Induced Glucogenic and Orexigenic Protein Hormone

**Atul Chopra, M.D., Ph.D.** | Caroline Wiess Law Scholar, Assistant Professor, Department of Molecular and Cellular Biology, Department of Molecular and Human Genetics  
Baylor College of Medicine  
1 Baylor Plaza, DeBakey M824, Houston, TX 77030 | (713) 798-4951

The ability to survive periods of fasting is the cornerstone of evolution and life on earth. Mammals respond to fasting by activating an enormous cascade of interconnected processes that are precisely coordinated by an array of hormones. Two such processes are appetite stimulation and hepatic glucose release into the circulation, which together, ensure the drive to obtain food, and keep the brain nourished and alert while that is accomplished. Through study of a rare genetic condition in humans – Neonatal Progeroid syndrome, we have discovered a new fasting-induced protein hormone that is highly expressed in adipose tissue, and upon secretion, coordinately stimulates appetite and hepatic glucose release, and we name it Asprosin. Asprosin is a ~30 kDa C-terminal cleavage product of a larger pro-protein (profibrillin) and circulates in plasma and cerebrospinal fluid (CSF) at nanomolar levels. Its plasma and CSF levels rise with fasting, and in a cell-autonomous manner at the liver and arcuate nucleus, it activates the G-protein-cAMP-PKA pathway resulting in rapid glucose release into the circulation and stimulation of appetite. The cumulative effect of increased circulating asprosin concentration is hyperglycemia, hyperinsulinemia and an increase in appetite, adiposity and body weight. Asprosin loss-of-function results in the opposite – hypoglycemia, hypoinsulinemia, and a reduction in appetite leading to reduced adiposity and body weight. Humans and mice with obesity and insulin resistance show pathologically elevated plasma asprosin levels, and its immunologic or genetic depletion results in protection from obesity-associated hyperinsulinemia and hyperphagia. Thus, asprosin represents the first example of a circulating glucogenic and orexigenic protein hormone that is cleaved from a pro-protein that also generates an extracellular matrix component (fibrillin), and therapeutically targeting it may be beneficial in hyperinsulinemic and hyperphagic conditions like metabolic syndrome.

# 11th Annual Peptide Therapeutics Symposium

## New Therapeutics from the Human Microbiome: Discovery of Novel Proteins and Peptides for the Treatment of Gastrointestinal Barrier Related Disorders

**Karim Dabbagh, Ph.D. | Chief Scientific Officer**

Second Genome, Inc.

341 Allerton Avenue, South San Francisco, CA 94080 | (650) 440-4606

Humans, like other multicellular organisms, interact abundantly with microbes. This interaction is generally symbiotic and we now have ample evidence that microbes play a key role in the development and regulation of multiple physiological processes, ranging from nutrition and metabolism to the immune system. Alterations in the function of the mammalian microflora have been shown to result in the establishment of many pathological conditions. We sought to identify the role played by bacteria in the function of the gastro-intestinal (GI) tract, a key site of bacterial colonization, with a particular emphasis on identifying molecules that mediate intestinal barrier function homeostasis. To this effect, we characterized the microbiome of humans with chronic GI inflammation (inflammatory bowel disease) and compared it to those of healthy individuals. This enabled the identification of potentially beneficial bacterial strains associated with a healthy GI tract. Analysis of the secreted proteins and peptides from these bacteria helped us identify several novel proteins that displayed epithelial barrier protection and repair abilities *in vitro*. We further characterized the *in vivo* therapeutic potential of these molecules in an animal model of GI barrier dysfunction. These studies demonstrate that healthy human bacteria encode proteins that act on the GI epithelium by eliciting beneficial physiological responses that can be potentially developed as novel therapeutic agents. We further propose that the human microbiome can be mined for novel peptide-based medicines for many indications where a key role for the bacteria in health and disease has been demonstrated, opening a new era of biotechnology drug development.

## InGell® – Injectable Drug Depots for Short and Mid-term Release of Soluble and Non-soluble API's

**Mike G.W. de Leeuw, MSc, BBA | Founder and Chief Business Officer**

InGell Labs BV

L.J. Zielstraweg 1, 9713GX Groningen, The Netherlands | +31 6136 020 12

InGell® is a patent-protected, biodegradable polymer platform for localized or sustained release of API's. Injected as a liquid formulation through up to 30G needles, bio-compliant depots are rapidly formed for the retention and the slow release of API's. Release of the API coincides with the degradation and erosion of the depot, making it very suitable for repeat injections in chronic disease management.

InGell® consists of tri-block co-polymers, which are straightforward to scale-up, and which very easy to formulate with a broad variety of API's. Loading capacity can go up to 25% weight, and release has been measured up to 2 months *in vivo*, coinciding with the complete elimination of the soft, durable depots.

Current development projects for localized and/or sustained applications will be discussed. The presentation will introduce the formulation, protection and delivery of different classes of APIs such as non-soluble Small Molecule Drugs, soluble Peptides and Antibodies, with practical comments on in-vitro and in-vivo screening and testing.

# 11th Annual Peptide Therapeutics Symposium

## Structural Elucidation of Ligand Binding Sites in Class B GPCRs and Their Application in Drug Discovery

**Ali Jazayeri, Ph.D. | Chief Technology Officer**

Heptares Therapeutics

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G protein-coupled receptors (GPCRs) comprise one of the most important families of drug targets owing to the multitude of roles they fulfil across many different physiological processes. Despite the huge amount of investment in GPCR drug discovery there remains significant opportunities for identification of novel or better drugs. One way to achieve this goal is to utilise structure based drug design (SBDD) strategies. However, GPCRs are generally challenging proteins for crystallisation and structure determinations primarily due to their hydrophobic nature, low expression levels and conformational flexibility. To facilitate application of SBDD approaches to GPCRs Heptares uses its proprietary StaR technology to thermostabilise GPCRs in a single conformational state. The purified StaRs can then be used for crystallisation to yield X-ray structures with multiple ligands as well as used in biophysical screening techniques. Using the StaR approach, we have solved structures of multiple Class B GPCRs in both agonist and antagonist conformations. This has led to the elucidation of the orthosteric and allosteric binding sites. The structural insight into multiple ligand binding sites has significantly increased our ability to design novel drugs to modulate the activities of these receptors.

## Endocrine FGFs and Structure-Activity-Relationship

**Alexei Kharitononkov, Ph.D. | Professor, Department of Chemistry**

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Fibroblast growth factor 21 (FGF21) is an emerging regulator of energy homeostasis and a novel target for the development of therapies to treat diabetes, cardiovascular disease and obesity. FGF21 was discovered in an *in vitro* high throughput screen. It was later shown to have impressive metabolic effects including glucose, lipid and body weight lowering effects in a variety of animal models, including non-human primates. When tested clinically an FGF21-based analog demonstrated dramatic efficacy in management of circulating lipids but failed to produce a robust glycemic lowering. The basis of this discrepancy in human study relative to pre-clinical investigations remains a conundrum and an area of active investigation. More recently, FGF1 and related structural analogs have emerged as capable of providing impressive metabolic benefits when administered to mice, and as such proposed as an alternative to FGF21-based therapy. Our work is focused on enhancing the inherent properties of FGF21 by studying structure-activity-relationship to improve its potency and tissue-specific actions and further interrogating the mechanism for FGF1 signaling relative to FGF21.

## Tailoring Peptides for Less Frequent Dosing and Non-invasive Delivery

**Jesper Lau, Ph.D. | Vice President, Diabetes Protein and Peptide Chemistry, Diabetes Research Unit**

Novo Nordisk A/S

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The development within biotechnology has given access to various technologies that are applicable to optimize the properties of endogenous proteins and peptides to stable and efficacious drugs with improved pharmaceutical profiles. The *in vivo* parameters that often are being optimized includes renal clearance (by adding mass and charge to the protein), liver elimination (by removal or hiding of specific receptor signatures), and plasma elimination (by elimination of proteolytic sites with amino acid substitutions). In addition the biophysical properties addressing stability of drug product is often being optimized to obtain a successful drug candidate. At Novo Nordisk the attachment of fatty acids to proteins and peptides has been an important focus area to change not only the *in vivo* properties, but also the biophysical properties of the drug candidates. These matters will be discussed using examples from GLP-1 and insulin to meet patients' needs for less frequent dosing and for non-invasive delivery.

# 11th Annual Peptide Therapeutics Symposium

## Engineering Potent and Selective Inhibitors of Nav1.7 Sodium Channel

**Les Miranda, Ph.D. | Director Research, Therapeutic Discovery**

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Nav1.7 is a voltage-gated sodium ion channel implicated by human genetic evidence as a therapeutic target for the treatment of pain. Screening fractionated venom from the tarantula *Grammostola porteri* led to the identification of a 34-residue peptide, termed GpTx-1, with potent activity on Nav1.7 (IC<sub>50</sub> = 10 nM) and promising selectivity against key Nav subtypes (20x and 1000x over Nav1.4 and Nav1.5, respectively). NMR structural analysis of the chemically synthesized three disulfide peptide was consistent with an inhibitory cystine knot motif. Here we report the results of efforts to efficiently identify potent and selective inhibitors of Nav1.7 from GpTx-1, and improve our understanding of the peptide-Nav1.7 interaction model.

## Peptide-based Strategies to Treat Inflammatory and Autoimmune Diseases: From the Peptide P140 to Lupuzor™

**Sylviane Muller, Ph.D. | Professor and CNRS Director**

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Nowadays, pharmacologic treatments of inflammatory diseases and autoimmune diseases are largely palliative rather than curative. Most of them result in non-specific immunosuppression, which can be associated with disruption of natural and induced immunity with significant, sometimes dramatic, adverse effects. Among the novel strategies that are under development, tools that target specific molecular pathways and cells, and more precisely modulate the immune system to restore normal tolerance mechanisms, are central. In these approaches, peptide therapeutics represent a class of agents that display many physicochemical advantages. Within this class of potent drugs, the phosphopeptide P140 is very promising for treating patients with systemic lupus, and probably patients with other chronic inflammatory diseases. In a multicenter, randomized, placebo-controlled phase IIb study for lupus, P140/Lupuzor™ was found to be safe and met its primary efficacy end points, confirming pre-clinical data generated in lupus-prone mice. It is currently evaluated in a phase III clinical trial. We recently discovered that P140 targets autophagy, a finely orchestrated catabolic process, involved in the regulation of inflammation and in the biology of immune cells. P140 acts directly on a particular form of autophagy called chaperone-mediated autophagy (CMA), which is hyperactivated in lupus. The modulating effect of P140 on CMA results in a change of MHC-peptide presentation to autoreactive T cells, leading to a significant improvement of physiopathological status of treated mice. These results open very new avenues of therapeutic intervention in other inflammatory conditions in which reduction of CMA activity would be desired.

## Unlocking the Mysteries of Amyloid Diseases with Macrocyclic $\beta$ -Sheet Peptides

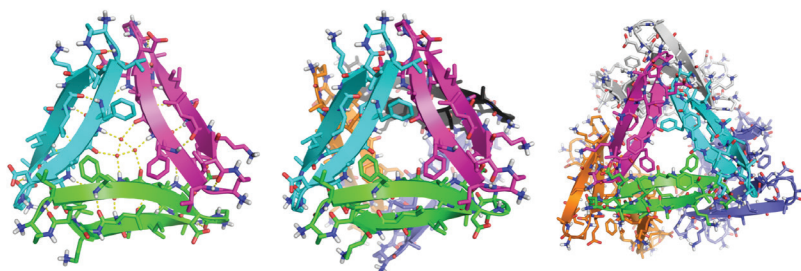
**James S. Nowick, Ph.D.** | Professor of Chemistry and Department Chair

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

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



X-ray crystallographic structure of oligomers of a peptide derived from A $\beta_{17-36}$ . Trimer (left). Hexamer — a dimer of trimers (center). Dodecamer — a tetramer of trimers (right). (*JACS* **2014**, 136, 5595. PDB 4NTR)

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# 11th Annual Peptide Therapeutics Symposium

## Chemo-enzymatic Peptide Synthesis (CEPS): A Generally Applicable, Traceless Ligation Technology for the Synthesis of Peptide-to-peptide and Peptide-to-protein Conjugates

**Timo Nuijens, Ph.D. | Lead Scientist**

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Enzymatic peptide fragment condensation is a procedure for assembling peptides. The procedure, also referred to as chemo-enzymatic peptide synthesis (CEPS), is fast, free of racemization and does not require side-chain protecting groups. Until recently, chemo-enzymatic approaches to fragment synthesis were typically accompanied by extensive hydrolytic side-reactions, i.e. hydrolysis of the acyl donor ester and the peptide backbone. The synthesis to hydrolysis ratio (S/H ratio) of proteases can be improved by engineering. For example, subtiligase developed by Wells et al. can be used for peptide fragment condensation in aqueous solution.<sup>1</sup> Although the S/H ratio is improved, hydrolysis is still substantial. Subtiligase is not suited as a tool for commercial production because the process requires 10 equivalents of one fragment. It is also a very unstable enzyme.

Peptiligase is a novel, hyper-stable, engineered enzyme with an exceptional S/H ratio that has been specifically developed for CEPS. This enzyme can ligate unprotected peptide fragments in an aqueous environment very efficiently without the need of a recognition sequence (traceless ligation technology) and can tolerate detergent agents, e.g. urea and guanidiniumchloride, organic co-solvents such as DMF and DMSO, and reducing agents such as DTT. Ligation is fast (10–60 min) and efficiency is usually greater than 90% (product) using only 1.1 equivalents of one fragment. The enzyme has been further engineered both to broaden the substrate scope for universal applications as well as tightening the substrate scope to permit only the coupling of defined peptide sequences.

The synthesis of some peptides of pharmaceutical interest (e.g. exenatide and thymosin- $\alpha_1$ ) has been demonstrated. Peptiligase is a particularly useful tool for the assembly of head-to-tail macrocyclics, addressing chain-specific conjugation to heterodimeric peptide substrates and, perhaps surprisingly, for coupling peptide esters to proteins. The latter will be demonstrated for the coupling of exenatide and other peptides to human serum albumin (HSA). Conjugation of peptides to HSA usually proceeds in a matter of minutes and in quantitative yield when 5 equivalents of peptide ester are being used. When using sequence-specific ligases, peptides such as exenatide can also be attached to HSA by CEPS using a sequential fragment approach. Purification of the exenatide-HSA conjugate was straightforward using a single ultrafiltration step.

The conjugation technology broadens the scope of CEPS to include the assembly of fusion proteins with unnatural amino acids, to provide a peptide-protein conjugation technology that maintains peptide bond integrity, and to act as an adjunct to NCL methods in the synthesis of post-translationally modified proteins.

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### Collaborators:

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# 11th Annual Peptide Therapeutics Symposium

## AB103: Host-Oriented Therapeutics to Control Life Threatening Infections

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Atox Bio is developing novel immunomodulators to treat critically ill patients with severe infections. AB103, the lead candidate, is a short synthetic peptide that modulates an excessive acute immune response by interfering with generation of the immunological synapse, without affecting the adaptive or normal humoral immune response. As a result, it modulates T-cell hyper-activation and the associated cytokine storm. This approach denotes a platform technology representing a treatment paradigm based on modulating the host immune response, and is applicable in situations where an excessive cytokine response and acute inflammation are responsible for disease pathology.

AB103 has a broad spectrum activity (pathogen agnostic) and as such it has a potential to address the public health issue of antibiotic resistance. Pre-clinical studies in multiple experimental models of gram positive, gram negative or poly-microbial infections, indicated that one dose of AB103 (administered after infection was already established), significantly improved survival and reduced inflammation. In animal models of severe inflammation (irradiation injury or acute pancreatitis), consistent reduction of systemic inflammation as well as inflammation across several key tissues/organs was evident. A phase 2 study was conducted in patients with Necrotizing Soft Tissue Infections (NSTI). NSTI is a rare life threatening disease caused by uncontrolled inflammatory response to bacteria and have significant unmet need with no approved pharmacologic treatments.

The study was a multicenter, randomized, placebo controlled study of NSTI patients treated with AB103 that was conducted at several high quality trauma sites across the US. Results from this study indicated that AB103 had a dose dependent positive effect on multiple clinical relevant end points, both local and systemic. Patients (infected by various pathogens) that received AB103, had statistically significant improvement in organ function, higher recovery rate from acute kidney injury, reduced need for critical care support and needed less debridements to control the infection. The data demonstrate internal consistency of all the measured components with a correlation between utilization of hospital related resources and organ dysfunction. AB103 is currently being evaluated in a phase 3 study (ACCUTE study), for patients with NSTI. The study is randomized, placebo controlled clinical trial with the primary objective of demonstrating efficacy of AB103 as compared to Placebo, using a clinical composite end point. The study is conducted in 60 clinical sites in the US, with a target enrollment of 290 patients. NSTI serves as model for a range of other diseases characterized by an excessive inflammatory response to pathogens and/or toxins and as such, AB103 is considered in additional indications where acute inflammation is a key cause of morbidity.

## Discovery and Clinical Development of Elamipretide

**Hazel H. Szeto, M.D., Ph.D. | Adjunct Professor**

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Mitochondria play a central role in energy generation in the cell, providing ATP to carry out essential biological functions. As energy output declines, the most energetic tissues are preferentially affected, including the skeletal muscles, heart and eyes. Age-related decline in mitochondrial function is associated with numerous complex diseases, including heart failure, kidney disease, neurodegenerative diseases, loss of visual function, and muscle weakness. This talk will describe the discovery of a family of mitochondria-targeted tetrapeptides (Szeto-Schiller peptides) that preserve mitochondrial structure and restore cellular bioenergetics by targeting cardiolipin, a unique phospholipid on the inner mitochondrial membrane. Preclinical and clinical development of the first drug candidate (Elamipretide, Bendavia™) for rare genetic mitochondrial diseases and common age-related mitochondrial diseases will be presented.



# 11th Annual Peptide Therapeutics Symposium

## Targeting Novel Polysaccharide-binding Proteins to Block Tissue Fibrosis

### Eva Ann Turley, Ph.D. | Co-Founder

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Fragmentation of polysaccharides such as hyaluronan (aka hyaluronic acid) are early danger signals warning of tissue injury. They play such key roles in initiating the inflammatory response as providing chemotactic and chemokinetic signals for macrophages, mesenchymal progenitor cells and fibroblasts. Hyaluronan is also emerging as a player in mesenchymal lineage decisions and as an example regulates myofibroblast differentiation. Hyaluronan fragments signal through a number of receptors including toll-like receptors and CD44 as well as a co-receptor RHAMM. Although the first two receptors are ubiquitously expressed, RHAMM is unique in that it is primarily expressed during response to injury processes and not during homeostasis. It therefore is an interesting therapeutic target that is predicted to have low toxicity profile. We first isolated RHAMM as a motogenic protein released from rapidly migrating mesenchymal progenitor cells. Later our analyses of RHAMM<sup>-/-</sup> mice established that RHAMM expression is required for fibrotic repair of excisional skin wounds. Using these mice, other laboratories demonstrated that RHAMM loss blunted fibrosis following vascular and lung injury. RHAMM is a cytoplasmic and unconventionally secreted protein that partners with integral receptors including CD44, TLR2,4 and key fibrogenic growth factor receptors (e.g. PDGFR). Its partnership with these receptors together with its binding to specific sizes of hyaluronan fragments is required for robust activation of signalling pathways such as MAPK and PI3 kinase cascades, which results in expression of pro-inflammatory and pro-fibrosis genes. These RHAMM mediated associations are required for the expression of a subset of MAP kinase/PI3 kinase target genes including IL-1 $\beta$ , RANTES, TGF $\beta$ -1 and CTGF. In collaboration with Dr. Len Luyt, we therefore developed peptide mimics of RHAMM designed to block its binding to hyaluronan fragments, which are present in small amounts even in damaged or repairing tissues. Several of these peptides have now been assessed for their ability to block RHAMM induced pro-inflammatory and fibrogenic cytokine expression, to reduce macrophage and fibroblast migration and to reduce TGF $\beta$ -1 expression by lung fibroblasts. These have also been assessed for their ability to reduce fibrosis in a bleomycin lung injury model and shown to reduce damage to lung architecture as assessed by Ashcroft scoring and reduce lung tissue fibrosis as measured by hydroxyproline levels and collagen1/3 mRNA ratio. The potential therapeutic uses of these peptides and utility of RHAMM as therapeutic target for other diseases is discussed.

# 11th Annual Peptide Therapeutics Symposium

## Non- Peptide GLP-1 Receptor Agonists; From Idea to Medicine

**Carmen Valcarce, Ph.D. | Senior Vice President and Chief Scientific Officer**

vTv Therapeutics

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GLP-1R is a well validated target for the treatment of T2DM, with multiple marketed injectable GLP-1 analogues/mimetics that provide glycemic control and weight loss in T2DM patients. Although several of these peptides targeting GLP-1 receptor have reached blockbuster status, their use has been limited by two major factors: 1) route of administration - injections and 2) unfavorable tolerability profile - nausea and vomiting. Recognizing these drawbacks, scientists at vTv Therapeutics (vTv) have designed orally bioavailable small molecule (non-peptide) GLP-1R agonists (TTP054 and TTP273) that have a favorable tolerability profile compared to the injectable GLP-1 analogues/mimetics.

The vTv compounds are stand-alone agonists of the GLP1-receptor and were discovered using vTv's proprietary drug discovery engine, Translational Technology®, in silico modeling, cellular binding and activation studies. In contrast to marketed GLP-1 peptides mimetics/analogues, these small molecules are orally administered, i.e. delivered near the site of secretion of GLP-1 (the gut), which may better resemble the physiological site of action of native GLP-1 and might provide a better safety profile.

Based on in silico modeling, TTP054 and TTP273 share the same putative “allosteric” binding site and are specific for GLP-1 receptor activation. Evaluation of receptor signaling through G protein and ERK pathways show a relative selectivity of TTP273 for cAMP with no significant activation of ERK at clinically relevant concentrations.

The vTv GLP-1R agonists have demonstrated efficacy in nonclinical and clinical studies by improving glycemic control and reducing body weight. Additionally, vTv's compounds have shown an impressive tolerability profile in the clinic as compared to the published tolerability profiles of the peptide GLP1 analogues/mimetics.

The vTv oral small molecule GLP1R agonists confirm that Class-B receptors can be activated by non-peptide small molecules and could provide an alternative to expand the use of GLP-1R therapies. vTv is currently advancing its GLP-1R program in a phase 2 clinical trial.

## Homogeneous Glycoproteins for Structural and Functional Study

**Chi-Huey Wong, Ph.D. | Professor of Chemistry, Department of Chemistry**

The Scripps Research Institute

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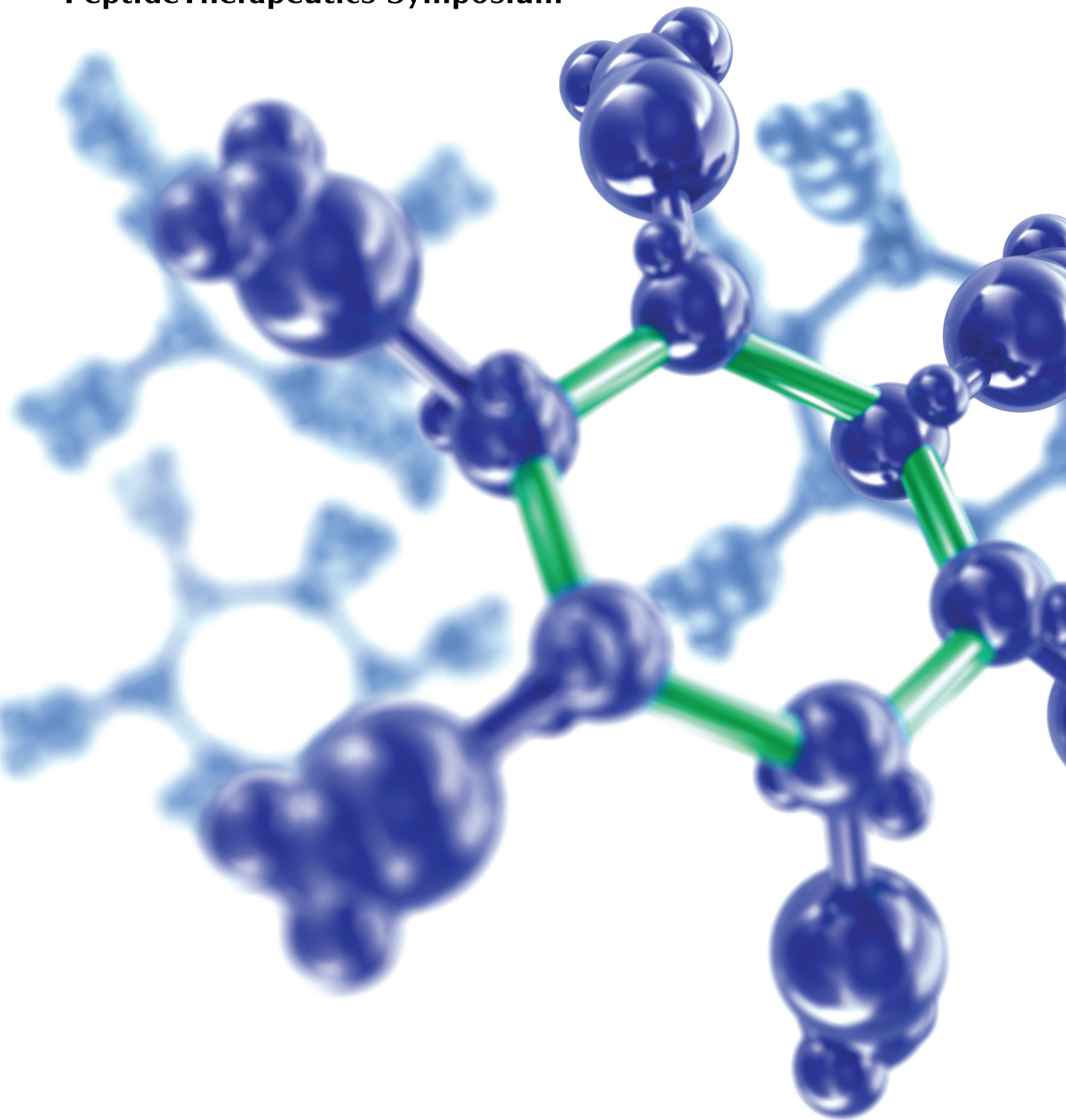
Protein glycosylation is a complex post- and co-translational event used by nature to modulate the structure and function of proteins. However; it has been difficult to understand the role of this process as it always creates a mixture of heterogeneous glycoforms during the process. This lecture will present several strategies developed in our laboratory for the preparation of homogeneous glycoproteins with well-defined glycan structures at the glycosites, and illustrate the use of synthetic homogeneous glycoforms to investigate the effect of glycan structure on the folding, stability and function of glycoproteins as well as their implication in biomedical development.

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**Abstracts of Poster Presentations  
11th Annual  
Peptide Therapeutics Symposium**



## 11th Annual Peptide Therapeutics Symposium

**PO1 Protein Kinase C Epsilon Peptide Inhibitor Exerts Cardioprotective Effects in Myocardial Ischemia/Reperfusion Injury**

Christine Adekayode, Anahi McIntyre, Israel Benjamin, Joseph Heron, Stephanie Liu, Ifeanyi James, Qian Chen, Robert Barsotti, [Lindon H. Young](#).

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During myocardial ischemia/reperfusion (I/R), the generation of reactive oxygen species (ROS) contributes to post-reperfusion cardiac injury and contractile dysfunction. Activation of protein kinase C epsilon (PKC  $\epsilon$ ) has been shown to increase ROS release, in part, by its stimulation of increased uncoupled endothelial nitric oxide synthase activity during I/R. We hypothesize that using a cell permeable PKC  $\epsilon$  peptide inhibitor (PKC  $\epsilon$ -) (N-myr-EAVSLKPT, MW=1054 g/mol, 10 $\mu$ M) will improve post-reperused cardiac function and attenuate infarct size compared to untreated controls in isolated perfused rat hearts subjected to I(30 min)/R(90 min). Male Sprague-Dawley rats (275-325 g) were anesthetized with sodium pentobarbital (60 mg/kg) and anticoagulated with heparin 1000 units IP. PKC  $\epsilon$ - was dissolved in Krebs' buffer and infused for the first 10 min of reperfusion. PKC  $\epsilon$ - treated hearts exhibited significant improvement in post-reperused cardiac function at 90 min (left ventricular developed pressure (LVDP): 63 $\pm$ 5%; maximal rate of LVDP (+dP/dt<sub>max</sub>): 55 $\pm$ 6%; n=5) compared to untreated controls (n=5) which only recovered to 42 $\pm$ 6% and 34 $\pm$ 6% of baseline values for LVDP and +dP/dt<sub>max</sub> respectively (p $\le$ 0.05). Furthermore, PKC  $\epsilon$ - treated hearts showed significant reduction in infarct size (26 $\pm$ 2% compared to controls 38 $\pm$ 3%; p $\le$ 0.05). The results suggest that PKC  $\epsilon$ - is effective in improving cardiac function and reducing infarct size and is a putative treatment that could aid in clinical myocardial infarction/organ transplantation patient recovery.

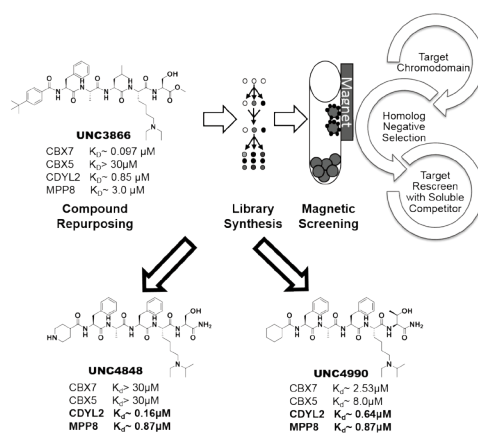
This study was supported by the Center for Chronic Disorders of Aging, the Division of Research and the Department of Bio-Medical Sciences at Philadelphia College of Osteopathic Medicine.

**PO2 A Combinatorial Repurposing Platform for Chromodomain Chemical Probe Discovery**

[Kimberly Barnash](#), Kelsey Lamb, Jacob Stuckey, Jacqueline Norris, Stephanie Cholensky, Dmitri Kireev, Stephen Frye, Lindsey James

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Peptidomimetic inhibitors of protein-protein interactions offer the advantage of a rational starting point for ligand optimization, but face a steep, uphill battle to potent and selective inhibitors capable of permeating the cell membrane. Strategies for the rapid optimization of peptide properties hold the potential to drastically increase the rate of chemical probe discovery for these interactions. Our lab has developed a target class strategy for the rapid and cost-effective discovery of peptidic inhibitors of methyl-lysine readers via combinatorial chemistry and on-bead screening. Our discovery of UNC3866 as an effective cellular chemical probe for Polycomb repressive complex 1 (PRC1) chromodomains has demonstrated the efficacy of peptidic inhibitors against these reader proteins, but this inhibitor has limitations due to off-target affinity for the chromodomains CDYL/CDYL2 and MPP8. Additionally, the SAR driven approach employed to develop UNC3866 is inefficient as a target class strategy; however, the peptidic nature of UNC3866 enables rapid diversification using split-and-pool synthesis. We developed a targeted UNC3866-derivative library to repurpose this class of inhibitors for CDYL/CDYL2 and MPP8. By applying stringent negative selections and soluble competitor, pools of hit compounds were characterized on-bead to provide inhibitors with new selectivity profiles. The highest redundancy hits were resynthesized yielding submicromolar compounds, UNC4990 (CDYL/CDYL2-selective) and UNC4848 (CDYL/CDYL2 and MPP8-selective). The in vitro selectivity profiles of these ligands demonstrates the effectiveness of this combinatorial chemistry target class approach and highlights the efficiency of this platform in optimizing chromodomain inhibitors.



## **P03 Evaluation of a Novel, Broadly Conserved Antigen of *Borrelia burgdorferi* as a Lyme Disease Vaccine**

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*Borrelia burgdorferi* is the causative agent of the most commonly reported arthropod-borne illness in the United States, Lyme disease. Outer surface proteins are the most common target of vaccination efforts due to their exposure to the immune system. However, surface antigens presently employed in Lyme disease vaccines are heterogeneous and thus require the utilization of several copies of each epitope to generate effective protection. As such, the identification of an antigen that is highly conserved in all pathogenic *Borrelia* would allow for a vaccine against Lyme to be more effective while at the same time require fewer antigen molecules. To accomplish this goal, genome analysis was performed to identify a set of highly conserved genes across the genus *Borrelia*, the vonWillebrand Factor A (VWFA) domain containing proteins. To identify new potential antigens, *in silico* analysis and *in vitro* assays were used to determine cellular localization, functional domains, and antigenicity. One VWFA domain containing protein, BB0172, was determined to be extracellularly exposed and expressed during transmission of *B. burgdorferi* from tick to mammalian host. BB0172 also binds host integrin  $\alpha 3\beta 1$ , and taken together, likely functions during infection and dissemination in the mammalian host — making it a strong vaccine candidate. Peptide antigens derived from BB0172 were evaluated as candidates for a novel, broadly protective Lyme disease vaccine. One particularly promising peptide, PepB, was found to offer 50% protection against tick challenge with *B. burgdorferi* in the murine model. Building on that work, the PepB antigen is being reformulated to enhance antigenicity and protection through the utilization of an outer surface protein derived scaffold.

## **P04 Investigating the Endosomolytic Activity of Branched, Multivalent Variants of the Cell-Penetrating Peptide TAT**

Dakota J. Brock, Alfredo Erazo-Oliveras, Kristina Najjar, Ting-Yi Wang and Jean-Philippe Pellois\*

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Cell-penetrating peptides (CPPs) are beneficial tools that can be exploited to mediate the delivery of biologically active, macromolecular cargoes into cells. Even though this behavior has been well-established, the usefulness of many existing CPPs, such as the prototypical CPP TAT, has been greatly depreciated due to the suboptimal release of CPPs and cargoes into the cytosolic space of cells. On a cellular level, this event stems from the low propensity of endocytosed CPPs to escape from endosomes ultimately leading to endosomal entrapment. Recently, new classes of highly-efficient CPPs have been developed that evade the problem of endosomal entrapment. These compounds share a universal trait: they are multivalent or include multiple CPP copies or branches in close proximity. Here we investigate the effect that multivalency has on cell-penetrating activity. To perform this study, a new generation of branched, multivalent CPPs (mCPPs), which differ only in CPP copy number, have been designed and synthesized. By using *in cellulo* and model lipid bilayer systems, these mCPPs have served as tools to assess the extent of cytosolic penetration, route of cellular internalization, and mechanism of endosomal escape in live cells. Our findings would thus provide a fundamental understanding of the contribution of multivalency towards the efficiency and mechanism of TAT cell-penetration. The knowledge obtained from these studies can then be implemented as an innovative approach to designing highly deliverable or cell-permeable probes and drugs, thereby furthering the fields of cellular biology and therapeutics.



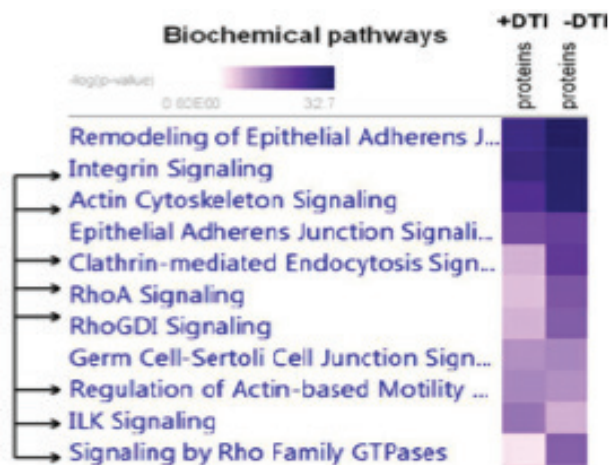
# 11th Annual Peptide Therapeutics Symposium

## P05 Label Free Proteomics Profiling of Thrombin-activated Platelets Highlights the Down-regulation of the Integrin and RhoA/ILK Signaling Pathways in Response to the Treatment with Peptidic Thrombin Inhibitors

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Thrombin is known to induce the activation of platelet aggregation by binding to and cleaving the extracellular N-terminal domains of protease-activated receptors 1 and 4 (PAR1 and PAR4). To date, many direct thrombin inhibitors (DTI) have proved to be potent inhibitors of thrombin-activated platelet aggregation. Such DTI can be used as pharmacological agents for the management of Acute Coronary Syndrome (ACS). This research presents the structure-based drug design (SBDD), synthesis and evaluation of novel tetrapeptide DTI inhibitors of thrombin-activated platelet aggregation, all done by means of proteomics. Analogs of the lead DTI, [D-Phe(P<sub>3</sub>)-Pro(P<sub>2</sub>)-D-Arg(P<sub>1</sub>)-D-Thr-(P<sub>1</sub>')-CONH<sub>2</sub>], have been designed to improve the hydrophobic driven interactions with the S3 pocket of thrombin by replacing the D-Phe (in P3) with D-3,3-diphenylalanine (DIP). The newly DTI lead compounds completely inhibited threshold  $\checkmark$ -thrombin-induced platelet aggregation at concentrations of 25-800 nM. The global changes in the protein expression profiles in the human platelets treated with the lead DTI in presence of thrombin were further analyzed by label free quantification (LFQ) using nanoLC-MS/MS on a Q-Exactive-Orbitrap mass spectrometer. Ingenuity Pathways Analysis (IPA) highlighted that many proteins involved in the actin, ILK, rhoA, rhoGDI and integrin signaling pathways are down-regulated in the lead DTI-treated platelets. These data sustain the new designed DTIs as potent inhibitors of thrombin-activated platelet aggregation and further advocate for the use of the label free proteomics profiling as a reliable assay for monitoring the efficacy of selected drug treatment during ACS management.





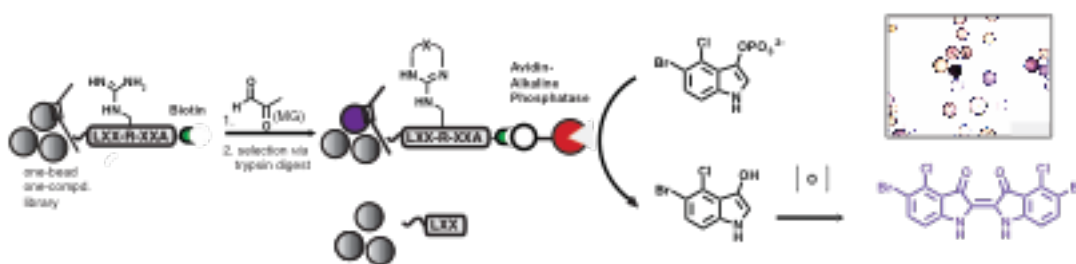
# 11th Annual Peptide Therapeutics Symposium

## P06 Defining the Scope of Selective Glycation, a Native Non-enzymatic Posttranslational Modification

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Detection of persistent and elevated levels of non-enzymatic posttranslational modification (nPTM) in disease models has prompted a concerted effort to understand their role at the molecular level. One of the most prevalent and diverse nPTM is glycation, which occurs through the addition of sugars and sugar metabolites to protein to give rise to an array of chemical protein modifications collectively termed advanced glycation end-products (AGEs).<sup>1</sup> Although AGEs are formed spontaneously, the distribution of the products *in-vivo* is not random.<sup>2,3</sup> As a result, controlling selective AGE modification experimentally remains a fundamental challenge. Our work focuses on understanding the underlying factors that drive selective glycation. Central to our approach is the hypothesis that the protein local environment, a combination of sequence and structure surrounding the reaction site, is responsible for dictating the distinct glycation outcome. To explore this idea we employed a one-bead one-compound peptide library to select for peptide sequences that are able to promote glycation. Using this strategy, we have seen the emergence of acidic and charged motifs as one of the primary variables for increased glycation. This work will lay the foundation to map glycation networks under mild conditions such as those found in a living cell.



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## P07 The Development and Validation of QC Analytical Techniques for the Study of Peptides and Complex Peptide Mixtures

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Tepnel has a wealth of knowledge and history of performing method development. Our strategy is to employ the principles of QbD to ensure that the most applicable separation solution is created and tailored to the need of our clients.

For >10 years our experience has been extensive in the development and application of separation techniques for peptides. Typically we have likened peptides to small molecules to influence the approach we have taken. This has been successful for over 40+ separations utilising UHPLC with UV and MS detection.

The development and validation of QC analytical techniques for the study of peptides and complex peptide mixtures is not trivial. In many cases, methods are either non-existent and require full development or are commonly not fit for purpose having been developed for research purposes and need re-evaluation and development before verification and validation.

Three case studies will be presented to highlight the challenges faced providing analytical support to a number of projects.

One example study demonstrates the development of a stability indicating method for purity measurement of a therapeutic peptide drug substance. The development was completed within a design protocol to build quality and reliability into the method. Advantages gained by development were realised in cost and time savings coupled with confidence and accuracy in the data produced. Method robustness was displayed with successful validation and adoption for long term stability projects.

## 11th Annual Peptide Therapeutics Symposium

**P08 Intracellular Uptake of Cysteine-rich Peptide Derived from *Hibiscus sabdariffa***

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Cysteine-rich peptides (CRPs) are underexplored bioactive compounds in medicinal plants. They are stable and conformational-constrained mini-proteins of 2-8 kDa. Our laboratory has identified a novel family of CRPs derived from *Hibiscus sabdariffa*, designated roseltides. The prototypic roseltide rT1 is a knottin-type neutrophil elastase inhibitor. Here, we show the intracellular uptake mechanisms of roseltide rT1. Using chemical biology approach, we fluorescently-labeled rT1 at its N-terminus using Cy3-NHS ester. Our results revealed that Cy3-rT1 can be internalized by human endothelial cells (HUVEC-CS) and lung fibroblast cells (WI38) using flow cytometry and live-cell confocal microscopy. Using glycosaminoglycan-deficient cell line (PgsA-745), the efficiency of Cy3-rT1 internalization was significantly lowered compared to CHO-K1 (wild-type) cells ( $P < 0.05$ ), indicating that glycosaminoglycan is important for the cellular entry of Cy3-rT1. The cellular uptake was also demonstrated to be temperature-dependent, suggesting endocytosis. Endocytosis of rT1 is achieved through dynamin-mediated endocytosis as shown by the attenuation of cellular uptake using specific endocytosis inhibitor. Cy3-rT1 has endosomal escape properties which was supported by its subcellular localization at the mitochondria, observed using live-cell confocal microscopy. Taken together, our results showed that roseltide rT1 is the first-in-class, dynamin-mediated internalized cysteine-knot mitochondria-targeting peptide with potentials in the development of mitochondrial-targeting therapeutics. Our results also suggested that roseltide rT1 may act on the “undruggable” intracellular molecular targets.

**Acknowledgement**

This project is supported in part by the National Research Foundation (NRF-CRP8-2011-05) of the Prime Minister's Office of Singapore.

**P09 A Hunter-killer Peptide Targeting Adipose Stromal Cells Inhibits Cancer Progression**Fei Su<sup>1</sup>, Achinto Saha<sup>2</sup>, Alexes Daquinag<sup>1</sup>, Brad Snyder<sup>1</sup>,John DiGiovanni<sup>2</sup> and Mikhail G. Kolonin<sup>1</sup><sup>1</sup>The University of Texas Health Science Center at Houston, Houston, TX, 77030, <sup>2</sup>The University of Texas at Austin, Austin, TX, 78723

Cancer is a promising indication for emerging peptide therapeutics. Adenocarcinoma progression is promoted by infiltrating mesenchymal stromal cells (MSC). Adipose stromal cells (ASC) are MSC that serve as adipocyte progenitors and endothelium-supporting cells in white adipose tissue (WAT). We have reported that ASC trafficking to tumors promote human prostate cancer progression (T. Zhang et al., Nature Comm. 2016). To test if cancer progression can be blocked by ASC targeting, we have used a proteolysis-resistant targeted hunter-killer peptide D-CAN composed of a cyclic domain CSWKYWFGEK homing to ASC expressing PDGFR $\beta$  and of a pro-apoptotic domain KFAKFAK<sub>2</sub>. We reported that depletion of PDGFR $\beta$ + ASC with D-CAN suppresses obesity development and tumor growth in mouse allograft models (Daquinag et al., Mol. Therapy 2016). Here, we investigated the effects of D-CAN in a spontaneous genetic mouse model of prostate cancer, Hi-Myc. We show subcutaneous D-CAN administration depletes prostate stromal cells expressing a chemokine CXCL12. We provide evidence that the epithelial-mesenchymal transition (EMT) is reduced upon depletion of CXCL12-expressing cells. Our results suggest that PDGFR $\beta$ + ASC promote the EMT, at least in part, via CXCL12 paracrine signaling. By analyzing cells derived from WAT of bariatric surgery patients, we demonstrate that, like in mice, D-CAN selectively depletes PDGFR $\beta$ + ASC. We propose that peptide-based drugs targeting ASC can be developed as a therapy suppressing cancer progression to complement conventional cancer treatments.

**P10 Development Trends for Peptide Therapeutics: Status in 2016**

Jolene L. Lau and Michael K. Dunn

Ferring Research Institute Inc.

Peptides are an important class of therapeutics, with over 50 peptide drugs now approved in the US and other major markets. Peptides continue to enter clinical development at a steady pace. We have compiled and maintain a comprehensive dataset on >500 peptides that have entered human clinical studies (~250 currently approved or in active development by pharmaceutical companies). Here we present data on the molecular and pharmacologic characteristics of peptides in development; key therapeutic indications; and success rates for various phases of clinical development.

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## P11 Myristoylation of Protein Kinase C Isoform Peptide Inhibitors Exert Differential Regulation of Endothelial Derived Nitric Oxide Release

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Protein kinase C (PKC) phosphorylation of endothelial nitric oxide synthase (eNOS) by PKC beta II ( $\beta$ II) or zeta ( $\zeta$ ) on Thr-495 leads to decreased eNOS activity whereas phosphorylation by PKC epsilon ( $\epsilon$ ) on Ser-1177 leads to increased eNOS activity. Myristoylation (myr) of peptides enables rapid entry into cells to affect biochemical processes. However, the efficiency of myr-PKC  $\beta$ II peptide inhibitor (10  $\mu$ M) (N-myr-SLNPEWNET), myr-PKC  $\zeta$  peptide inhibitor (10  $\mu$ M) (N-myr-SIYRRGARRWRKL), and myr-PKC  $\beta$ II peptide inhibitor (10  $\mu$ M) (N-myr-EAVSLKPT) have not been compared to their native peptide sequences in regulating NO release. We hypothesized that myr-PKC  $\beta$ II and  $\zeta$  peptides would enhance eNOS derived NO release; however, myr-PKC  $\epsilon$  would decrease this effect. In addition, native peptide inhibitors would exhibit minimal effects on NO release. Thoracic rat aortas were extracted from male Sprague-Dawley rats (275-325 g), cut into four equivalent segments (~10 mg/segment), and pinned into a 24 well culture dish with the endothelial surface facing upwards containing 1 mL of Krebs's buffer maintained at 37°C. NO release was measured using a calibrated NO electrode. PKC  $\beta$ II and  $\zeta$  peptide inhibitors significantly increased basal NO release by  $5.86 \pm 0.76$  and  $8.58 \pm 2.46$  pmol/mg respectively compared to their native peptides which only showed an increase of  $1.99 \pm 0.78$  and  $2.00 \pm 0.66$  pmol/mg respectively. By contrast, PKC  $\epsilon$  peptide inhibitor decreased basal release by  $6.01 \pm 1.02$  pmol/mg. The effects of myr-conjugated peptides were significant compared to their non-myristoylated native peptides ( $p < 0.05$ ). These results suggest that myr-conjugated PKC peptides facilitate regulation of PKC function as compared to native peptides.

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.

## P12 Knottin-type Neutrophil Elastase Inhibitor Derived from *Hibiscus Sabdariffa*

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Cysteine-rich peptide (CRP) is an underexplored class of bioactive principles in medicinal plants. They are miniproteins which are structurally stable and have a myriad of biological activities<sup>1,2</sup>. Knottins is a class of CRP characterized by their cystine-knot topology (disulfide connectivity: C<sub>I</sub>-C<sub>IV</sub>, C<sub>II</sub>-C<sub>V</sub> and C<sub>III</sub>-C<sub>VI</sub>)<sup>3</sup>. Here, we report the isolation, identification and characterization of a knottin-type CRP with inhibitory activities against neutrophil elastase from *Hibiscus sabdariffa*. Using reversed-phase high performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), we identified a CRP with molecular weight 2620 Da, designated as roseltide rT1. S-reduction and S-alkylation by dithiothreitol (DTT) and iodoacetamide (IAM), respectively, revealed that roseltide rT1 contains six cysteine residues with three disulfide bonds. NMR structural studies showed that roseltide rT1 adopts a knottin-type fold. Stability assays demonstrated that roseltide rT1 is resistant against proteolytic, acid and human serum-mediated degradation. Roseltide rT1 displayed human neutrophil elastase inhibition in a dose-dependent manner. However, this activity was not observed in porcine pancreatic elastase. To demonstrate the cellular effects of elastase inhibition, we established a cellular model overexpressed with protease activated receptor 2 (PAR2) and cAMP biosensor. Results showed that roseltide rT1 ameliorated neutrophil elastase stimulated cAMP level. Taken together, this study highlights the discovery of roseltide rT1, a knottin-type neutrophil elastase inhibitor and its therapeutic potentials for the treatment of neutrophil elastase-related diseases including chronic obstructive pulmonary disease (COPD), pain and inflammation<sup>5</sup>.

### Acknowledgement

This project was supported in part by the National Research Foundation (NRF-CRP8-2011-05) of the Prime Minister's Office of Singapore.

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## 11th Annual Peptide Therapeutics Symposium

**P13 An Entirely SPPS-Based Strategy for Synthesis of GBAP Analogue Libraries: Towards the Attenuation of *Enterococcus faecalis* Quorum Sensing-Dependent Pathogenicity**

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The increasing prevalence of multiple drug resistant bacteria such as vancomycin-resistant enterococci (VRE) has prompted investigation into alternative therapeutics that are not bactericidal. The dependency of virulence in most pathogens on communication pathways, specifically quorum sensing (QS), has attracted significant attention to these pathways as a potential anti-infective target. Generally, QS circuits are centered on the production, secretion and detection of signal molecules to assess the bacterial population density. In Gram-positive bacteria, these signals are oligopeptides termed autoinducing peptides (AIPs). The development of analogues of the AIP signals, capable of inhibiting the QS circuitry, can provide an alternative means of treating infections by these pathogens. Because these signal analogues would potentially eradicate infections by disrupting or attenuating the infectivity of the bacteria, rather than by killing them directly, the selective pressure favoring development of resistance would be dramatically reduced. *E. faecalis* is the predominant enterococci responsible for clinical infections, and has been implicated in granting vancomycin resistance to Methicillin-resistant *Staphylococcus aureus* (MRSA). The gelatinase biosynthesis activating pheromone (GBAP) is a lactonebased cyclic AIP signal that regulates virulence factor production and the initiation of infections in *E. faecalis*. In this study, we aim to target the GBAP-mediated QS circuit, termed *fsr*, as a non-bactericidal strategy to treat *E. faecalis* infections. To facilitate the development of GBAP analogue libraries, we have developed an entirely solid-phase peptide synthesis (SPPS)-based strategy for the construction of GBAP and analogues. Our approach allows multiple alternative attachment sites to the resin, thus permitting the preparation of a wide variety of analogues. This approach accelerates the preparation of peptides by avoiding solution-phase synthetic steps with their concomitant intermediary purification steps. Furthermore, this approach is also compatible with automation. We have used this synthetic approach to prepare bioactive GBAP and several preliminary analogues and are currently testing them for QS modulation using Beta-galactosidase *E. faecalis* QS reporter assays.

**P14 Peptide as a Promising Tool for Self Assembled Monolayers in Label-Free Capacitive Diagnostics**

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Capacitance derived electroanalytical assays are sensitive indicators of interfacial change, such as target binding at an appropriate receptor. Most of the researches are concerned in alkanethiol SAMs. However, peptides have proved to be a promising tool for SAMs, improves the affinity, specificity, and stability of molecular recognition components for the development of self-assembled monolayers. Herein, we described synthesis of redox tagged peptide with self-assembling capability, for the C Reactive Protein detection (indicative of risk of diabetes, hypertension, and cardiovascular disease). Peptide containing ferrocene (fc) was synthesized by solid phase peptide synthesis (Fc-E-A-A-C-NH<sub>2</sub>). To obtain the electrochemically capacitive interface, the side chain of Cys was covalently bound to the gold electrode, the N-terminus group was used to attach the ferrocene in the peptide chain and the anti-CRP was attached to the peptide using the side chain of glutamate. The self-assembly and redox capability was characterized by cyclic voltammetry and electrochemical impedance based capacitance spectroscopy techniques. The surface coverage was found to be  $\Gamma = (2.8 \pm 0.15)10^{-10}$  mol cm<sup>-2</sup>, and the limit of detection was estimated to be 149 pmol L<sup>-1</sup>, which is comparable with alkanethiolate monolayers (240 pmol L<sup>-1</sup>)<sup>1</sup>. The electron transfer rate of the peptide SAM (12 s<sup>-1</sup>) is in good agreement with the alkanethiolate (13 s<sup>-1</sup>)<sup>2</sup>. Exemplified here with a clinically important target, it is clear that redox-tagged peptide have their advantages. In addition, the design of redox active peptides self-assembly is predictably useful in the development of biosensor devices, making use of the electrochemical capacitance signal intrinsically existing in redox-active monolayers<sup>3</sup>.

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# 11th Annual Peptide Therapeutics Symposium

## P15 Expanding the Scope of Microwave Assisted Solid Phase Peptide Synthesis (SPPS): R&D Scale to Pilot Plant

Keith Porter, Jonathan Collins

CEM Corporation, Matthews, NC, USA

Peptide therapeutics are an attractive alternative to their small molecule drug counterparts <sup>[1]</sup>. With several high revenue peptide drugs on the market and a pipeline full of potential candidates <sup>[2]</sup>, the demand for highly robust and efficient synthetic methods is of great importance. Microwave assisted SPPS has established itself as the primary chemical method to produce high quality peptides while drastically reducing synthesis time and waste <sup>[3]</sup>. In this presentation, the most recent advances in microwave assisted SPPS are reported. Topics include: (1) unpublished research in the development of a highly active, universal resin for preparing C-terminal peptide acids and (2) a protocol for scale up production using microwave assisted SPPS for the synthesis of peptides in pre-clinical and early phase studies. This protocol incorporates 24 – 48 amino acids per 24hr with a demonstrated scale of 500g purified peptide. Crude purity from R&D to production scale is preserved if not improved and unwanted side reactions such as epimerization and aspartimide formation are easily controlled. The result, easier purification and reduced labor cost.

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## P16 Novel Synthetic Therapeutic Peptides Targeting Both Untreatable Cancer and Fibrosis

Bruce L. Riser

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Nearly half of the deaths in the developed world are associated with fibrosis, which can affect any organ; and the fibrotic-like stroma is likely key to progression and metastases in cancer. Over the last decade, targeting the CCN family of the matricellular signaling proteins, particularly CCN2, has emerged as a highly promising and attractive approach to the treatment of fibrotic diseases and cancer. In 2009, we showed that another CCN family member, CCN3, downregulated by TGF- $\beta$ , uniquely acts as a natural antagonist of CCN2 and fibrosis development in kidney cells (*Amer J Path*). More recently, our proof of principle study in an established mouse model of human type 2 diabetic nephropathy with obesity (*Amer J Path*, 2014), showed that treatment with rhCCN3 both halted and reversed key hallmarks of established fibrosis and loss of kidney function. In cancer, to date, the clinical work targeting CCN2 has not focused on peptides, despite their inherent potential advantages (e.g. good efficacy, safety and tolerability, high target selectivity, potency, and predictable metabolism- collectively resulting in lower attrition rates). To address this need, BLR Bio has developed proprietary peptides capable of not only halting fibrosis initiation and stromal support of cancer progression, but also of re-establishing tissue homeostasis. In cancer, these peptides will have efficacy alone- both by removing the cancer-microenvironment and as direct inhibitors of cancer cell replication. However, perhaps more importantly, they will greatly augment the efficacy of conventional chemotherapy and immunotherapy (and at lower doses) when used in combination.

## P17 The Importance of Peptide-Membrane Interactions in Toxin Inhibition of Therapeutically Relevant Sodium Channels

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Peptide toxins isolated from spiders and cone snails are potent inhibitors of human voltage-gated sodium channels (NaV). Some of these peptides are selective against subtype Na<sub>v</sub>1.7, reported to be involved in nociception, and may thus have potential as pain therapeutic leads. Peptide toxins can inhibit Na<sub>v</sub> activity by blocking the pore domain (i.e. pore blockers) or by binding to the membrane-embedded voltage sensor domain of the sodium channel (i.e. gating-modifier toxins). It is still not known how and if gating-modifier toxins also interact with lipid membranes where voltage-sensor domains are located and whether peptide-lipid interactions are relevant for their inhibitory activity at the sodium channel. Using a range of biophysical techniques, we have examined the importance of membrane binding on the inhibitory activity of a subset of peptides, showing for the first time a direct correlation between membrane binding affinity and Na<sub>v</sub>1.7 inhibition, highlighting the importance of considering potential membrane-binding events when designing sodium channel voltage-gating modifier inhibitors as future therapeutic lead molecules.



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**P18 Discovering the Mechanism of Action of an Ocular Therapeutic Peptide using Transcriptomics and Proteomics**Zixuan Shao<sup>1</sup>, Dan Zhou<sup>2</sup>, Julia Kornfield<sup>2</sup><sup>1</sup> Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA<sup>2</sup> Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA

Age-related macular degeneration (AMD) and diabetic retinopathy (DR) are leading causes of blindness in the developed world. The current standards of care, anti-VEGF pathway antibody injections, require monthly doctor visits and are ineffective for a significant portion of the patient population. We are currently investigating a promising oligopeptide drug, ALG-1001, that has shown efficacy in clinical studies — including patients who are non-responsive to anti-VEGF treatments. While the drug shows efficacy in the clinic, the mechanism of action remains unclear. To elucidate the molecular basis of the drug's effects, we employ genomics and proteomics methods including RNA-seq and LC Mass Spectrometry to search for the regulatory pathways involved. Preliminary results suggest the drug may act on pathways linked to the immune response, which is implicated as a driver of degenerative ocular diseases. Results from this study will shed light on the pathophysiology of the relevant ocular diseases and may lead to better therapeutic treatments.

**P19 Development of CSP-based Therapeutics Against Pneumococcal Infections**Yifang Yang, Bimal Koirala, Naiya R. Phillips, Lucia A. Sanchez, Sally R. Hamry, and Yftah Tal-Gan

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*Streptococcus pneumoniae* (pneumococcus) is a deadly human pathogen that is the leading cause of invasive disease in children 2 years or younger. This bacterium is involved in a variety of chronic and acute human illnesses including pneumonia, bacteremia and meningitis. The rapid increase in resistance development in pneumococcus is of major concern and is attributed to the ability of this pathogen to acquire genetic information from the environment through a process termed competence. The competence stimulating peptide (CSP)-based quorum sensing (QS) circuitry, a cell-cell signaling mechanism that enables bacteria to assess their population density through the production, secretion and detection of signal molecules, governs competence in *S. pneumoniae*, along with biofilm formation and virulence factor production. Thus, interception of this QS circuitry can lead to attenuated infectivity as well as reduced antibiotic resistance development rate in this notorious pathogen. Since this QS circuitry is centered on a secreted peptide signal (CSP) and its interaction with a transmembrane histidine-kinase receptor (ComD) to trigger the QS pathway, we aim to develop CSP-based QS modulators that would be applied to prevent and clear pneumococcal infections, and presented here are our recent structure-activity relationship analyses of the CSP signal.

**P20 Simultaneous Membrane Translocation of Peptide Monomers with Cell-penetrating Peptide Oligonucleotide Complexes**Luis Daniel Vasconcelos<sup>1</sup>, Fatemeh Madani<sup>2</sup>, Tonis Lehto<sup>3</sup>, Vlad Radio<sup>2</sup>, Vladana Vukojevic<sup>2</sup>, Ulo Langel<sup>3</sup><sup>1</sup>Stockholm University<sup>2</sup> Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden<sup>3</sup> Department of Neurochemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, Sweden

Secondary amphipathic cell-penetrating peptides, as the N-terminal stearylated PepFacts, are delivery systems, which efficiently mediate cellular transduction of potential therapeutic oligonucleotides, such as siRNA and pDNA, after non-covalent complexation.

Identified as a major bottleneck to the *in vivo* application of PepFacts is the heterogeneity of the particles formed during complexation. The uncomplicated preparations of PepFact – oligonucleotide complexes are efficiently used *in vitro*, generally with low toxicity and high transfection yields. However a closer look at the formed supramolecular entities, reveal high polydispersity and unordered distribution of the cargo among the peptide particles, which limits their success for *in vivo* applications.

Using biophysical methods to study one of the most efficient PepFact members, we found that the size of its particles varies and is dependent of the type of cargo, the peptide sequence and the pH. Circular dichroism showed predominance of  $\alpha$ -helical peptide structures in the presence of oligonucleotide cargo and large unilamellar vesicles, a simple cell membrane mimic. Fluorescence Correlation Spectroscopy (FCS) measurements demonstrated the heterogeneity of particles that coexist in distinct equilibria in solution, namely free peptide, peptide particles and peptide – oligonucleotide complexes. Additionally, in cell FCS measurements using a U87 human primary glioblastoma cell line, have shown simultaneous plasma membrane translocation of these entities, what might explain the diversity of cellular uptake mechanisms usually found for these delivery systems.

## P21 The Dual Glucagon-Like Peptide-1 (GLP-1)/Glucagon Receptor Agonist MEDI0382 Reduces Body Weight in Diet-Induced Obese Mice via Inhibition of Food Intake and Increased Energy Expenditure

Sarah Will<sup>1</sup>, Louise Lantier<sup>2</sup>, Staci Bordash<sup>2</sup>, Simon Henderson<sup>3</sup>, Lutz Jermutus<sup>3</sup>, Anish Konkar<sup>1</sup>, James L. Trevaskis<sup>1</sup>.

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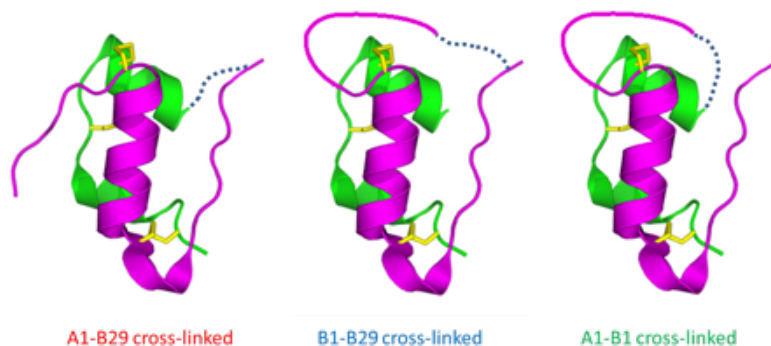
MEDI0382 is a dual GLP-1 and glucagon receptor agonist currently being explored for the treatment of obesity and diabetes. Here we explored the mechanism(s) via which MEDI0382 reduces body weight in diet-induced obese (DIO) mice. C57BL/6 mice on high-fat diet (60% kcal/fat) for 18 weeks were randomized to one of three treatment groups: vehicle, MEDI0382 (10 nmol/kg, s.c., q.d.), or vehicle and pair-fed to MEDI0382-treated mice. MEDI0382 significantly inhibited food intake (17.6%) and reduced body weight (16.0%) relative to vehicle controls at day 16. Pair-fed mice, with similarly inhibited energy intake (19.1%), demonstrated equal weight loss up until day 10 at which point the weight loss plateaued such that weight loss in pair-fed mice at day 16 was 9.5% ( $p < 0.05$  vs. MEDI0382 group). Weight loss induced by MEDI0382 administration, but not pair-feeding, was associated with a significant reduction in fat mass, whereas lean mass was not different between groups. Indirect calorimetry assessment for a consecutive 72 h period (day 17-20) revealed that MEDI0382 treatment increased rate of oxygen consumption adjusted for lean mass compared to vehicle controls with a trend for an increase vs. pair-fed mice ( $p = 0.077$ ). The respiratory exchange ratio (RER) was reduced by MEDI0382 treatment vs. vehicle group and tended to be lower vs. pair-fed controls ( $p = 0.064$ ). These data are consistent with increased metabolic rate and preferential fat utilization with MEDI0382 treatment. Physical activity levels were not different between treatment groups. In summary, MEDI0382 reduces body weight in obese mice via both appetitive and metabolic mechanisms.

## P22 Conformational Constrained Insulins Formed by Cross-linking with a Bifunctional Bridge

Fa Zhang, Fa Liu, John P. Mayer, and Richard D. DiMarchi\*

Department of Chemistry, Indiana University Bloomington, IN 47405

Molecular cross-links within peptide hormones constitute a valuable approach to interrogate secondary and tertiary structure-function relationship. Insulin analogs bearing intramolecular cross-links have historically served to define bioactivity as a function of restrictions to conformational mobility in select regions of the hormone. The A1-B29 analogs are easiest to prepare and as such they have been appreciably studied, while other regions of the peptide having received little to no attention. We have broadened the molecular space within insulin where cross-linking can be deployed using orthogonal chemistry and advanced analytical tools to further establish the relationship in conformational change to bioactivity, and physical stability. A set of novel, asymmetrical cross-linked insulin analogs were synthesized and studied by *in vitro* and *in vivo* methods. Starting from three intermediates where initial acylation occurs at three different amines, a second intramolecular reductive alkylation provided four unique cross-linked insulin analogs. It was determined that the epsilon-amino group is poorly reactive under our reductive amination conditions. Consequently to more fully use this chemical approach more aggressive conditions to alkylation at side-chain lysine will need to be developed or a structural mimetic recruiting the higher reactivity of alpha-amines will need to be used. Separately, the four analogs that were prepared proved to be of sizably, but differentially reduced potency when biologically studied. We conclude that this chemical approach using orthogonal cross-links to explore higher-order structure-function relationships is powerful, but needing of additional chemical refinement to achieve its full potential.





## 11th Annual Peptide Therapeutics Symposium

**P23 Finding the Binding Site of Luminat®<sup>®</sup>, a Therapeutic Peptide for Blinding Retinal Disease**

Dan Zhou, Zixuan Shao, Julia A. Kornfield

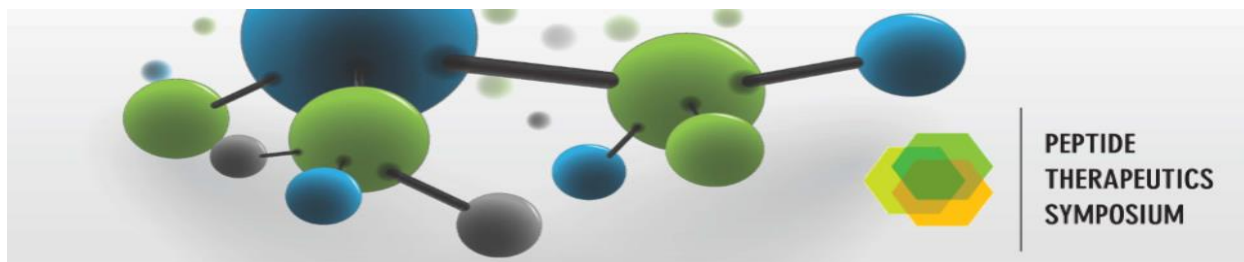
Division of Chemistry and Chemical Engineering, California Institute of Technology

Diabetic and age-related retinopathies, both associated with growth of abnormal blood vessels, are leading causes of blindness in the developed world. Current treatments, such as Laser Photocoagulation and anti-Vascular Endothelial Growth Factor (VEGF) drugs, have limited efficacy and undesirable side effects. A recently discovered therapeutic peptide Luminat®<sup>®</sup> (Allegro Ophthalmics, LLC) has proven to be effective in human clinical trials. Over half of the patients in Phase I Human studies demonstrated vision improvement with 4 lines or over, and in some patients, the macular thicknesses was reduced back to the healthy state. It has shown significantly longer lasting benefits than anti-VEGF treatments and shows synergistic effects when used with them. Thus, the peptide appears to act through a different pathway than anti-VEGF agents. Finding that pathway might give new insights into the retinopathies and their managements. Therefore, we are using the peptide as a tool to find the molecular mechanism of its clinically observed therapeutic effects.

The peptide and its scrambled counterparts are used to prepare fluorophore-peptide conjugates and peptide-directed coupling reagents. Results will be presented from in-vitro and ex-vivo experiments to visualize the distribution of Luminat binding using fluorophore-peptide conjugates. Progress toward enrichment of binding using ligand-directed receptor “pull down” will be described. Our goal is to enrich and identify the associated receptors by drug-directed crosslinking and immunoprecipitation. If successful, identification of the receptor that binds Luminat could provide further insight into the molecular basis of retinopathies, which could guide novel therapeutic agents to prevent vision loss.







## ERRATA

Friday, October 28, 2016

### Plenary Lectures

9:10 a.m. – 9:50 a.m.



### Novel Synthetic Strategies for Insulin and Related Peptides

**Fa Liu, Ph.D. | Director of Chemistry**

Novo Nordisk Research Center-Indianapolis

5225 Exploration Drive, Indianapolis, IN 46241 | (317) 518-8616

### Speaker Biography

Fa Liu is currently the Director of Chemistry at Novo Nordisk Research Center at Indianapolis. Dr. Liu received his Ph.D. from the Shanghai Institute of Organic Chemistry in 2004 followed by a five year stay at the National Cancer Institute as a postdoctoral fellow (2004-2007) and a scientist (2007-2009). He started his industrial career at Eli Lilly and Co. in 2009 where he spent five years. He joined Calibrium Biotech as Director of Chemistry in 2014 and started his current role in Novo Nordisk in Jan 2016. His research focuses on peptide therapeutics and the development of novel peptide chemistries.

### Lecture Abstract

The challenges involved in the chemical synthesis of insulin and insulin-like peptides hinder the exploration of the structure-function relationships of this class of biologically important hormones. Problematic features such as the hydrophobicity of individual chains and the complexity of the disulfide architecture both require the development of specialized strategies. We have successfully utilized the isoacyl peptide concept to overcome the hydrophobicity of the insulin A-chain and relaxin B-chain. In addition, we have modified the iodine/Acm-directed disulfide bond forming conditions to iodine-free methods via chemical or enzymatic means. These conditions are fully compatible with oxidation-sensitive residues including tryptophan and methionine. Furthermore, we have integrated the isoacyl peptide concept and the single-chain redox folding approach to provide a method which is broadly applicable to the synthesis of two-chain insulin-like peptides and their analogs.

### Poster Session Additions

#### **P24 Development of a Multi-Step Synthetic Peptide Purification Process Utilizing a Single Stationary Phase**

J Preston<sup>1</sup>, Guido Krautz<sup>2</sup>, and Marc Jacob<sup>1</sup>

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

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

## **P25 In Situ COMU Activation to Facilitate Long Peptide Syntheses On Prelude X**

Daniel Martinez<sup>1</sup>, Elizabeth Restituyo-Rosario<sup>1</sup>, James P Cain<sup>1</sup>, and Fernando Albericio<sup>2</sup>

*1 Gyros Protein Technologies, Tucson, United States*

*2 Institute for Research in Biomedicine, Barcelona, Spain*

The success of the coupling steps during solid phase peptide synthesis (SPPS) has always been dependent on the efficiencies of the reagents used. Many efforts have been directed to the discovery of different coupling reagents and additives in order to increase peptide synthesis yield and purities, including the recent development of the highly efficient COMU and Oxyma Pure. Despite the number of different coupling reagents available, even optimized coupling reagents may have drawbacks, such limited stability in solution over prolonged times.

The creation of COMU as a coupling reagent bridged the low racemization efficiencies seen in carbodiimide chemistry with the reactivity of uronium based chemistries. Unfortunately, the high reactivity made COMU more unstable in the presence of solvents making it difficult to use in long syntheses running on automated peptide synthesizers.

Here we tested the ability to, in essence, make COMU in situ in the reaction vessel by adding HDMC and Oxyma Pure separately. This allows the use of COMU's chemical properties in the synthesis of difficult peptides in an automated fashion without jeopardizing the reactivity over the course of the synthesis. The use of the Prelude X facilitated the screening of a set of different conditions and peptide sequences simultaneously, with increased yields and productivity. This included a screening of multiple temperatures simultaneously, utilizing the independent, parallel heating capacity of the Prelude X.

