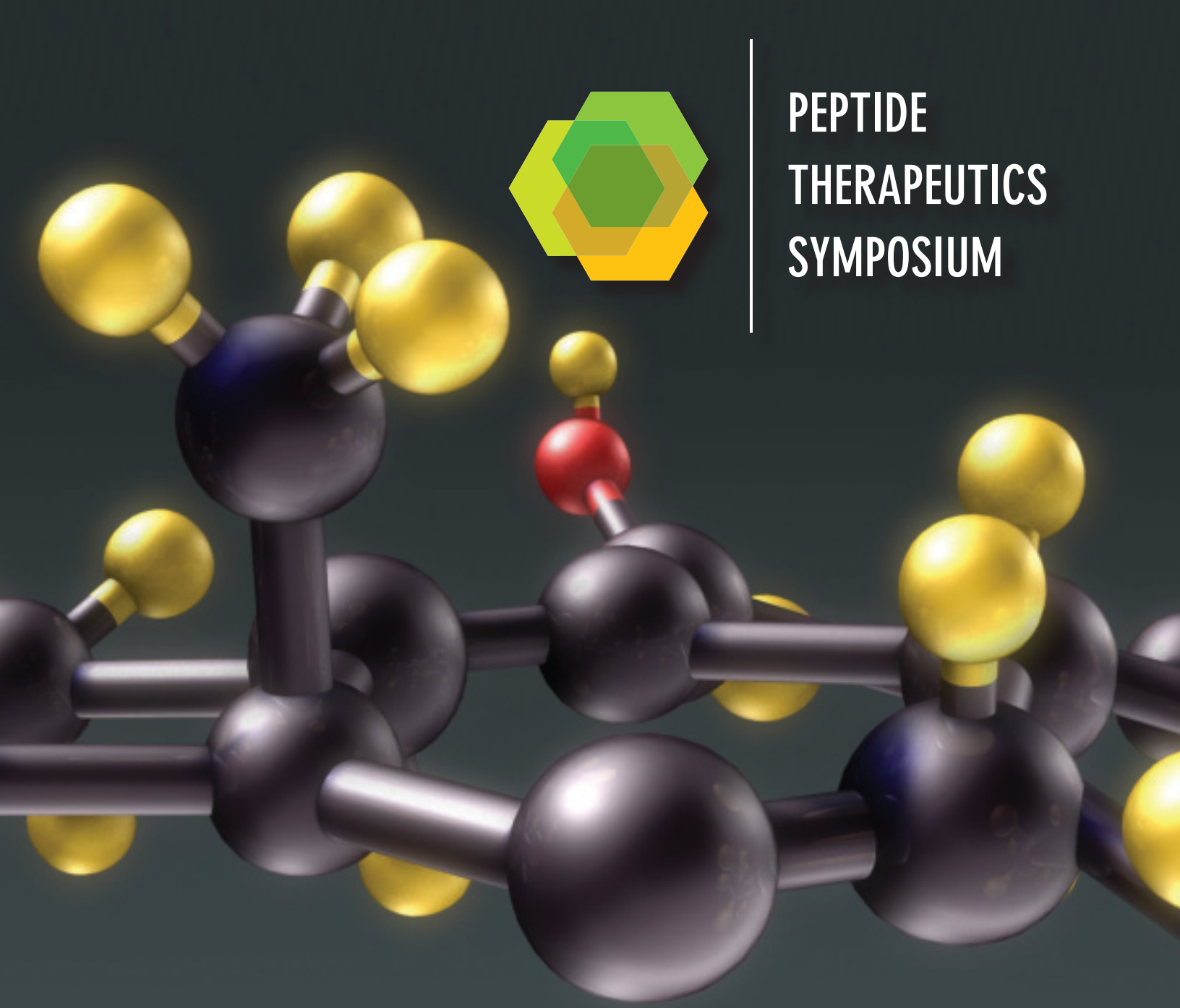


PEPTIDE THERAPEUTICS SYMPOSIUM

Program and Proceedings
13th Annual Peptide Therapeutics Symposium

October 25 – 26, 2018
Salk Institute for Biological Studies, La Jolla. CA

www.peptidetherapeutics.org

13th Annual Peptide Therapeutics Symposium

Table of Contents

Table of Contents

13th Annual Peptide Therapeutics Symposium

Symposium Sponsors

13th Annual Peptide Therapeutics Symposium

Dear Colleagues,

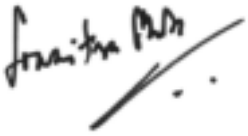
We are pleased to welcome you to the Salk Institute for Biological Studies, for the 13th Annual Peptide Therapeutics Symposium. The Symposium, as in past years, will highlight the discovery and development of peptide-based drug candidates.

The conference will open on Thursday afternoon with Plenary Lectures from Professors Jim Wells of the University of California at San Francisco, and Tom Muir of Princeton University. A session highlighting advances in chemoenzymatic peptide synthesis will follow. To conclude Thursday's program, please join us for the Poster Session and Opening Reception.

The Friday morning session will feature Keynote Lectures by Professors Paul Schimmel of The Scripps Research Institute and Sir David Lane from A*STAR Singapore. Dr. Michael Dunn from Ferring Research Institute, Inc., will provide an overview of recent changes in the status of peptides in clinical development. The remainder of the day will be devoted to lectures pertaining to clinical advances of peptide therapeutics and cutting-edge research supporting the next generation discovery of drug candidates.

We are excited to provide what we believe is a high quality program along with the opportunity to meet with fellow attendees to foster the exchange of scientific information and informed peer-review. As always we are grateful for your participation which has historically been central to rendering this annual scientific event so successful.

Sincerely,



Richard DiMarchi
Chairman of the Board
Peptide Therapeutics Foundation

Soumitra Ghosh
President
Peptide Therapeutics Foundation

Sponsors, Peptide Therapeutics Foundation

ChemPartner
Ferring Research Institute, Inc.
MedImmune/AstraZeneca
Novo Nordisk
The PolyPeptide Group
Zealand Pharma
Zydus Cadila
Peptide Therapeutic Foundation

13th Annual Peptide Therapeutics Symposium

ChemPartner

ChemPartner is a leading research organization with over 2000 scientists providing high-quality and cost-effective integrated partnerships for the biopharmaceutical industry. Our dedication to life science can been seen in all of our services from discovery biologics, discovery peptide and medicinal chemistry, discovery biology, and preclinical development through pharmaceutical development and manufacturing services for small molecules and biologics, DMPK, CMC, and biologics manufacturing.

We are a true partner in therapeutic peptide drug discovery and we offer a fully integrated capability. Our company serves a diverse global client base and has laboratories, business offices, and representatives in the US, Europe, China, and Japan. ChemPartner has a center of excellence located in South San Francisco, California. This group of talented scientists has extensive industry experience in peptide, medicinal, synthetic, analytical and computational chemistry as well as biologics.

Our chemists have designed and synthesized millions of molecules and peptides for hundreds of pharmaceutical and biotechnology companies. A large number of these have contributed to the clinical development pipelines of our biopharmaceutical partners, including an array of early-phase clinical candidates, and a recent entry into Phase III clinical studies for a major pharmaceutical client.

Ferring Research Institute, Inc.

Headquartered in San Diego, California, Ferring Research Institute, Inc., (FRI) is a critical component of Ferring Pharmaceutical's global therapeutics research and discovery engine. 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: reproductive medicine & women's health, urology and gastroenterology/hepatology. Our state-of-the art facility includes peptide and protein drug design, chemistry, pharmacology, biology, and preclinical ADME capabilities. Historically FRI has focused on the discovery of amino acid-based therapeutics utilizing the body's signaling hormones. Today FRI is committed to building a portfolio of novel, innovative therapeutics using a wide array of modalities in order to address areas of high unmet medical need in our core therapeutic areas. Driving value through personalized medicine.

Ferring Pharmaceuticals is a research-driven, specialty biopharmaceutical group committed to helping people around the world build families and live better lives. Headquartered in Saint-Prex, Switzerland, Ferring is a leader in reproductive medicine and women's health, and in specialty areas within gastroenterology/hepatology and urology. 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. Ferring has been developing treatments for mothers and babies for over 50 years and has a portfolio covering treatments from conception to birth. Founded in 1950, privately-owned Ferring now employs approximately 6,500 people worldwide, has its own operating subsidiaries in nearly 60 countries and markets its products in 110 countries.

MedImmune/AstraZeneca

MedImmune is the global biologics research and development arm of AstraZeneca, a global, innovation-driven biopharmaceutical business that focuses on the discovery, development and commercialization of small molecule and biologic prescription medicines. MedImmune is pioneering innovative research and exploring novel pathways across Oncology, Respiratory, Cardiovascular, Renal and Metabolic Diseases, and Infection and Vaccines. Peptide therapies are a significant part of both MedImmune's and AstraZeneca's marketed, clinical and pre-clinical portfolio. The MedImmune headquarters is located in Gaithersburg, MD., one of AstraZeneca's three global R&D centers, with additional sites in Cambridge, UK and South San Francisco, CA. For more information, please visit www.medimmune.com.

Novo Nordisk

Novo Nordisk is a global healthcare company with more than 90 years of innovation and leadership in diabetes care. This heritage has given us experience and capabilities that also enable us to help people defeat obesity, haemophilia, growth disorders and other serious chronic diseases. Headquartered in Denmark, Novo Nordisk employs approximately 41,700 people in 77 countries and markets its products in more than 165 countries. Novo Nordisk's B shares are listed on Nasdaq Copenhagen (Novo-B). Its ADRs are listed on the New York Stock Exchange (NVO). For more information, visit novonordisk.com.

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.

Foundation Sponsors

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 and Boehringer Ingelheim and a proprietary pipeline of product candidates, which primarily target specialty diseases with significant unmet needs.

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® and in the U.S. as Adlyxin®. Sanofi has also developed iGlarLixi, a fixed-ratio combination of lixisenatide and Lantus® (insulin glargine) marketed in U.S. as Soliqua® and Europe as Suliqua®.

Zealand's proprietary pipeline includes; glepaglutide*, a GLP-2 analog for the treatment of short bowel syndrome which will initiate Phase III studies in 1H18; dasiglucagon*, a glucagon analog in Phase III as a single-dose rescue therapy for severe hypoglycemia and in Phase II 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 130 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 therapies, including small molecule drugs, biologic therapeutics and vaccines. The group employs over 20,000 people worldwide, including 1200 scientists engaged in R&D, and is dedicated to creating healthier communities globally. www.zyduscadila.com

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 seven corporate sponsors; ChemPartner, Ferring Research Institute, Inc., MedImmune, Novo Nordisk, 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.

2018 Travel Grant Awardees

Christopher Apostol, University of Arizona
Mohd Shamoon Asmat, Aligarh Muslim University, Aligarh, India
Dakota Brock, Texas A&M University
Anthony Harrington, University of Nevada, Reno
Daphne Metellus, Philadelphia College of Osteopathic Medicine
Jonathan Rittichier, Harvard Medical School
Marcus Van Engen, Dordt College
Daniel Wiegand, Wyss Institute for Biologically Inspired Engineering at Harvard University
Sheng Zhang, University of California, Irvine
Dan Zhou, California Institute of Technology

13th Annual Peptide Therapeutics Symposium

Thursday, October 25, 2018

11:00 a.m. – 5:30 p.m.	**Registration Check-in**
1:00 p.m. – 1:15 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* *VP and Site Director, Novo Nordisk Research Center, Indianapolis*
1:15 p.m. – 2:45 p.m.	**Plenary Lectures** **Moderator:** Andrew Parker, MBA, Ph.D. *Secretary and Director, Peptide Therapeutics Foundation* *CSO and EVP, Head of Research & External Innovation* *Zealand Pharma*
1:15 p.m. – 2:00 p.m.	**Modulating Biological Signaling Through Protein and Small Molecule Design for Therapeutic Purposes** Jim Wells, Ph.D. *Professor and Chair, Department of Pharmaceutical Chemistry* *University of California, San Francisco*
2:00 p.m. – 2:45 p.m.	**Houdini Proteins: Discovery and Applications of Ultrafast Inteins** Tom W. Muir, Ph.D. *Chair, Department of Chemistry* *Princeton University*
2:45 p.m. – 3:30 p.m.	**Beverage Break & Poster Viewing**
3:30 p.m. – 5:30 p.m.	**Session I** **Moderator:** Claudio D. Schteingart, Ph.D. *Director, Peptide Therapeutics Foundation* *Vice President, Science & Technology - Research* *Ferring Research Institute, Inc.*
3:30 p.m. – 4:00 p.m.	**Efficient Chemo-enzymatic Synthesis (CEPS) of Incretin Peptides** Timo Nuijens, Ph.D. *CSO, EnzyPep, B.V.*
4:00 p.m. – 4:30 p.m.	**Extending the Circulating Half-life of Clot Factors by Conjugation to the Natural Carbohydrate Polymer Heparosan** Carsten Behrens, Ph.D. *Principal Scientist* *Novo Nordisk A/S*
4:30 p.m. – 5:00 p.m.	**Chemically Synthesized Glycosylated Somatostatin Analogs with High Metabolic Stability and Native-like Binding Affinity to all Five Receptor Subtypes** Sofia Elouali, Ph.D. *Researcher* *GlyTech, Inc.*
5:00 p.m. – 5:30 p.m.	**State-of-the-Art Assessment of Chemo-enzymatic and Enzymatic Synthesis** Rodney Lax, Ph.D. *Business Development Consultant* *Rodney Lax Consulting* AND James P. Tam, Ph.D. *Director of the Synzymes and Natural Products Center* *School of Biological Sciences, Nanyang Technological University*
5:30 p.m. – 7:00 p.m.	**Poster Session & Reception**

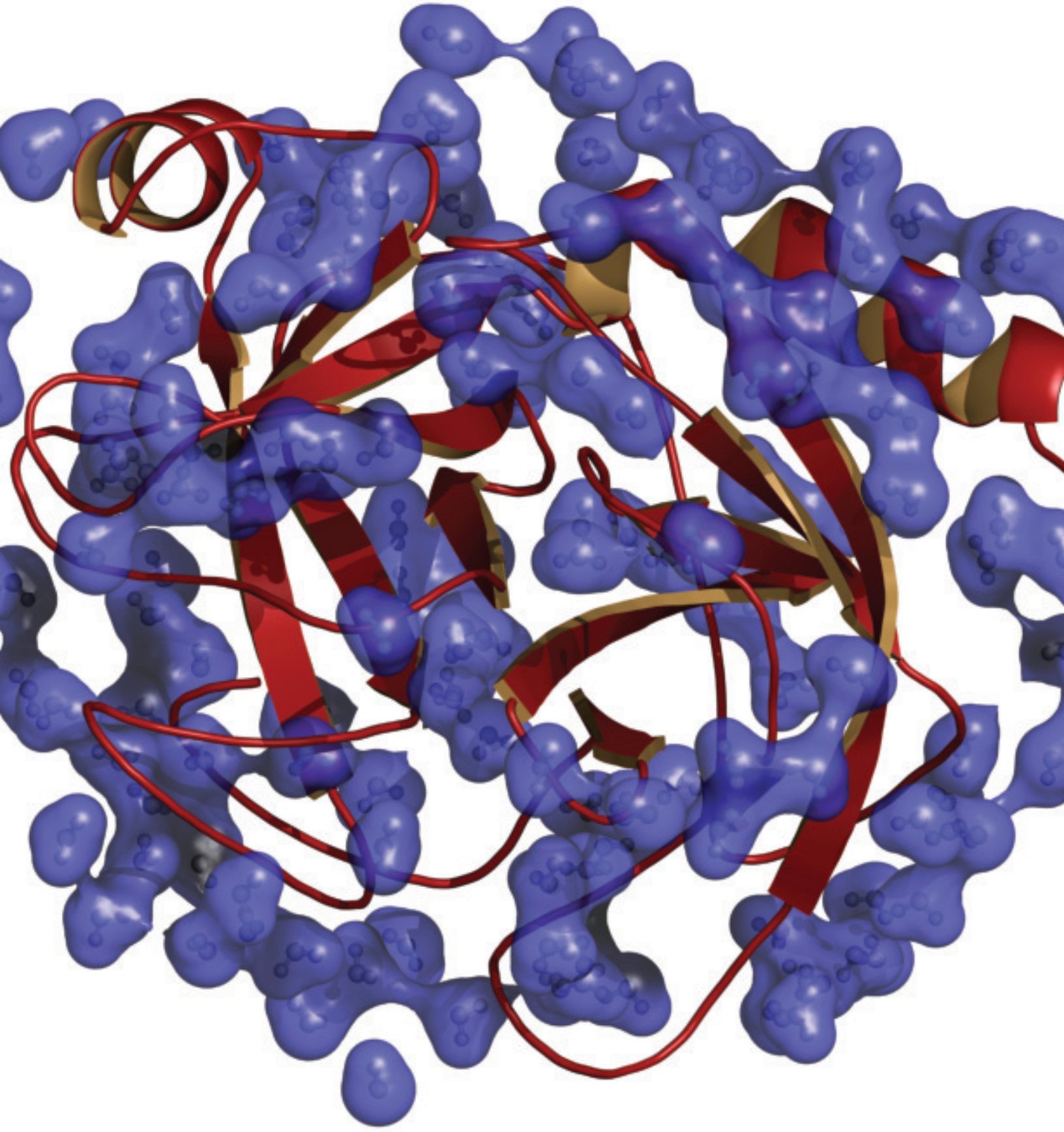
Friday, October 26, 2018

7:00 a.m. – 11:30 a.m.	**Registration Check-in**
7:00 a.m. – 8:15 a.m.	**Breakfast & Poster Viewing**
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:** Yvonne Angell *Director, Peptide Therapeutics Foundation* *Director and Head of Peptide Chemistry* *ChemPartner*
8:30 a.m. – 9:10 a.m.	**Alternative Forms and Activities of Human tRNA Synthetases in Biology and Disease** Paul Schimmel, Ph.D. *Ernest and Jean Hahn Professor* *The Scripps Research Institute*
9:10 a.m. – 9:50 a.m.	**Constrained Peptides and Mini Proteins as Novel Therapeutics Targeting p53** Sir David Lane, FRS *Chief Scientist; Director, p53Laboratory, BMSI* *A*STAR*
9:50 a.m. – 10:30 a.m.	**Beverage Break & Poster Viewing**
10:30 a.m. – 11:00 a.m.	**Introduction** Adrienne Day, Ph.D. *Treasurer and Administrator, Peptide Therapeutics Foundation* *Senior Director, Business Development* *Ferring Research Institute, Inc.* **Peptide Therapeutics Update** Michael K. Dunn, Ph.D. *Senior Director, Scientific Information and Intelligence* *Ferring Research Institute, Inc.*
11:00 a.m. – 12:30 p.m.	**Session II** **Moderator:** Fa Liu, Ph.D. *Director, Peptide Therapeutics Foundation* *Director Chemistry* *Novo Nordisk Research Center*
11:00 a.m. – 11:30 a.m.	**Glepaglutide, a Novel Long-acting Glucagon-like Peptide-2 Analog for the Treatment of Patients with Short Bowel Syndrome** Andrew Parker, MBA, Ph.D. *Secretary and Director, Peptide Therapeutics Foundation* *CSO and EVP, Head of Research and External Innovation* *Zealand Pharma*
11:30 a.m. – 12:00 p.m.	**Development of Avexitide for Treatment of Post-Bariatric Hypoglycemia** Colleen M. Craig, M.D. *Director of Clinical Development, Metabolic Diseases* *Eiger BioPharmaceuticals*
12:00 p.m. – 12:30 p.m.	**ALRN-6924, a Stapled α-helical Peptide, Reactivates Wild-type p53 by Inhibiting MDMX and MDM2 in *in vitro* Cell Assays, *in vivo* Animal Models, and in Patients with Cancer** Manuel Aivado, Ph.D., M.D. *President and Chief Executive Officer* *Aileron Therapeutics, Inc.*

13th Annual Peptide Therapeutics Symposium

12:30 p.m. – 1:30 p.m.	**Lunch & Poster Viewing**
1:30 p.m. – 3:00 p.m.	**Session III** **Moderator:** Rajiv Sharma, Ph.D. *Director, Peptide Therapeutics Foundation* *Head, Discovery Chemistry and Senior Vice President* *Zydus Cadila*
1:30 p.m. – 2:00 p.m.	**Identifying and Targeting Tumor Neoantigens** Catherine J. Wu, M.D. *Professor of Medicine, Department of Medical Oncology,* *Dana-Farber Cancer Institute and Harvard Medical School*
2:00 p.m. – 2:30 p.m.	**About the High Resolution Aβ(1-42) Fibril Structure – and the Orally Available Clinical Stage all-D-Enantiomeric Peptide PRI-002 that Reverts Cognition Deficits and Decelerates Neurodegeneration in Transgenic AD Mouse Models** Dieter Willbold, Ph.D. *Director, Institut fur Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf* *Director, Institute of Complex Systems in the Research Centre Jülich*
2:30 p.m. – 3:00 p.m.	**Targeting Eph Receptors with Agonist and Antagonist Peptides** Elena B. Pasquale, Ph.D. *Professor, Tumor Initiation and Maintenance Program* *Director, Academic Affairs* *Sanford Burnham Prebys Medical Discovery Institute*
3:00 p.m. – 3:30 p.m.	**Beverage Break & Poster Viewing**
3:30 p.m. – 5:00 p.m.	**Session IV** **Moderator:** Waleed Danho, Ph.D. *Distinguished Research Leader (Retired)* *Hoffman-La Roche, Inc.*
3:30 p.m. – 4:00 p.m.	**Injectable Peptide Gels for the Local Delivery of Drugs** Joel Schneider, Ph.D. *Deputy Director, Center for Cancer Research* *Chief, Chemical Biology Laboratory* *National Cancer Institute, NIH*
4:00 p.m. – 4:30 p.m.	**BioChaperone®: A Platform to Improve Solubility and Stability of Therapeutic Proteins and Peptides** Stephen Daly *US General Manager* *Adocia*
4:30 p.m. – 5:00 p.m.	**Definition of Endocrine FGF Structure as a Means to Super-agonism** Sebastian Parlee, Ph.D. *Senior Scientist* *Novo Nordisk Research Center Indianapolis*
5:00 p.m. – 5:15 p.m.	**Closing Remarks** Adrienne Day, Ph.D. *Treasurer and Administrator, Peptide Therapeutics Foundation* *Senior Director, Business Development* *Ferring Research Institute, Inc.*
5:15 p.m. – 6:15 p.m.	**Closing Reception**

Speaker Biographies
13th Annual
PeptideTherapeutics Symposium



13th Annual Peptide Therapeutics Symposium

Manuel Aivado, Ph.D., M.D. | President and Chief Executive Officer, Aileron Therapeutics, Inc.
ALRN-6924, a Stapled α-helical Peptide, Reactivates Wild-type p53 by Inhibiting MDMX and MDM2 in in vitro *cell Assays,* in vivo *Animal Models, and in Patients with Cancer*

Manuel Aivado, MD, Ph.D. began serving as President and CEO of Aileron Therapeutics in September, 2018. Previously he was SVP, CMO & CSO since September 2014. From March 2012 until September 2014, Dr. Aivado served as Vice President of Clinical Development and Pharmacovigilance at Taiho Oncology, Inc., a pharmaceutical company. From October 2006 until March 2012, Dr. Aivado served as Senior Medical Director in the clinical development group at GlaxoSmithKline, Inc., a global pharmaceutical company. In addition, Dr. Aivado was an instructor in medicine at Beth Israel Deaconess Medical Center/Harvard Medical School. Prior to his industry experience, Dr. Aivado practiced clinical medicine in Germany for ten years, during which time he was awarded the Dr. Mildred Scheel cancer research scholarship award in 2002. Dr. Aivado is a German board-certified physician for internal medicine, hematology and medical oncology, and he received an M.D. and Ph.D. from the Medical School of the University of Dusseldorf, in Germany.

Carsten Behrens, Ph.D. | Principal Scientist, Novo Nordisk A/S
Extending the Circulating Half-life of Clot Factors by Conjugation to the Natural Carbohydrate Polymer Heparosan

Carsten Behrens obtained his M.Sc in bioorganic chemistry in 1992 and after a brief research stay with Professor Leo Paquette at Ohio State University from 1993 to 1994 obtained his Ph.D. in organic chemistry from University of Copenhagen in 1997 with Professor Peter E. Nielsen. He has since then been employed at Novo Nordisk where he have worked with both chemical and enzymatic protein modification in most of the company's core research areas including diabetes, haemophilia and growth disorders. His current research is focused on half-life extension technologies, in particular albumin binder and polymer conjugation technologies for prolonging the circulation time of large proteins in blood. Carsten Behrens is named inventor and co-author on +60 research articles and patents.

Colleen M. Craig, M.D. | Director of Clinical Development, Metabolic Diseases, Eiger BioPharmaceuticals
Development of Avexitide for Treatment of Post-Bariatric Hypoglycemia

Dr. Colleen Craig is Director of Clinical Development, Metabolic Diseases at Eiger BioPharmaceuticals, a clinical-stage biopharmaceutical company located in Palo Alto, CA focused on the development and commercialization of targeted therapies for rare diseases.

Prior to joining Eiger, Dr. Craig was a research fellow in the Division of Endocrinology at Stanford University, where her research centered on incretin physiology, post-bariatric metabolism, and the development of therapeutic approaches for treatment of post-bariatric hypoglycemia (PBH). Funded by an NIH Career Development Award and several prestigious institutional awards, including Stanford's SPARK and TRAM translational research awards, Dr. Craig was co-investigator with her mentor Tracey McLaughlin, M.D. on clinical studies that helped establish the critical role for glucagon-like peptide-1 (GLP-1) in mediating PBH. Dr. Craig demonstrated proof-of-concept in clinical investigations involving the first-in-human subcutaneous administration of the GLP-1 receptor antagonist avexitide (formerly exendin 9-39) in patients with PBH. oThis work resulted in related publications and patents, paving the way for the further development of avexitide by Eiger BioPharmaceuticals.

Dr. Craig obtained her M.D. at Brown University School of Medicine and completed her postdoctoral research fellowship at Stanford University School of Medicine.

Stephen Daly | US General Manager, Adocia
BioChaperone®: A Platform to Improve Solubility and Stability of Therapeutic Proteins and Peptides

In December 2014, Steve Daly joined Adocia in the newly created role of United States General Manager. Adocia is a clinical-stage, French biotech focused on affordable innovation by unlocking novel clinical attributes of already-approved peptides and proteins. Aside from day-to-day operations at Adocia USA, Steve is responsible, in whole or in part, for Business Development, Investor Relations, and Scientific Community Engagement. Before joining Adocia, Steve served in senior roles for the commercialization of therapeutics in the diabetes and metabolism fields at companies such as Amylin Pharmaceuticals (exenatide, pramlintide), Halozyme (hyaluronidase), and Affymax (peginesatide). Prior to nearly 14 years of experience in diabetes and metabolism, Steve held portfolio planning and commercialization roles in the generic and biosimilar marketplace for Baxter International and Sicor, a division of Teva Pharmaceuticals.

Adrienne Day, Ph.D. | Treasurer and Administrator, 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 Conforma Therapeutics, Senior Director of Business Development at Molecumetics Ltd., Associate Director of Corporate Development at Ligand Pharmaceuticals. She was Ligand Pharmaceuticals' first Project Manager, and began her biotechnology career at Invitrogen Corporation where she held various positions.

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

13th 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; VP and Site Director, Novo Nordisk Research Center, Indianapolis
Opening Remarks

Dr. DiMarchi contributions in peptide & protein sciences consists of three decades of work in academia, the pharmaceutical industry and biotechnology companies. He is co-founder of Ambrx, Marcadia, Assembly, Calibrium 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 Vice President at Novo Nordisk Research Laboratories and a former Group Vice President at Eli Lilly and 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 rDNA-derived Humalog® (LisPro-human insulin). Dr. DiMarchi also significantly contributed to the commercial development of Humulin®, Humatrope®, rGlucagon®, and Forteo®. His academic research has broadened the understanding of glucagon physiology while championing the discovery of single molecule mixed agonists for the treatment of diabetes and obesity.

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, and identified as a top-five translation researcher by Nature Biotechnology for the years 2014 and 2015.

Michael K. Dunn, Ph.D. | Senior Director, Scientific Information & Intelligence, Ferring Research Institute, Inc.
Peptide Therapeutics Update

Michael Dunn is the Senior Director of Scientific Intelligence and Information at Ferring Research Institute, Inc., the drug discovery affiliate of Ferring Pharmaceuticals. He leads a diverse group that is responsible for scientific competitive intelligence, patents, research reporting, and data integrity. Dr. Dunn also serves on the Board of Directors of the BioNova Institute, a non-profit research and educational organization.

He holds a Ph.D. from the Division of Medical Sciences at Harvard Medical School, an MBA from Cornell University's Johnson Graduate School of Management, and a B.S. in Pharmacology from University of California, Santa Barbara.

Sofia Elouali, Ph.D., | Researcher, GlyTech, Inc.
Chemically Synthesized Glycosylated Somatostatin Analogs with High Metabolic Stability and Native-like Binding Affinity to all Five Receptor Subtypes

Dr. Sofia Elouali is a researcher working on glycopeptide synthesis at GlyTech, Inc. (Kyoto, Japan). The company is focused on the application and supply of glycans and chemical glycosylation technologies for the development of peptide, protein and oligonucleotide therapeutics.

Dr. Elouali received her MChem at the University of Sheffield and a Ph.D., from University College London. Following a secondment at the former UK Department for Business, Innovation and Skills, she undertook postdoctoral studies at Queen's University Belfast before joining GlyTech, Inc.

Soumitra Ghosh, Ph.D. | Director and President, Peptide Therapeutics Foundation; President, Doon Associates LLC
Welcoming Remarks

Soumitra Ghosh is a biopharmaceutical industry consultant and entrepreneur with extensive experience in drug development, technology licensing and in formulating and implementing R&D strategy. He is a co-founder of Avexegen Therapeutics, Abvance Therapeutics and Aquros Bio, start-ups focused on GI indications, diabetes and urological disorders, respectively. His experience includes R&D leadership positions at Amylin Pharmaceuticals and MitoKor, where he led research programs for the development of small molecule, peptide and protein-based drug candidates for the treatment of metabolic diseases and CNS disorders. Multiple drug candidates were advanced to the clinic during his tenure, or were partnered with companies for clinical development. He has been a recipient of several SBIR and California state grants for his work in the industry. 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 work at the Rockefeller University in New York.

Sir David Lane, FRS | Chief Scientist of A*STAR; Director, p53Laboratory, BMSI, A*STAR
Constrained Peptides and Mini Proteins as Novel Therapeutics Targeting p53

Sir David is the Chief Scientist of A*STAR, where his main role is to advise and engage in scientific development across the Biomedical Research Council (BMRC) and the Science and Engineering Research Council (SERC) at the strategic level. He also runs a research lab primarily focusing on research on p53 using both mammalian and zebrafish systems.

Sir David completed undergraduate and postgraduate degrees at University College London where he studied auto-immunity. He did his postdoctoral training at the Imperial Cancer Research Fund in London (where he discovered p53 as a T antigen binding protein) and then at the Cold Spring Harbor Labs in New York. On his return to UK, he set up his independent research lab at Imperial College London and at the ICRF Clare Hall labs before moving to the University of Dundee.

Sir David has published more than 400 research articles in international peer reviewed journals many of which have been very highly citied and has co-authored a successful practical guide to the use of immunochemical methods called “Antibodies” with Ed Harlow, selling more than 40,000 copies. He is the founder of Cyclacel, a NASDAQ listed company with three drugs in Clinical trial. Sir David established the Experimental Therapeutics Centre at A*Star in 2007. He was formerly the Chief Scientist at Cancer Research UK and for three years the Scientific Director of the Ludwig Institute. He has been recognized for his work by many international awards including the Paul Ehrlich Prize, the Brucbacher Prize, the Buchanan Medal of the Royal Society and the Royal Medal of the Royal Society of Edinburgh. Last year he received the Presidents Science and Technology Medal in Singapore for his contribution to the development of Science in the Republic.

13th Annual Peptide Therapeutics Symposium

Rodney Lax, Ph.D. | Business Development Consultant, Rodney Lax Consulting
State-of-the-Art Assessment of Chemo-enzymatic and Enzymatic Synthesis

Rodney Lax is an independent business development consultant active in the field of peptide therapeutics. Most of his time is currently spent supporting EnzyPep B.V., a Dutch company developing enzymatic processes for manufacturing peptides and bioconjugates. Until 2014, he was Senior Director of Business Development for the PolyPeptide Group in North America. Before moving into peptides in 1986, Rodney Lax was lecturer, senior research assistant and associate professor at the Institute of Physiological Chemistry at the University of Essen in Germany, where his work focused on the function of hepatic steroid hormone receptors and the regulation of hepatic steroid metabolism. Dr. Lax received his B.Sc. from the University of Birmingham (UK) in 1967 and his Ph.D. from University of London (UK) in 1972.

Tom W. Muir, Ph.D. | Chair, Department of Chemistry, Princeton University
Houdini Proteins: Discovery and Applications of Ultrafast Inteins

Tom W. Muir received his B.Sc in Chemistry in 1989 and Ph.D. in Chemistry in 1993 from the University of Edinburgh. After postdoctoral studies with Stephen B.H. Kent at the Scripps Research Institute, he joined the faculty at the Rockefeller University in New York City in 1996. In 2011, Dr. Muir joined Princeton University as the Van Zandt Williams, Jr. Class of '65 Professor of Chemistry. He currently serves as Chair of the Princeton Department of Chemistry. He has published over 180 scientific articles in the area of chemical biology and is best known for developing methods for the preparation of proteins containing unnatural amino acids, posttranslational modifications and spectroscopic probes. These approaches are now widely employed in academia and industry. His currents interests lie in the area of epigenetics, where he tries to illuminate how chemical changes to chromatin are linked to different cellular phenotypes. Professor Muir has won a number of honors for his research, including; the Burroughs-Wellcome Fund New Investigator Award, the Pew Award in the Biomedical Sciences, the Alfred P. Sloan Research Fellow Award, the Leonidas Zervas Award from the European Peptide Society, the Irving Sigal Award from the Protein Society, the Vincent du Vigneaud Award in Peptide Chemistry, the Blavatnik Award from the New York Academy of Sciences, the Jeremy Knowles Award from the Royal Society of Chemistry, the Arthur C. Cope Scholar Award from the American Chemical Society, the Breslow Award in Biomimetic Chemistry from the American Chemical Society, and the E.T. Kaiser Award in Protein Chemistry from the Protein Society. Dr. Muir is a Fellow of American Association for the Advancement of Science, the Royal Society of Chemistry and the Royal Society of Edinburgh.

Timo Nuijens, Ph.D. | CSO, EnzyPep. B.V.
Efficient Chemo-enzymatic Synthesis (CEPS) of Incretin Peptides

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 and he was involved in the founding of EnzyPep mid 2012. At EnzyPep he currently holds the position of CSO and co-ordinates the R&D team in Geleen.

Andrew Parker, MBA, Ph.D. | Secretary and Director, Peptide Therapeutics Foundation; CSO and EVP, Head of Research and External Innovation, Zealand Pharma

Glepaglutide, a Novel Long-acting Glucagon-like Peptide-2 Analog for the Treatment of Patients with Short Bowel Syndrome

Andrew Parker joined Zealand in July 2016 as Chief Science Officer and Head of Research & External Innovation. Andrew has more than twenty years of experience from senior leadership and managerial positions in international pharmaceutical, biotech and start-up companies, including Shire Pharmaceuticals, Opsona Therapeutics, and AstraZeneca. Prior to joining Zealand, Andrew spent several years in venture capital with Eclosion2 as a General Partner and Scientific Director.

He holds a Ph.D. from the National Institute for Medical Research at Mill Hill, London, conducted post-doctoral research at Johns Hopkins Medical School, Baltimore, USA, and also has an MBA from the University of Warwick Business School, UK.

Sebastian Parlee, Ph.D. | Senior Scientist, Novo Nordisk Research Center, Indianapolis

Definition of Endocrine FGF Structure as a Means to Super-agonism

Dr. Sebastian Parlee is a Senior Scientist at the Novo Nordisk Research Center in Indianapolis, Indiana. Dr. Parlee completed his Bachelor of Science in Neuroscience at Dalhousie University in Canada, continuing on in the university to complete his PhD in Pharmacology under the guidance of Dr. Kerry Goralski. Sebastian subsequently brought this expertise to the prestigious Brehm Center for Diabetes Research at the University of Michigan where he completed his postdoctoral fellowship with Dr. Ormond MacDougald.

Sebastian has dedicated his career to understanding the fundamental physiological and pharmacological mechanisms that underlie adipose (fat) tissue development, adipocyte-derived protein (adipokine) production, and their contribution to the pathophysiology of obesity and diabetes. Sebastian now uses this knowledge in the discovery and development of novel therapeutics in one of the world's leading pharmaceutical companies.

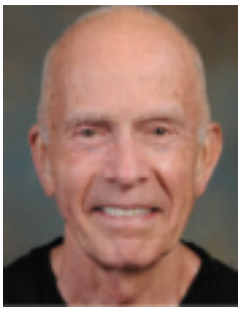
Sebastian's success is well documented within numerous peer-reviewed publications alongside a multitude of honors and awards.

Elena B. Pasquale, Ph.D. | Professor, Tumor Initiation and Maintenance Program and Director of Academic Affairs, Sanford Burnham Prebys Medical Discovery Institute

Targeting Eph Receptors with Agonist and Antagonist Peptides

Elena Pasquale is a Professor in the Tumor Initiation and Maintenance Program in the Cancer Center of the Sanford Burnham Prebys Medical Discovery Institute in La Jolla. During her postdoctoral training in the laboratory of Dr. John Singer at the University of California, San Diego, Dr. Pasquale was among the first to study protein tyrosine phosphorylation in normal cells and tissues. She discovered that tyrosine phosphorylation is particularly elevated during early stages of embryonic development and devised a strategy to uncover the tyrosine kinases responsible. This led to the identification of novel receptor tyrosine kinases, including multiple members of the fibroblast growth factor (FGF) receptor and Eph families. A major focus of Dr. Pasquale's laboratory has been to elucidate key signal transduction pathways involved in Eph receptor-dependent regulation of neural function and cancer cell malignancy. Translational aspects of Dr. Pasquale's research include the development of peptides that target Eph receptors and modulate their function, and have resulted in 4 patents and 3 patent applications. Dr. Pasquale has co-authored nearly 200 publications, including highly cited reviews on the Eph/ephrin system as an important regulator of physiological and pathological processes and as a promising therapeutic target.

13th Annual Peptide Therapeutics Symposium



Paul Schimmel, Ph.D. | Ernest and Jean Hahn Professor of Molecular Medicine and of Chemistry, The Scripps Research Institute
Alternative Forms and Activities of Human tRNA Synthetases in Biology and Disease

Paul Schimmel is Ernest and Jean Hahn Professor of Molecular Medicine and of Chemistry at The Scripps Research Institute. Prior to joining The Scripps, he was John D. and Catherine T. MacArthur Professor of Biochemistry and Biophysics at MIT (Massachusetts Institute of Technology). Author or coauthor of many scientific research publications, he is also coauthor of a widely used 3-volume textbook on biophysical chemistry. His research interests have focused on aminoacyl tRNA synthetases as fundamental interpreters of the genetic information. Through career-long investigations of this ancient and universal set of essential enzymes, his laboratory has worked on a universal mechanism for correcting errors in the interpretation of genetic information, and went on to show how this mechanism is essential for maintaining cellular homeostasis and for preventing serious pathologies and disease. His laboratory also discovered what others have referred to as a tRNA synthetase-directed primordial, or 'second', genetic code that eventually was incorporated into the modern code. In a separate line of research published back in 1983, Schimmel developed the concept of what are now known as ESTs (expressed sequence tags) and the strategy of shotgun sequencing, approaches that several years later were adopted for the human genome project. Nature magazine listed Schimmel's work on the development of ESTs as one of the four key developments that launched the human genome project (Nature volume 409, p. 862 (2001)). Lastly, his laboratory established connections of synthetases to disease and, most recently, they reported the structural and functional metamorphosis of these proteins, whereby they are repurposed with novel activities, both inside and outside the cell, in a variety of cell signaling pathways.

Honors: Named to various society and university awards and honorary degrees, and elected to membership in the American Academy of Arts and Sciences, the National Academy of Sciences, the American Philosophical Society, the Institute of Medicine (National Academy of Medicine) and National Academy of Inventors. Active in many scientific and academic organizations and committees, including past service as President of the Division of Biological Chemistry of the American Chemical Society (presently with over 7,000 members) and as an editorial board member of numerous scientific journals.

Contributions to Translational Medicine: Holds a portfolio of patents and is a cofounder or founding director of numerous enterprises that developed new medicines that flowed out of academic research. These enterprises have created FDA-approved medicines and are developing new therapeutics to treat patients for infections, mental disorders, cancers, diabetes, and inflammatory conditions.

Joel Schneider, Ph.D. | Deputy Director, Center for Cancer Research
Chief, Chemical Biology Laboratory, National Cancer Institute, NIH
Injectable Peptide Gels for the Local Delivery of Drugs

Joel Schneider is Deputy Director of the Center for Cancer Research (CCR) and Chief of the NCI's Chemical Biology Laboratory. He received a Ph.D. in Organic Chemistry with Dr. Jeffery Kelly from Texas A&M University, and then joined the laboratory of Dr. William DeGrado at the University of Pennsylvania School of Medicine to study protein design. In 1999, he started his independent career at the University of Delaware as an assistant professor of Chemistry and Biochemistry and was promoted to associate and then full professor in 2009 with a secondary appointment in Materials Science and Engineering. He joined the NCI in 2010 to build their newly established Chemical Biology Laboratory. He also served as Editor-in-Chief and now Executive Editor of Peptide Science, the official journal of the American Peptide Society. In his own research, he develops injectable hydrogel materials, bioadhesives, and cellular delivery vehicles capable of delivering therapeutics locally to tissue that help to overcome the limitations of parenteral administration. He is particularly interested in peptide and protein-based hydrogel materials formed by self-assembly mechanisms. His work spans molecular conception, materials synthesis, nano- and bulk mechanical materials characterization, cell-material interactions, biocompatibility, and assessment of performance efficacy. In addition to his interests in developing potential applications for these materials, he studies the mechanisms by which they are formed, their structures at all length-scales and determines how their structures influence their mechanical and biological properties. His basic research establishes how material composition and structure influences material function.

James P. Tam, Ph.D. | Director of the Synzymes and Natural Products Center,
School of Biological Sciences, Nanyang Technological University, Singapore
State-of-the-Art Assessment of Chemo-enzymatic and Enzymatic Synthesis

James P. Tam is the Lee Wee Nam Professor and Director of the Synzymes and Natural Products Center. He served as the founding Dean of the School of Biological Sciences, the founding director of Biological Research Center and the founding director of the double-degree program in Biomedical Science and Chinese Medicine at Nanyang Technological University, Singapore.

He received his Ph.D. in Medicinal Chemistry from the University of Wisconsin, Madison, USA and held appointments as Associate Professor at The Rockefeller University, Professor at Vanderbilt University, and The Scripps Research Institute. His research work focuses on the discovery, design and development of therapeutics, particularly orally active biologics, immunologics and anti-infectives. The laboratory's efforts generally involve the discovery, synthesis, and study of novel peptides, particularly metabolical-stable peptides as orally-active peptide leads, and work in the subfields of biomedicine, synthetic enzymes, and chemical biology.

In addition to his scientific research, he has also been active in the peptide community. Besides serving on many editorial boards, he organizes international peptide and protein symposia and was co-founder of the past ten International Chinese Peptide Symposia. He received the Cathay Award from the Chinese Peptide Society, China in 1996. He was also honored as Honorary Professor by Peking University and Peking Union Medical College.

13th Annual Peptide Therapeutics Symposium

Jim Wells, Ph.D. | Professor and Chair, Department of Pharmaceutical Chemistry University of California, San Francisco
Modulating Biological Signaling Through Protein and Small Molecule Design for Therapeutic Purposes

James "Jim" Wells is Professor and Chair of the Department of Pharmaceutical Chemistry at the University of California San Francisco. He joined UCSF in 2005 in both the Department of Pharmaceutical Chemistry at the School of Pharmacy and the Department of Cellular and Molecular Pharmacology at the School of Medicine. His research is focused on understanding and modulating signaling processes in human cells through protein and small molecule design. His lab seeks activators as well as inhibitors of signaling molecules, including proteases and kinases, and uses them to study the consequences of triggering specific nodes of signaling circuits. He is especially interested in the inter-protein circuitry of pathways involved in cell death and inflammation, and the intra-protein allosteric circuitry that governs how distant functional sites in one protein communicate.

He began his independent research career as a founding member of the Protein Engineering Department at Genentech Inc. His group pioneered gain-of-function engineering of enzymes (such as subtilisin), growth factors (hGH), and antibodies by site-directed mutagenesis and protein phage display. The group also discovered hot-spots in protein-protein interfaces by developing alanine-scanning, and with Kossiakoff Lab (University of Chicago) they revealed the first cytokine-receptor mechanism for dimerization and built a new antagonist for human growth hormone, now used for treating acromegaly (Somavert, sold by Pfizer). They also helped humanize the anti-VEGF antibody for treating cancers (Avastin, sold by Genentech).
Jim then became founder, CSO, and President of Sunesis Pharmaceuticals. At Sunesis, the group developed a novel technology for site-directed fragment-based drug discovery, Tethering®, and applied it to cancer targets. Several of the compounds discovered with Tethering are now in clinical development. They also discovered the anti-inflammatory drug Lifitegrast, which was subsequently developed by SarCODE and is now sold by Shire for dry eye syndrome.

Jim Wells received his BA degrees in biochemistry and psychology from University of California, Berkeley and a PhD in biochemistry from Washington State University with Ralph Yount, PhD. He completed his postdoctoral studies at Stanford University School of Medicine with George Stark, PhD.

Dieter Willbold, Ph.D. | Director, Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany; Director, Institute of Complex Systems, ICS-6: Structural Biochemistry, Research Centre Jülich, Jülich, Germany
About the High Resolution Aβ(1-42) Fibril Structure - and the Orally Available Clinical Stage all-D-enantiomeric Peptide PRI-002 that Reverts Cognition Deficits and Decelerates Neurodegeneration in Transgenic AD Mouse Models

Dieter Willbold studied biochemistry in Tübingen (Germany), Bayreuth (Germany) and Boulder (Colorado, USA). He completed his PhD in 1994 at the University of Bayreuth. After some more years in Bayreuth and a couple of research visits, e.g. at the Sackler School of Medicine of the Tel-Aviv University, he headed a junior research group at the Institute for Molecular Biotechnology in Jena. In 2001 Willbold became an associate Professor at the Heinrich Heine University in Düsseldorf. Since 2004, he is full professor at the Institute of Physical Biology in Düsseldorf and director of the Institute of Complex Systems in the Research Centre Jülich. His main interests are protein interactions with physiological and artificial ligands, high resolution structural biology, neurodegeneration and autophagy.

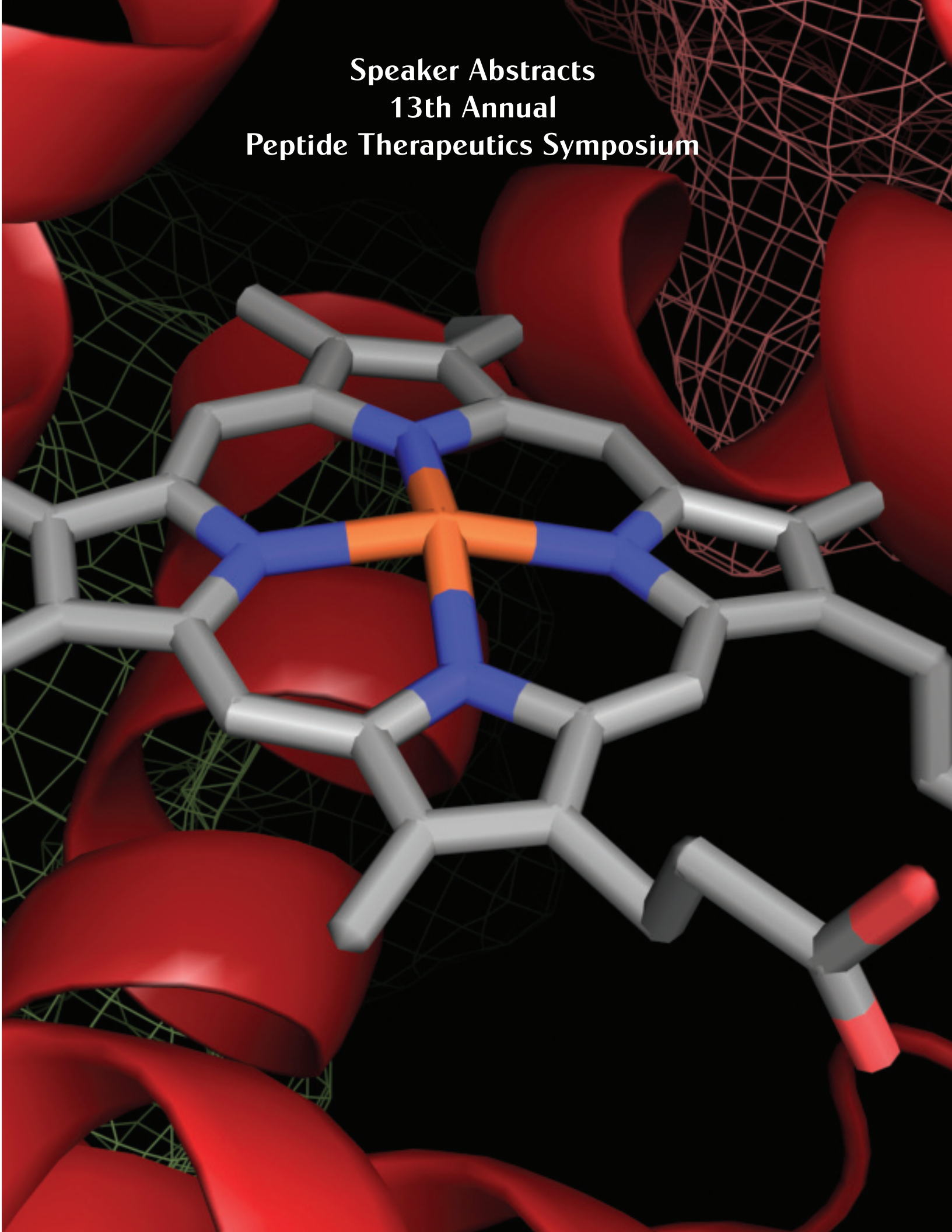
Catherine J. Wu, M.D. | Professor of Medicine, Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School

Identfying and Targeting Tumor Neoantigens

Catherine J. Wu, MD is a Professor of Medicine at the Dana-Farber Cancer Institute, Boston. She received her M.D. from Stanford University School of Medicine and completed her clinical training in Internal Medicine and Hematology-Oncology at the Brigham and Women's Hospital and Dana-Farber Cancer Institute in Boston, MA. She joined the staff at the Dana-Farber Cancer Institute in 2000. At DFCI, she has initiated an integrated program of research and clinical activities that focuses on dissecting the underlying mechanisms of pathobiology of chronic lymphocytic leukemia (CLL), including the understanding of clonal heterogeneity and kinetics in this disease. She has been Principal Investigator of several center-initiated clinical trials of cancer vaccination. A major priority of her studies is the identification of tumor-specific antigens that would allow effective tumor targeting without collateral toxicity. She has been using exome and transcriptome sequencing technologies to identify unique mutated leukemia antigens that arise from individual-specific genetic alterations within a tumor and that could be potentially targeted immunologically, thus paving the way for developing personalized tumor vaccines across malignancies, which she is testing in melanoma, glioblastoma, renal cell carcinoma and indolent lymphomas.

Notes

Speaker Abstracts
13th Annual
Peptide Therapeutics Symposium



ALRN-6924, a Stapled α-helical Peptide, Reactivates Wild-type p53 by Inhibiting MDMX and MDM2 in *in vitro* Cell Assays, *in vivo* Animal Models, and in Patients with Cancer

Manuel Aivado, Ph.D., M.D. | President and Chief Executive Officer
Aileron Therapeutics, Inc.
490 Arsenal Way, Watertown, MA 02472

Mouse double minute 4 homolog (MDM4; also known as MDMX) and mouse double minute 2 homolog (MDM2) are endogenous inhibitors of the tumor suppressor protein p53. MDMX and MDM2 are frequently overexpressed in cancer, which renders their inhibitory protein-protein interactions with p53 an important pharmacological target. This is due to the central role of p53 in inducing cell cycle arrest, apoptosis and senescence in response to cellular stress and oncogenic signals. In addition to its intrinsic anti-tumor activity, p53 plays an important role in the regulation of innate and adaptive immunity and can elicit anti-tumor immunity.

Pharmacological disruption of the protein-protein interactions between MDMX/MDM2 and p53 has long been pursued as a strategy to restore p53-dependent tumor suppressor activity in cancers with wild-type p53. Until recently, selective targeting of this pathway has been limited to MDM2-only small-molecule inhibitors, which lack affinity for MDMX.

ALRN-6924 is the first synthetic macrocyclic peptide against intracellular targets that has successfully been tested in the clinic (Meric-Bernstam et al., ASCO 2017). ALRN-6924 has been structurally stabilized ("stapled") in an α-helical configuration to mimic the α-helical domain of p53 that is responsible for the protein-protein interaction between p53 and MDMX/MDM2. ALRN-6924 robustly activates p53-dependent transcription in live cells and exhibits biochemical and molecular biological on-target activity in leukemia cells *in vitro* and i*n vivo*. Dual MDMX/MDM2 inhibition by ALRN-6924 inhibits cellular proliferation by inducing cell cycle arrest and apoptosis in cell lines and primary AML patient cells, including leukemic stem cell–enriched populations, and disrupts functional clonogenic and serial replating capacity. Notably, ALRN-6924 exhibited greater inhibition of clonogenicity compared to the MDM2-only inhibitor RG-7388 (idasanutlin), suggesting that dual MDMX and MDM2 inhibition targets clonogenic potential of immature, leukemia-driving cells better than inhibition of MDM2 alone. Furthermore, ALRN-6924 markedly improves survival in AML xenograft models.

The existing data provides mechanistic insight to support further development of ALRN-6924 for patients with Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS), Non-Hodgkin Lymphomas (NHL) and several other cancers with wild-type p53. ALRN-6924-mediated activation of p53 has shown promising anti-tumor responses as monotherapy in clinical trials, and should be tested in combination with other therapies such as chemotherapy, targeted therapies including cyclin-dependent kinase 4/6-inhibitors, and immune checkpoint inhibitors.

Extending the Circulating Half-life of Clot Factors by Conjugation to the Natural Carbohydrate Polymer Heparosan

Carsten Behrens, Ph.D. | Principal Scientist
Novo Nordisk A/S
Novo Nordisk Park 1, Maaloev, DK-2760, Denmark | 453 079 8572

INTRODUCTION

While recombinant FVIIa is a safe and reliable option for the treatment of bleeding episodes in haemophilia patients with inhibitors, a longer acting molecule would be desirable for routine prophylaxis. Polyethylene glycol (PEG) conjugation is effective in prolonging the in vivo half-life of FVIIa, however, at the expense of a reduction in activity.[1] Here, we have explored a new half-life extension principle based on the conjugation of heparosan using the HEPtune™ technology from Caisson Biotech.[2] Heparosan is a naturally occurring linear polysaccharide composed of [-β1,4-N-acetylglucosamine-α1,4-glucuronic acid-] repeats. It can be produced chemo-enzymatically, which allows for tailoring of size and incorporation of chemical handles for conjugation to payloads.[3] In the present study, we have investigated the size-dependent effect of heparosan-conjugation on the pharmacokinetics and i*n vitro* activity of FVIIa.

RESULTS AND DISCUSSION

Heparosan was produced in sizes from 13 to 157 kDa with a maleimide moiety at the non-reducing end, which allowed for site-specific conjugation to a FVIIa variant containing a free C-terminal cysteine (FVIIa 407Cys). For reference, similar size PEG-FVIIa 407Cys conjugates were prepared. *In vitro* activity was determined using a TF-independent FX activation assay. Pharmacokinetics was determined in Sprague Dawley rats after intravenous administration of 20 nmol/kg (n=3).

For both the heparosan and PEG-conjugates, the half-life of FVIIa was observed to increase with increasing polymer size up to 40-kDa. Above this size no further extension of half-life was observed. For both 40-kDa polymer conjugates, the half-life of functional FVIIa was 6.5 ± 0.4 h in comparison to 0.8 ± 0.1 h for FVIIa. In contrast, the heparosan conjugate retained a higher specific proteolytic activity of (48 ± 4)% as compared to (27 ± 1)% for the corresponding PEG conjugate. From these studies it was concluded that the naturally occurring polysaccharide heparosan was as effective as PEG in prolonging the half-life of FVIIa, while retaining a significantly higher activity at an optimal size of 40 kDa.

The use of heparosan as a potentially half-life extension principle for FVIII and FIX was also explored. It was found that 40 kDa heparosan-conjugation resulted in 1.8-fold prolonged half-life of rFVIII and 2.5-fold prolonged half-life of rFIX potentially enabling prophylaxis in haemophilia with less frequent dosing and/or higher through levels.

REFERENCES

1. Stennicke HR, Ostergaard H, Bayer RJ *et al*. (**2008**) Generation and biochemical characterization of glycoPEGylated factor VIIa derivatives. *Thromb Haemost*, 100: 920–928.
2. DeAngelis P.L. (**2013**) HEPtune: A Process of conjugating a naturally occurring sugar molecule, heparosan, to a drug for enhanced drug delivery. *Drug Development and Delivery*: January 2013.
3. Sismey-Ragatz AE, Green DE, Otto NJ, Rejzek M, Field RA, DeAngelis PL. (**2007**) Chemoenzymatic synthesis with distinct Pasteurella heparosan synthases: monodisperse polymers and unnatural structures. *J Biol Chem*. 282(39):28321-28327.

Development of Avexitide for Treatment of Post-Bariatric Hypoglycemia

Colleen M. Craig, MD | Director of Clinical Development, Metabolic Diseases
Eiger BioPharmaceuticals
2155 Park Blvd., Palo Alto, CA 94306

Post-bariatric hypoglycemia (PBH) is an increasingly reported severe complication of bariatric surgery, most often following Roux-en-Y Gastric Bypass. Characterized by frequent episodes of postprandial hypoglycemia with neuroglycopenic symptoms, patients with PBH suffer quality of life implications and are at risk for severe neuroglycopenic outcomes, such as loss of consciousness, seizures, and comas. A central role for exaggerated meal-induced secretion of glucagon-like peptide-1 (GLP-1) has been demonstrated, leading to the clinical investigation of GLP-1 receptor antagonism as a targeted therapeutic approach. Eiger BioPharmaceuticals has demonstrated positive proof-of-concept with a novel formulation of the GLP-1 receptor antagonist avexitide (formerly exendin 9-39) in Phase 2 clinical studies in patients suffering from PBH. An overview of the avexitide clinical development program for treatment of PBH will be presented.

BioChaperone®: A Platform to Improve Solubility and Stability of Therapeutic Proteins and Peptides

Stephen Daly | US General Manager
Adocia
115 Avenue Lacassagne
Lyon, France 69003 | Email: s.daly@adocia.com

The growing field of peptide therapeutics holds promise to improve treatments in a multitude of disease states. Many peptides, however, may not achieve their full therapeutic potential due to low solubility, propensity to aggregation, chemical instability, and/or a short half-life.

Adocia (EuroNext Paris: ADOC) is a clinical-stage biotechnology company focused on the development of innovative biologics based on already-approved peptides and proteins. Adocia's proprietary technology, BioChaperone, unlocks the potential of single agents and enables the combination of previously un-combinable agents to deliver better outcomes for patients, providers, and payers.

BioChaperone is a clinical-stage, excipient-based platform designed to increase the solubility and stability of target therapeutic proteins and peptides as well as to enable novel stable combinations. By forming physical and reversible molecular complexes, BioChaperone reduces the intrinsic self-association of peptides in solution. The complexes formed between BioChaperone and the underlying active agent(s) are fully dissociated following *in vivo* administration.

Adocia's proprietary portfolio of injectable treatments for diabetes is one of the largest and most well-differentiated in the industry, including 5 clinical products and four preclinical products. Adocia is now expanding its presence, leveraging the proven versatility and strong track-record of BioChaperone Technology to help other companies better serve patients in therapeutic areas with high unmet need.

Peptide Therapeutics Update

Michael K. Dunn, Ph.D. | Senior Director, Scientific Information & Intelligence
Ferring Research Institute, Inc.
4245 Sorrento Valley Boulevard
San Diego, CA 92121

Peptide therapeutics have played a notable role in medical practice since the advent of insulin therapy in the 1920s. Over 60 peptide drugs are approved in the United States and other major markets, and peptides continue to enter clinical development at a steady pace. Peptide drug discovery has diversified beyond its traditional focus on endogenous human peptides to include a broader range of structures identified from other natural sources or through medicinal chemistry efforts. We maintain a comprehensive dataset on peptides that have entered human clinical studies that includes over 150 peptides in active development today. Here we provide an overview of the peptide therapeutic landscape, including historical perspectives, molecular characteristics, regulatory benchmarks, and a therapeutic area breakdown. We will also highlight some emerging and evolving cases in the field.

Chemically Synthesized Glycosylated Somatostatin Analogs with High Metabolic Stability and Native-like Binding Affinity to all Five Receptor Subtypes

Sofia Elouali, Ph.D. | Researcher
GlyTech, Inc.
KRP-1-109, 134 Chudoji Minamimachi, Kyoto 600-8813, Japan | E-mail: sofia-elouali@glytech.jp

Somatostatin (somatotropin release-inhibiting factor, SRIF) is produced throughout the body in the form of a 14- or 28-amino acid peptide, both of which inhibit the release of numerous secondary hormones by binding to the five G-protein-coupled SRIF receptor subtypes (sst1–5). The short blood half-life of native SRIF (<2 min) severely limits its clinical applications. Although longer-acting synthetic analog peptides such as octreotide (t1/2 ≈ 90 min) are clinically established for a number of indications, they are mainly subtype selective agonists for sst2 and sst5. This prevents their application towards octreotide-resistant diseases and adenomas/tumors in which multiple receptor subtypes are involved. Development of a metabolically stable SRIF analog that is capable of targeting all five receptor subtypes should therefore offer a useful clinical alternative.

Among the various approaches that can be used to lengthen the half-life of biologically-active peptides, glycosylation with conserved mammalian sugars most closely mimics nature, and is known to be capable of enhancing solubility and half-life while reducing biodegradation, aggregation, and immunogenicity. In the present study, we synthesized glycosylated SRIF-14 and -28 analogs to improve their pharmacokinetic properties without affecting their receptor subtype selectivity. Haloacetamidyl glycan(s) derived from our established Asn-linked glycan library were readily coupled with SH group(s) on the free peptides.[1] Cys-scanning was used to determine the optimum glycosylation profile to obtain SRIF analogs with a prolonged circulation lifetime as well as high binding affinity and agonistic activity towards all five receptor subtypes. An exploratory clinical study on one of the analogs, GT-02037, revealed no adverse effects at up to 2 mg/body. These results indicate that glycosylated SRIF analogs could be potent therapeutics for acromegaly, octreotide-resistant diseases and adenomas/tumors that involve several receptor subtypes.

References

1. Yamamoto N., Sakakibara T., Kajihara Y., *Tetrahedron Lett.* **2004**, 45, 3287-3290.

Constrained Peptides and Mini Proteins as Novel Therapeutics Targeting p53

Sir David Lane, FRS | Chief Scientist of A*STAR; Director, p53Laboratory, BMSI, A*STAR
Agency for Science, Technology and Research
Singapore | Email: dplane@p53Lab.a-star.edu.sg

Many important targets for human therapy are deemed “difficult“ because of their intracellular location and lack of binding sites for small molecules. This problem is now being addressed by developing new larger molecules that can for example act as excellent inhibitors of protein interactions or promote the correct folding of mutant proteins by acting as molecular chaperons. The p53 pathway provides an outstanding target and test system for these new approaches and we have used synthetic biology methods to develop powerful reporter systems. The challenge of these larger molecules that includes stapled and cyclic peptides, monobodies and other mini-protein domains is ensuring their effective entry into the correct intracellular compartment. The study of natural products and toxins is providing novel insights into this process and the ability of synthetic biology and protein evolution methods to access huge libraries of variants of mini proteins and constrained peptides suggest that a whole new synthetic pharmacy is within reach. Most progress has been made using hydrocarbon stapled peptides where the first generation of molecules that bind and inhibit Mdm2 and Mdm4 and thus activate p53 as a transcription factor are in clinical trial. In this model second generation molecules of great specificity and potency are being developed using novel stapling methods, unnatural amino acids and new delivery methods.

Brown, C.J., Quah, S.T., Jong, J., Goh, A.M., Chiam, P.C., Khoo, K.H., Choong, M.L., Lee, M.A., Yurlova, L., Zolghadr, K., Joseph, T.L., Verma, C.S., Lane, D.P. (2012) Stapled Peptides with Improved Potency and Specificity That Activate p53. *ACS Chem Biol.* **2013** Mar 15;8(3):506-12

Wei, S.J., Joseph, T., Chee, S., Li, L., Yurlova, L., Zolghadr, K., Brown, C., Lane, D., Verma, C., Ghadessy, F. (2013) Inhibition of Nutlin-Resistant HDM2 Mutants by Stapled Peptides. PLoS One. 2013 Nov 20;8(11) Yurlova, L., Derks, M., Buchfellner, A., Hickson, I., Janssen, M., Morrison, D., Stansfield, I., Brown, C.J., Ghadessy, F.J., Lane, D.P., Rothbauer, U., Zolghadr, K., Krausz, E. (2014) The Fluorescent Two-Hybrid Assay to Screen for Protein-Protein Interaction Inhibitors in Live Cells: Targeting the Interaction of p53 with Mdm2 and Mdm4. *J Biomol Screen.* **2014** Apr;19(4):516-25.

Thean D, Ebo JS, Luxton T, Lee XC, Yuen TY, Ferrer FJ, Johannes CW, Lane DP, Brown CJ. (2017) Enhancing Specific Disruption of Intracellular Protein Complexes by Hydrocarbon Stapled Peptides Using Lipid Based Delivery. *Sci Rep.* **2017** May 11;7(1):1763. doi: 10.1038/s41598-017-01712-5.

Houdini Proteins: Discovery and Applications of Ultrafast Inteins

Tom W. Muir, Ph.D. | Chair, Department of Chemistry
Princeton University
Frick Laboratory, 325, Princeton, NJ 08544 | 609-258-5778

Inteins are auto-processing domains found in organisms from all domains of life. These proteins are consummate molecular escape artists that spontaneously excise themselves, in a traceless manner, from proteins in which they are embedded. Chemical biologists have long exploited various facets of intein reactivity to modify proteins in myriad ways for both basic biological research as well as therapeutic applications. While many thousands of inteins have been identified at the sequence level, only a handful of these proteins have been characterized in any biochemical detail. Here I discuss our recent efforts to mine this genomic database, leading to the discovery of ultrafast split inteins that ligate proteins together several orders of magnitude faster that any inteins previously known. I will also discuss the remarkable folding properties of these split proteins, and how this has led to the development of new methods for the manipulation of protein structure in the test tube and in cells.

Efficient Chemo-enzymatic Synthesis (CEPS) of Incretin Peptides

Timo Nuijens, Ph.D. | CSO
EnzPep, B.V.
Brightlands Campus, Building 93. Room 093.3.220, Urmonderbaan 22, 6167 RD Geleen, Netherlands.

Many incretin mimic peptide therapeutics, used for the treatment of diabetes type II and obesity (e.g. exenatide, liraglutide), will come off patent in the near future and become available as generic drugs. Most of these will need to manufactured at multi-10 kg or even >100 kg quantities. The development of these generic therapeutics and the scale of manufacture offer a unique opportunity to introduce novel and more cost-effective routes of production. The use of peptiligases for the efficient enzymatic fragment assembly of peptides represents a promising strategy that provides an elegant link between chemistry and biology, and typically leads to less impurities and a higher overall yield as compared to linear SPSS.[1-3]

The linear SPPS manufacture of generic forms of acylated GLP-1 analogs such as liraglutide and semaglutide is especially demanding because the innovators use recombinant expression, which inherently generates challenging impurity profiles that in turn lead to low overall yields.

In our presentation, we report efficient chemo-enzymatic peptide synthesis (CEPS) manufacturing processes for the block-buster incretin peptides such as exenatide, liraglutide and semaglutide. Specific items discussed will be the optimal ligation strategy, SPPS of the fragments, enzymatic ligation, overall efficiency of the CEPS process and scale-up. In general, the overall yield obtained from the production of incretin peptides using CEPS is more than doubled as compared to linear SPPS. These procedures can also be applied to many of the acylated incretin candidates in clinical and pre-clinical development.

1. M. Schmidt, A. Toplak, P. JLM Quaedflieg, J. H. van Maarseveen, T. Nuijens, *Drug Discov. Today Technol.* **2017**, 26, 11–16.
2. M. Schmidt, A. Toplak, P. J. Quaedflieg, T. Nuijens, *Curr. Opin. Chem. Biol.* **2017**, 38, 1–7.
3. A. Toplak, T. Nuijens, P. J. L. M. Quaedflieg, B. Wu, D. B. Janssen, *Adv. Synth. Catal.* **2016**, 358, 2140–2147.

Glepaglutide, a Novel Long-acting Glucagon-like Peptide-2 Analog for the Treatment of Patients with Short Bowel Syndrome

Andrew Parker, MBA, Ph.D. | Secretary and Director, Peptide Therapeutics Foundation; CSO and EVP, Head of Research and External Innovation
Zealand Pharma
Smedeland 36, Glostrup, Denmark, 2600 | 458-8773-600

Glucagon-like peptide (GLP-2) is an intestine-derived peptide that is rapidly deactivated and cleared from the circulation (T½ = ~7 min). GLP-2 positively modulates the morphology and function of the small intestine in both healthy and diseased conditions.

Short bowel syndrome (SBS) is a condition characterized by malassimilation in part due to reduced intestinal surface area following resection of the intestines primarily as a result of cancer, Crohn's disease or trauma. SBS patients need to supplement their food intake with long periods of time receiving parenteral nutrition. Glucagon-like peptide-2 (GLP-2) exerts mucosal growth-promoting effects mediated via stimulation of crypt cell proliferation and inhibition of villus cell apoptosis. In SBS patients, treatment with GLP-2 has been shown to improve intestinal absorption by increasing intestinal mucosal surface area and possibly by slowing gastrointestinal emptying and reducing gastrointestinal secretions.

Zealand is developing glepaglutide (ZP1848) a novel, potent and biologically stable GLP-2 receptor agonist in a ready-to-use liquid formulation for the treatment of SBS. Glepaglutide has successfully completed Phase II clinical studies using daily administration, demonstrating significant effects on reducing fecal wet weight output, increasing fluid absorption and reducing urine output, a secondary endpoint that guides reduction in parenteral support. In addition gelpaglutide has been shown to have a T1/2 of approximately 50 hours indicting the potential for a reduced dosing frequency. A pivotal Phase III study is planned to start in 2018. The 26-week trial will enroll approximately 130 patients with short bowel syndrome to test efficacy and safety of once- and twice weekly dosing with glepaglutide.

Definition of Endocrine FGF Structure as a Means to Super-agonism

Sebastian Parlee, Ph.D. | Senior Scientist
Novo Nordisk, Research Center Indianapolis
640 East Michigan Street B 128, Indianapolis, Indiana 46202 | 734-272-9680

FGF21 has demonstrated curative pharmacology in murine models of obesity and diabetes that include ameliorating elevated lipids, glucose and insulin while simultaneously reducing body weight. In subchronic studies in humans, FGF21-therapy has demonstrated clinically meaningful reductions in triglycerides and other markers of lipid metabolism, without any significant effect on hyperglycemia. Assessment of FGF21 in non-human obese and diabetic primates suggests this glycemic outcome requires more intensive dosing. Accordingly, we explored the molecular elements within FGF21 responsible for receptor activity as a potential means for identifying a super-agonist. The endocrine FGFs (19 and 21) biochemically signal through a bipartite FGFR/KLB co-receptor complex, with the C-terminus believed to be of seminal importance to this interaction, and thus biological function. The 25-terminal amino acids of either FGF21 or 19 were determined to be necessary and sufficient for binding KLB and capable of antagonizing the action of each endocrine FGF. Amino acid scanning identified a common structural basis for this binding despite only partial sequence identity among FGF19 and 21. While the majority of alanine-mutations in the C-terminus peptide resulted in detrimental or neutral effects on KLB binding, a similar substitution for the terminal lysine of native FGF19 significantly improved its potency. When incorporated to full-length FGF21, the structure-activity relationship of these peptide-based antagonists translated to full length agonists, of correlative potency. A specific FGF21 analog with a Cterminal FGF19-based peptide proved pharmacologically superior to either hormone when studied in vitro, and in dietary obese mice. Collectively, these results define the structural signature for KLBmediated signaling by endocrine FGFs and provide a directed approach to design of more efficacious FGF-based therapy for treatment of metabolic diseases.

Targeting Eph Receptors with Agonist and Antagonist Peptides

Elena B. Pasquale, Ph.D. | Professor, Tumor Initiation and Maintenance Program; Director Academic Affairs
Sanford Burnham Prebys Medical Discovery Institute
10901 North Torrey Pines Rd, La Jolla CA 92037 | 858-646-3100

The Eph receptors are the largest family of receptor tyrosine kinases and have widespread roles in tissue development and homeostasis. In addition, aberrant expression and activity of various Eph receptors have been implicated in disease processes ranging from inhibition of neural repair and neurodegeneration to pathological forms of angiogenesis, cancer malignancy, inflammation and infections. A long-standing interest of our laboratory has been to develop peptides that bind to Eph receptors and modulate their function. In phage display screens, we identified both linear and cyclic dodecameric peptides that bind to Eph receptors and inhibit the binding of their natural ligands, the ephrins. The crystal structures of four of these peptides in complex with the ligand-binding domain of their target Eph receptors reveal that the peptides all bind to the ephrin-binding pocket. However, in contrast to the promiscuous ephrin ligands, each peptide binds specifically to a single Eph receptor.

These Eph receptor-targeting peptides have been characterized and improved through collaborative efforts, and are used by other investigators for a variety of applications. Peptides binding to the EphA2, EphA4 and EphB4 receptors are the most widely used as selective tools to elucidate the function of each receptor in various biological processes and as potential starting points towards therapeutic leads and for medical imaging applications. The linear KYL antagonist peptide, which targets EphA4 with low micromolar affinity, has been reported to counteract neurodegenerative processes and promote neural repair in cell culture and in vivo preclinical models of amyotrophic lateral sclerosis (ALS), Alzheimer's disease and nerve injury. Our recent efforts have focused on the structure-guided improvement of another EphA4 antagonist peptide, APY, which is cyclized through a disulfide bond and is thus particularly suitable for pharmacological development. We have obtained APY derivatives with low nanomolar EphA4 binding affinity and dramatically increased resistance to plasma proteases, which are suitable for intracerebroventricular administration with osmotic minipumps. Ongoing work focuses on further increasing EphA4 inhibitory potency and on improving peptide lifetime in the blood circulation. The linear YSA peptide, which targets EphA2 with low micromolar affinity, can function as an agonist that induces EphA2 activation and trafficking to the cell interior. YSA and its derivatives have been used to deliver siRNAs, nanoparticles and conjugated drugs to cells, tumors and diseased tissues with high EphA2 expression. Structure-guided improvements are underway to increase the potency and plasma stability of YSA derivatives. We have also developed TNYL-RAW as a low nanomolar EphB4 antagonist peptide. TNYL-RAW can inhibit EphB4-dependent angiogenic responses, mobilization of hematopoietic stem/progenitor cells from the bone marrow, and differentiation of bone forming cells. Furthermore, TNYL-RAW has been used for targeted in vivo delivery of chemotherapeutics, imaging agents and theranostic nanoparticles to tumor xenografts with high EphB4 expression. Thus, while targeting the ephrin-binding pocket of Eph receptors with small molecules has proven challenging, these studies support the feasibility and value of developing a collection of peptides targeting each of the 14 Eph receptors with high potency and selectivity.

Alternative Forms and Activities of Human tRNA Synthetases in Biology and Disease

Paul Schimmel, Ph.D., Ernest and Jean Hahn Professor of Molecular Medicine and of Chemistry
Scripps Florida Research Institute
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037

Aminoacyl tRNA synthetases catalyze the first step of protein synthesis, in a reaction that establishes the rules of the genetic code. These universal enzymes have been extensively investigated for their catalytic activities and for their ability to pick out specific transfer RNAs, to match each of the 20 amino acids with the cognate triplet anticodons of the genetic code. Significantly, several dozen dominant and recessive mutations are known in the human population and have been associated with diseases. In many instances these mutations do not disrupt protein synthesis. In addition, the enzymes in animals are also found outside the cytoplasm, where protein synthesis normally occurs. These include nuclear and extra-cellular circulating forms, which have specialized novel activities and specific receptors and binding partners. In addition, over 200 splice variants have been annotated, with most disrupting the catalytic domain, and yet retaining the novel additions. These alternative forms and activities of tRNA synthetases reveal a new layer of biological complexity that integrates protein synthesis with cell signaling functions, and have pointed the way for ongoing applications to human diseases.

Injectable Peptide Gels for the Local Delivery of Drugs

Joel Schneider, Ph.D. | Deputy Director, Center for Cancer Research; Chief, Chemical Biology Laboratory
National Cancer Institute, NIH
376 Boyles Street, PO Box B, Frederickm MD 21702 | 301-846-5954

The beta-sheet is a ubiquitous protein fold whose propensity to self-assemble has made it a valuable building block for materials engineering. Sheets typically assemble into fibrils, which are themselves capable of higher order assembly that leads to the formation of hydrogel networks. We have designed a class of hydrogels from self-assembling beta-hairpin peptides that enable the direct three-dimensional encapsulation and subsequent localized syringe-based delivery of small molecules, nucleic acids, proteins or cells to tissue. Our recent progress in formulating gels to deliver cytokines important for adoptive cell transfer therapy, gels that deliver small molecule immune modulators that aid vascularized composite allotransplantation, and gels capable of delivering mRNA as adjuvants in mesothelioma resection surgery will be discussed.

About the High Resolution Aβ(1-42) Fibril Structure – and the Orally Available Clinical Stage all-D-Enantiomeric Peptide PRI-002 that Reverts Cognition Deficits and Decelerates Neurodegeneration in Transgenic AD Mouse Models

Dieter Willbold, Ph.D. | Director, Institut fur Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany; Director, Institute of Complex Systems, ICS-6: Structural Biochemistry, Research Centre Jülich, Jülich, Germany
Leo-Brandt-Str., Jülich, Germany 52425 | 492461612100 | E-mail: dieter.willbold@hhu.de

Oligomeric amyloid β (Aβ) is suspected of being the most toxic species in Aβ aggregation and responsible for development and progression of Alzheimer's disease (AD). Thus, development of compounds that are able to eliminate already formed, toxic Aβ oligomers is very desirable. In the recent years, we have developed all-D-enantiomeric peptides that have proven to be able to directly and specifically eliminate toxic Aβ oligomers in vitro. Such all-D-peptides combine the advantages of small molecules with the specificity of peptides. They are orally available and non-immunogenic. The *in vivo* proof of concept for "D3", the lead compound of this development, was accomplished in several treatment studies.[1-4] Here, we describe *in vivo* efficacy of the improved D3 derivative "PRI-002" (alias RD2).[5-7] PRI-002 is able to reverse the cognitive deficit and to significantly reduce Aβ pathology even in old-aged transgenic AD mice with full blown pathology and deficits, even after oral administration. PRI-002 has proven to be fully blood-brain-barrier penetrable and demonstrated target engagement in vitro and *in vivo*, in particular by showing significant reduction of Aβ oligomers in the brains of RD2-treated compared to placebo-treated mice. The correlation of Aβ elimination in vivo and the reversal of cognitive deficits in old-aged transgenic mice are in support of Aβ oligomers being relevant not only for disease development and progression, but also for Aβ oligomers as a promising target for the causal treatment of AD. I will summarize preclinical efficiency data with successful in vivo proof-of-concept in four treatment studies in three different transgenic animal models in three different laboratories. Based on very favourable properties in preclinical and safety and toxicology, we started a first-in-human phase I clinical trial to show safety also in humans. I will report the first clinical data and also the high resolution structure of the Aβ(1-42) fibril that will possibly allow rational drug design in the future.[8]

1. Funke *et al.*, *ACS Chem. Neurosci.* 1, 639-648 (**2010**).
2. Brener *et al.*, *Sci. Rep.* 5, 13222 (**2015**).
3. Jiang *et al.*, *PLoS One* 10, e0128553 (**2015**).
4. Elfgen *et al.*, *Eur. J. Pharm. Sci.* 107, 203-207 (**2017**).
5. Leithold *et al.*, *Pharm Res.* 33, 328-336 (**2016**).
6. van Groen *et al.*, *Sci. Rep.* 7, 16275 (**2017**).
7. Kutzsche *et al.*, *Molecules* 22, 1693 (**2017**).
8. Gremer *et al.*, *Science* 358, 116-119 (**2017**).

Identifying and Targeting Tumor Neoantigens

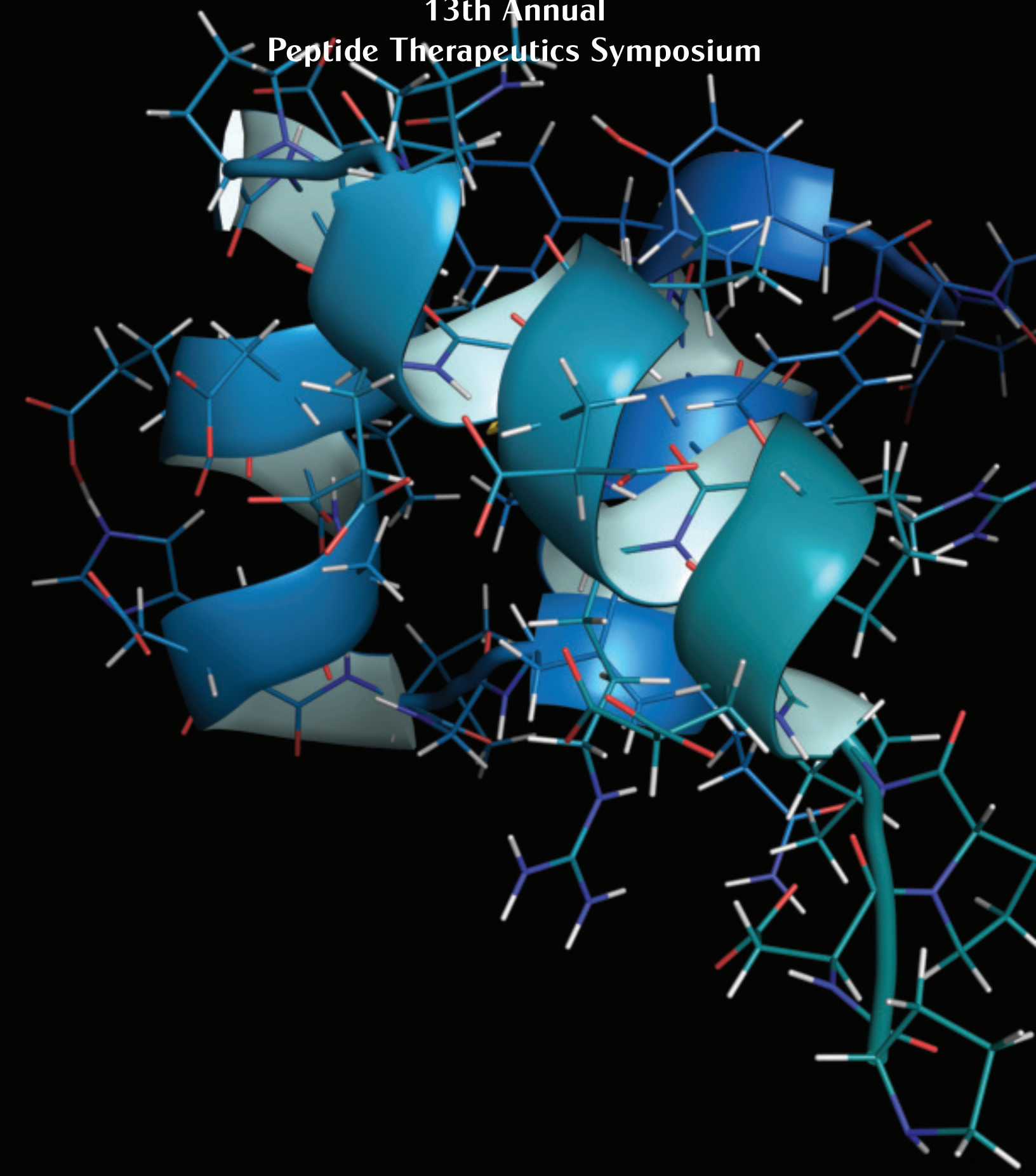
Catherine J. Wu, M.D. | Professor of Medicine, Department of Medical Oncology
Dana-Farber Cancer Institute and Harvard Medical School
Boston MA USA | 617-632-5943

With the recent availability of novel immunologic agents, priority has shifted to understanding the mechanisms of and predicting responses to each treatment. At the heart of cancer and host immune cell interactions is the tumor antigen and host antigen-specific T cell interaction, with the cytotoxic T cell-cognate antigen interaction forming the mechanistic basis for immune-mediated recognition and the killing of malignant cells. While the search for immunogenic tumor antigens has been the subject of decades-long studies, multiple lines of evidence have convincingly demonstrated tumor neoantigens as an important class of immunogenic tumor antigens. Neoantigens arise from amino acid changes encoded by somatic mutations in the tumor cell and have the potential to bind to and be presented by personal HLA molecules. Using next-generation sequencing approaches, we can now systematically identify mutations leading to amino acid changes that can be potentially recognized immunologically through the implementation of neoantigen discovery pipelines. In recent studies, we have demonstrated that neoantigen load is associated with clinical outcome to immune-based therapies, and neoantigens can be safely and feasibly targeted to generate customized cancer vaccines. We have been undertaking pilot clinical trials to develop personal cancer vaccines in melanoma and glioblastoma that utilize synthetic long peptides as delivery approach for this therapy. Recent results and new directions will be discussed.

Notes

Notes

Poster Abstracts
13th Annual
Peptide Therapeutics Symposium



P01 Early Development of a KISS1/GPR54 Receptor Agonist for Prostate Cancer

Yvonne Angell[a], Yan Wang[a], Yun Wu[b], Wen Jie[b], Chen Haixia[b], Yanfen Teng[b], Meng Xiangmin[b], Krishna Kodukula[c], and Robert Drakas[c]
[a]Department of Peptide Chemistry, ChemPartner SSF, 280 Utah Avenue, Suite 100, SSF, CA 94080; [b]ChemPartner Shanghai; [c]ShangPharma Innovation, 280 Utah Avenue, Suite 250, SSF, CA 94080

Metastin/kisspeptin is a C-terminal amidated peptide of 54 amino acid residues in length isolated from human placental tissue. It is a ligand of the orphan G-protein-coupled receptor KISS1R, also called GPR54, which is expressed throughout the central nervous system and in a variety of endocrine and gonadal tissues. Kisspeptin plays a critical role in controlling gonadotropin- releasing hormone (GnRH) neurons by acting directly on the GnRH neurons to stimulate GnRH release.[1] Compared to the full-length metastin protein, the N-terminally truncated peptide metastin(45–54) has 3–10 times higher receptor affinity and enhanced ability to increase intracellular calcium concentration, which is essential for activation of protein kinases involved in intracellular signaling in a number of pathways that affect reproduction and cell migration.[2] KISS1R receptor agonists and antagonists have been explored to investigate the biology of targeting the KISS1 receptor. Metastin(45–54) analogues with potent agonist activity and improved metabolic stability have been shown to suppress plasma testosterone in male rats with continuous subcutaneous administration.[3] KISS1R agonists have reached phase II in the clinic.[4]

We designed novel peptides with increased activity against KISS1R as well as enhanced plasma stability over previously reported KISS1R agonist compounds, and demonstrated in vivo efficacy of these novel peptides. The lead peptide, YA-156, demonstrated potent *in vitro* as well as in vivo efficacy in a subcutaneous, xenograft x1LnCAP BALB/c nude mouse prostate cancer model, excellent plasma stability, and a good *in vitro* ADME profile, aside from some unexpected receptor selectivity results.

1. Kotani, M. *et al.*, *J. Biol. Chem.*, **2001**, 276(37), 34631–34636.
2. Ohtaki, T. *et al.*, Nature, **2001**, 411, 613.
3. Matsui, H. *et al.*, *Eur. J. Pharmacol.*, **2014**, 735, 77–85.
4. MacLean, D.B. *et al.*, *J. Clin. Endocrinol Metab.*, **2014**, 99(8), E1445–E1453.

P02 Design and Synthesis of Brain Penetrant Glycopeptide Analogues of Pituitary Adenylate Cyclase Activating Peptide (PACAP) for the Treatment of Parkinson's Disease

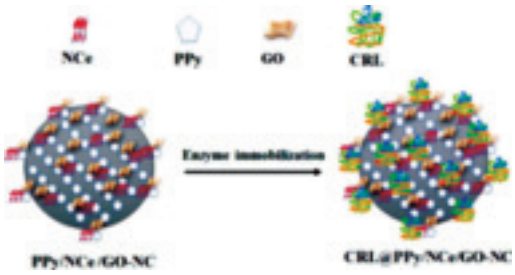
Christopher R. Apostol,[1] Chenxi Liu,[1] Lajos Z. Szabò,[1] Mitchell J. Bartlett,[2,3] Gabriella Molnar,[3] Torsten Falk[2,3] Michael L. Heien,[1] John Streicher,[3] and Robin Polt[1]*
Departments of Chemistry & Biochemistry,[1] Neurology,[2] and Pharmacology,[3] The University of Arizona, Tucson, AZ 85721
* polt@u.arizona.edu, phone (520) 370-2654

The pituitary adenylate cyclase activating polypeptide (PACAP) is an endogenous neuropeptide closely related to the two vasoactive intestinal peptides (VIPs). These peptides are members of the secretin family of peptide hormones that activate Class B GPCRs. PACAP binds and agonizes PAC1, VPAC1, and VPAC2 and inhibits neuronal apoptosis. It is considered to be neuroprotective in various pathological conditions in the CNS; making it a potential drug candidate for treating various neurodegenerative disorders. However, native PACAP exhibits poor pharmacokinetics as it is rapidly degraded by several proteases and peptidases; showing low bioavailability. Furthermore, activation of the VPAC2 receptor can lead to undesired peripheral side effects such as vasodilation and water retention. Therefore, it is critical that more stable and PAC1/VPAC1-selective agonists be developed, and that they can be targeted toward the CNS. One strategy that has not been extensively explored in the context of PACAP agonists is glycosylation. In other contexts, glycosylation of peptides has been shown to improve stability, enhance their original biological activities, and modulate their ability to cross cellular barriers like the BBB. To this end, we have designed and synthesized several PACAP glycopeptides containing C-terminal serine glycosides and additional amino acid substitutions. These glycopeptides were evaluated for their ability to stimulate cAMP production *in vitro* using individual CHO cell lines expressing PAC1, VPAC1, and VPAC2 receptors. A select number of the examined glycopeptides exhibited the desired pharmacological profiles. These compounds will be used as leads to further optimize their receptor selectivity, stability, and transport properties *in vivo*.

P03 A Nanoformulation Of Lipase With Cellulose Embodied Polypyrrole Functionalized Graphene Oxide Nanocomposite As Promising Nanobiocatalyst: Characterization, Stability Insights And Application

Mohd Shamoon Asmat and Qayyum Husain
Department of Biochemistry, Faculty of Life Sciences Aligarh Muslim University, Aligarh 202002, India

Nanoformulations have gained a significant attention because of their size-dependent properties and synthetic peptides represent a major share of the growing commercial market. However, the main challenge for their fabrication into commercial products is their inherent physicochemical and biological instability. The major hindrance faced by enzymes is their denaturation under various conditions. This work was performed to describe the facile procedure of a novel nanobiocatalyst, nano cellulose fused polypyrrole/graphene oxide for the efficacious immobilization of lipase, a versatile hydrolytic enzyme having potential applications in commercial industries. The fabricated peptide nanoformulation was characterized using Fourier transform infrared spectroscopy, differential thermal analysis, thermogravimetric analysis, X-ray diffraction, scanning electron microscopy, atomic force microscopy, transmission electron microscopy, and *Candida rugosa* lipase was immobilized onto nanocomposite through physical adsorption. The catalytic efficiency and operational stabilities of immobilized peptide nanoformulation were improved significantly compared to the free enzyme. The reusability profile outcomes showed that the peptide nanoformulation was an outstanding nanobiocatalyst as it retained 85% of its original catalytic activity after 10 cycles of application. The nanobiocatalyst was employed for the synthesis of the fruit flavour compound, ethyl acetoacetate. The peptide nanoformulation successfully synthesised flavour compound in solvent free media and n-hexane having 27.5% and 75.5% ester yields respectively. Moreover, these outcomes demonstrating graphene oxide modified carrier induced stabilization, amended solvent tolerance and operational stability of lipase nanoformulation, will have quintessential influence on practical scale up of commercial biotechnological industries.



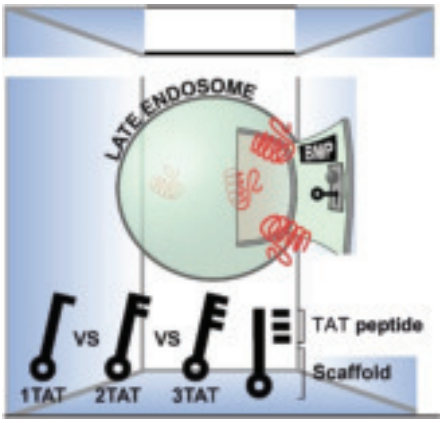
P04 Synthesis of a Competitive Stapled Peptide Inhibitor for CHP-NHE1 Protein-Protein

Bell, I[1]., Provost, J[1]., Gustafson, J[2].
[1]University of San Diego, San Diego, CA; [2]San Diego State University, San Diego, CA

The Sodium Hydrogen Exchanger (NHE1) is a membrane transporter that exchanges an intracellular proton for an extracellular sodium ion in order to maintain cellular pH. NHE1 is studied for its critical role interacting with, and activating migratory proteins along the leading edge of a mobile cell. Regulation of NHE1 is complex due to a large intracellular domain with many protein interactions and phosphorylation sites. Two NHE1 binding proteins, whose roles in regulating NHE, are not fully clear, are the calcineurin B homologous protein isoforms 1 and 2 (CHP1 and CHP2). Because CHP2 is primarily expressed in gut and transformed tumor cells, while CHP1 is present throughout the body, the interaction between the CHP isoforms and NHE1 is a potential anticancer target. Both CHP isoforms of interest bind to the same ~40aa region of NHE1, it is a candidate for a peptide inhibitor. Stapled peptides were synthesized to mimic the structure of NHE1 in order to create a more favorable binding partner for CHP2 over CHP1, these peptides were created based off of computational models of the binding of each CHP isoform to NHE conducted in approximated cellular conditions. These stabled peptides utilized a sulfenylation reaction with tryptophan amino acids placed within the native structure of a short NHE1 chain identified by the computational simulations to be the initial interaction site of the CHP2-NHE1 protein-protein interactions. This will allow for increased CHP1 activity in targeted cells (cancer cells) while also reducing CHP2 activity.

P05 Efficient Cytosolic Delivery of Molecular Cargo Mediated by Lipid-specific Endosomal Escape of Supercharged Branched Peptides

Dakota J. Brock[†], Lauren Kustigian[†], Mengqiu Jiang[†], Kristin Graham[†], Ting-Yi Wang[‡], Alfredo Erazo-Oliveras[§], Kristina Najjar[†], Junjie Zhang[†], Hays Rye[†], and Jean-Philippe Pellois[†,¤]
[†]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, USA; ‡Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, New York 14853, USA; §Department of Nutrition, Texas A&M University, College Station, Texas 77843, USA; ¤Department of Chemistry, Texas A&M University, College Station, Texas 77843, USA



Various densely-charged polycationic species, whether of biological or synthetic origin, have the ability to penetrate human cells and, concomitantly, carry macromolecular cargos into the intracellular milieu, albeit with variable efficiencies. The molecular underpinnings involved in such transport remain unclear. Herein, we assemble one, two, or three copies of the HIV peptide TAT on a synthetic scaffold to generate branched cell- permeable prototypes with increasing charge density. We establish that increasing TAT copies dramatically increases the cell penetration efficiency of the peptides while simultaneously enabling the efficient cytosolic delivery of macromolecular cargos. Cellular entry involves the leaky fusion of late endosomal membranes enriched with the anionic lipid BMP. Derivatives with multiple TAT branches induce the leakage of BMP- containing lipid bilayers, liposomal flocculation, fusion, and an increase in lamellarity. In contrast, while the monomeric counterpart 1TAT binds to the same extent and causes liposomal flocculation, 1TAT does not cause leakage, induce fusion or a significant increase in lamellarity. Overall, these results indicate that an increase in charge density of these branched structures leads to the emergence of lipid specific membrane-disrupting and cell-penetrating activities.

P06 Insulin Macrocyclization Explores the Relationship Between Conformation and Biological Activity

Brunel, FM[1], Mayer, JP[2], Gelfanov, VM[1], Zaykov AN[1], Finan, B, Perez-Tilve, D[3] and DiMarchi, RD[1,2]
[1]Novo Nordisk Research Center Indianapolis, Indianapolis, IN, USA electronic address: fob@novonordisk.com; [2]Department of Chemistry, Indiana University, Bloomington, IN, USA; [3]Department of Internal Medicine of Cincinnati College of Medicine, Cincinnati, OH

The chemical synthesis of insulin has been a historically challenging objective given the hydrophobic nature of the A-chain and its native two-chain, three-disulfide structure. The last decade has witnessed sizable progress driven by two independent synthetic strategies based on the oxidative folding of linear single-chain intermediates or disulfide bond formation by orthogonal cysteine protection. These synthetic advances have enabled the preparation of insulin analogs with additional disulfide bonds that had until recently not been accessible. The introduction of non-native bonds provides an opportunity to constrain the molecular flexibility of the hormone and explore the effect on biological action. With this as an objective, we have successfully synthesized 20 insulin analogs that contain a site-specific fourth disulfide in addition to the native three bonds. Each analog was prepared by folding of the linear DesDi single chain precursor. The non-native fourth disulfide, introduced by directed chemical oxidation, provided the unique opportunity to study the hormone in the constrained and unconstrained forms. A single analog with five disulfide bonds has also been prepared and represents the most structurally constrained insulin analog produced to date, with a twenty percent cysteine content. The biological activity within this novel series was highly dependent on the location of the additional disulfide, with close correlation between *in vitro* and *in vivo* potency. The chemical synthesis and biological characterization of these peptides constitute an important addition to the library of insulin analogs that have been studied over the last century, and demonstrate the current state-of-the art in insulin synthesis.

P07 Identification of Microprotein-Protein Interactions via APEX Tagging

Qian Chu[1], Annie Rathore[1], Jolene Diedrich[1], Cynthia Donaldson[1], John Yates[2], Alan Saghatelian[1]
[1]Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd, La Jolla, CA; [2]The Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla, CA 92037

Microproteins are peptides and small proteins encoded by small open reading frames (smORFs). Genomics and proteomics technologies have led to the recent discovery of hundreds to thousands of new microproteins. Emerging evidence demonstrates that these microproteins have fundamental roles in various biological processes including metabolism, apoptosis, and development, highlighting the value of characterizing these molecules. The identification of microprotein-protein interactions (MPIs) has proven to be a successful approach to the functional characterization of these genes; however, traditional immunoprecipitation methods result in the enrichment of non-specific interactions for microproteins. To address this issue, we tested and applied an in situ proximity tagging method that relies on an engineered ascorbate peroxidase (APEX) to elucidate MPIs. The results demonstrated that APEX tagging is superior to traditional immunoprecipitation methods for microproteins. Furthermore, the application of APEX-tagging to an uncharacterized microprotein, C11orf98-MP revealed that it interacts with nucleolar proteins nucleophosmin and nucleolin, demonstrating the ability of this approach to identify novel hypothesis-generating MPIs.

P08 Antibacterial and Non-cytotoxic Peptide Ferived from the p- BthTX-I

Norival A Santos-Filho[1], Julia P. Piccoli[1], Thainá C. Leal[1], Gabriela Marinho Righetto[2], Ilana L. B. C. Camargo[2], Eduardo M. Cilli[1]
[1]Institute of Chemistry, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil; [2]Instituto de Física de São Carlos, USP-Universidade de São Paulo, São Carlos, São Paulo, Brazil

Based on antimicrobial activity of the bothropstoxin-I (BthTX-I) we synthesized and characterized peptide derived from the C-terminal region of BthTX-I (p-BthTX-I, sequence: KKYRYHLKPFCKK) and its disulfide-linked dimeric form, obtained via air oxidation (p-BthTX-I)2. p-BthTX-I and (p-BthTX-I)2 =showed antimicrobial activity against both gram-negative and gram-positive bacteria, including multidrug-resistant strains and were not active against *C. albicans*, erythrocytes, epithelial cells, or macrophages, showing a possible specificity against prokaryotic cells. In addition, the dimeric form of the peptide was more active than the monomer. After serum incubation, our results showed that dimeric peptide are completely degraded after 25 min. However, mass spectrometry showed that the main degradation product was a stable peptide that had lost four lysine residues on its C-terminus region (des- Lys^{12}/Lys^{13}-$(p\text{-}BthTX\text{-}I)_2$). Antibacterial activities were evaluated against a variety of bacteria and exhibited similar or better activity than the $(p\text{-}BthTX\text{-}I)_2$. Aiming to analyze if the dimerization position could alter peptide activity, two others peptides were synthesized. The first was dimerized through a glutamic acid residue at the amino terminal [$E(p\text{-}BthTX\text{-}I)_2$] and another was dimerized through of the C-terminal lysine residue without the Lys^{12} and Lys^{13} residues (des-Lys^{12}/Lys^{13}-$(p\text{-}BthTX\text{-}I)_2K$). Both peptides presented antibacterial activity, but the best activity was found to (des-Lys^{12}/Lys^{13}-$(p\text{-}BthTX\text{-}I)_2K$), showing that the dimerization position is important to antimicrobial activity and that the Lys^{12} and Lys^{13} are not essential for antibacterial activity of this peptide. Summarizing, our results demonstrate that peptides analyzed are promising prototypes for new strategies to treat infections caused by multidrug- resistant bacteria.

P09 Structure-based Design, Synthesis and Evaluation of D-3,3-Diphenylalanine-based Tetrapeptides Inhibitors of Thrombin-activated Platelets Aggregation and Potent Anticoagulants

Cristina Clement,[1] Janet Gonzalez,[2] Morayma Reyes Gil,[1] Anna Babinska[3]
[1]Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461; [2]Natural Sciences, LaGuardia Community College, 31-10 Thomas Ave, Long Island City, NY 11101; [3]Department of Medicine, State University of New York, Downstate Medical Center, 450 Clarkson Ave, Brooklyn, NY 11203

Thrombosis-related disorders such as myocardial infarction, stroke, and pulmonary embolism remain a major cause of morbidity and mortality worldwide, a fact that is driving increasing interest in thrombin inhibitors as potential antithrombotic drugs. In 2012, Clement CC et al. published the biochemical and structural characterization of three noncovalent, direct thrombin inhibitors (DTI) that contain the common sequence D-Phe(P3)-Pro-(P2)-DArg(P1)-P1'-CONH2. Herein, we report the optimization of the tetrapeptide scaffold by replacing D-Phe in the P3 position with the un-natural Phe- analog, D-3,3-Di-Phenylalanine. We performed a structure-based drug design (SBDD) and structure-activity relationship (SAR) at the P1' position by replacing L-amino acids with their D-isomers and other unnatural amino acids analogs. Two types of binding experiments were employed to assess the inhibitory constant (Ki): (1) kinetics of alpha- thrombin inhibition of chromogenic substrate S2238; and (2) surface plasmon resonance (SPR) with immobilized alpha-thrombin. All D-3,3-Diphenylalanine-DTI analogs competitively inhibited alpha-thrombin's cleavage of the S2238 chromogenic substrate with K(i) of 500-20 nM that were further confirmed by the SPR assays. Remarkably, the novel DTIs inhibited the aggregation of human platelets in the "whole blood" thromboelastography (TEG) assay, as well as in the ex-vivo thrombin-activated platelets treatment. In addition, the peptidic DTIs showed potent inhibition of blood clotting monitored by aPTT, PT, and TT assays. These novel DTI tetrapeptides could be used as pharamacophore scaffolds for the development of inhibitors of thrombin- mediated platelets aggregation aiding the treatment of acute coronary syndrome (ACS). Moreover, the reported peptidic DTIs could be optimized as potential biomaterials with improved haemocompatibility for blood-contacting medical devices.

P10 Strategies Toward Optimizing Automated On-resin Disulfide Bond Formation in Disulfide Rich Peptides

Elizabeth Denton
Biotage, LLC, 10430 Harris Oaks Blvd, Charlotte, NC 28269

Disulfide rich peptides exhibit exquisite stability due to the covalent stabilization of their secondary structure mediated by disulfide bonds. While many of these compounds already display bioactivity, they have also demonstrated a unique plasticity when alternative sequences are grafted into various loop regions. After a particular sequence has been identified, these peptides are often folded in solution under redox conditions, assuming that the thermodynamically stable conformation will be the predominant species. However, there are many scenarios that multiple disulfide bonding patterns are observed upon folding completion, demanding additional purification and characterization before any biological assays can be performed.

A simplified on-resin synthesis strategy is attractive as the purification and characterization steps can be minimized, if not completely eliminated, likely increasing the overall yield of the peptide with the proper disulfide bond pattern. Herein we describe a fully automated, optimized solid phase synthesis of apamin, an 18 amino acid peptide conformationally constrained by two disulfide bonds. Using Branches™, the synthesis and on-resin disulfide bond formation was readily visualized and programmed, simplifying the total synthesis and ensuring that the proper disulfide bond pattern was achieved.

P11 Leveraging the Knorr Pyrazole Synthesis for the Facile Generation of Thioester Surrogates for use in Native Chemical Ligation

Dillon T. Flood, Jordi C. J. Hintzen, Michael J. Bird, Philip A. Cistrone, Jason S. Chen and Philip E. Dawson
The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Facile synthesis of C-terminal thioesters is integral to native chemical ligation (NCL) strategies for chemical protein synthesis. We introduce a new method of mild peptide activation, which leverages solid-phase peptide synthesis (SPPS) on an established resin linker and classical heterocyclic chemistry to convert C-terminal peptide hydrazides into their corresponding thioesters via an acyl pyrazole intermediate. Peptide hydrazides, synthesized on established trityl chloride resins, can be activated in solution with stoichiometric acetyl acetone (acac), readily proceed to the peptide acyl pyrazoles. Acyl pyrazoles are mild acylating agents and are efficiently exchanged with an aryl thiol, which can then be directly utilized in NCL. The mild, chemoselective, and stoichiometric activating conditions allow this method to be utilized through multiple sequential ligations without intermediate purification steps.

P12 Sequence Alignments and Peptide Optimization

Mark R. Hansen, Jane Razumovskaya, Jason Hodges, Hugo O. Villar
Altoris Inc., 600 W. Broadway Fl 7, San Diego, CA 92101

The discovery and optimization of peptide therapeutics involves nowadays hundreds of peptides and therefore structure activity relations based on three-dimensional molecular modeling will be hard to digest, even for short sequences. Information needs to be culled at the sequence level and thus the alignment of peptide sequences can be a critical step in the design and optimization of biopolymers for therapeutic purposes.

Sequence alignments are a critical step because without a clear understanding of which residues in a peptide sequence perform an equivalent function all subsequent analysis of correlations between structure and activity will be unworkable. The use of unnatural amino acids increases the challenge, since most sequence alignment techniques use substitution matrices derived for the 20 natural amino acids, while most projects rely on hundreds of different amino acids.

In this presentation we explore substitution matrices for the alignment of peptides that would be suitable to perform exploratory data analysis and the characterization of sequence activity relationships. We study different potential substitution matrices generated from computed properties to select those that show reliable alignments for different families of peptides. We show how those improved alignments facilitate analysis of trends for compound optimization and discuss ways to facilitate the identification of trends in the data and their association to sequence for natural and uncommon aminoacids.

P13 Discovery of a potential target for the development of therapeutic peptides for preventing bacterial-mediated colorectal cancer

Anthony Harrington and Yftah Tal-Gan
Department of Chemistry, University of Nevada, Reno, Las Vegas, NV 89123

Streptococcus gallolyticus subsp. *gallolyticus (Sgg)*, a member of the group D streptococci, is an opportunistic pathogen that was recently determined to be a bacterial driver of colorectal cancer (CRC), in addition to causing other diseases, such as infective endocarditis, bacteremia, neonatal meningitis, and septicemia. The mechanism by which *Sgg* causes CRC is not presently known. Our data show that *Sgg* utilizes quorum sensing (QS), a cell-density mechanism that allows bacteria to determine their population density and synchronize specific gene activation, to outcompete other gut microbes.[1] The *Sgg* QS circuitry may thus be involved in bacterial pathogenicity and the initiation of CRC. The identification and characterization of *Sgg* QS peptide signal can therefore be used as a potential pathway to target and attenuate this emerging human pathogen.

1. Harrington, A.; Tal-Gan, Y., Identification of Streptococcus gallolyticus subsp. gallolyticus (biotype I) competence stimulating peptide pheromone. *Journal of Bacteriology*, **2018**, JB. 00709-17.

P14 Chemical Syntheses of Teixobactin and Structural Analogs

Antoine Henninot, Daniel W. Carney
Ferring Research Institute, Inc., 4245 Sorrento Valley Blvd, San Diego, CA 92121

Teixobactin is a recently discovered nonribosomal peptidolactone antibiotic with potent and broad spectrum activity against Gram-positive bacteria. This natural product exhibits a novel mechanism of action that involves binding and sequestration of both lipid II and lipid III. Consequently, no observations of bacterial resistance to teixobactin have thus far been reported. Its potent activity and lack of resistance make teixobactin a highly attractive target for antibacterial drug discovery. In this poster we outline the key challenges to the total synthesis of teixobactin and describe two complementary synthetic approaches that were developed at the Ferring Research Institute. The first approach, which combines solid-phase peptide synthesis (SPPS) and a solution-phase macrocyclization, facilitated a scalable total synthesis of teixobactin and key analogs. The second approach, which utilizes lysine side chain anchoring, allowed teixobactin analog synthesis to be performed completely on-resin. The elimination of solution-phase chemistry facilitated the high-throughput parallel syntheses of several hundred teixobactin analogs for SAR studies.

P15 Development of a Multi-step Purification of Liraglutide

Marc Jacob, J. Preston, Venkat Thangella, Genevieve Hodson, Dani Xing
Phenomenex, 411 Madrid Ave, Torrance, CA 90501

Liraglutide is a human glucagon-like peptide-1 (GLP-1) analogue with a 31 amino acid sequence that is 97% similar to endogenous human GLP-1. Liraglutide was approved in the EU in 2009 and in the U.S. is 2010. Currently, Liraglutide is commercially available in more than 95 countries and has been approved for the treatment of type 2 diabetes and obesity in adults with related comorbidity.

The Pharmaceutical Industry requires high purities for active pharmaceuticals ingredient (APIs). In this study, a purification process is described that produced Liraglutide with a purity greater than 99%. In the work presented here, we describe the development of a multi-step purification process for Liraglutide.

Initially, a previously optimized analytical method (using an acidic buffer) was scaled up to PREP format for evaluation. These conditions were unable to meet the purity and yield results required for this project. Further method development was conducted and the chromatographic selectivity for this crude peptide was found to be dependent upon the pH of the aqueous component and the type of organic modifier used as the eluent. Several chromatographic stationary phases were evaluated and ultimately, the Luna C8(3) 10µm chromatographic media was chosen for the purification of this crude peptide sample. After significant method development including the evaluation of potential fraction pooling results, it was determined that a final polishing step was required to reach the necessary purity. The workflow of this multi-step purification was simplified utilizing the Luna C8(3) 10µm chromatographic media for both steps. The chromatographic selectivity for the polishing step was altered by changes in the eluent composition. The resulting two-step purification process was capable of meeting the required purity and yield requirements.

P16 Dissolution of β-sheet Stacking of Peptide Fibrils by Using Intense Mid-infrared Laser

Takayasu Kawasaki[1], Koichi Tsukiyama[1], and Kazuhiro Nakamura[2]
[1]IR Free Electron Laser Research Center, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan; 2Gunma University Graduate School of Health Sciences, 3-39-22, Showa-machi, Maebashi, Gunma 371-8511, Japan

Aggregation of peptides such as amyloid fibrils are closely associated with serious amyloidosis. In addition, the aggregation of the peptide drug such as insulin formulation is occasionally problematic in subcutaneous injection. Interestingly, most types of amyloid fibrils are stacked by β-sheet chains although the primary sequences of the amyloid peptides are various. One of effective approaches to suppress the progression of such diseases is to breakdown the fibril structures. However, dissociation of amyloid aggregates is difficult unless they are exposed to denaturants, and synthetic drugs and antibodies that inhibit β-sheet formation have yet to be developed. Instead, use of physical techniques with an electromagnetic field can potentially disrupt pathological biomolecules, and we show here that intense mid-infrared laser based on synchrotron radiation can dissociate the amyloid fibrils[1-4]. We applied the mid-infrared free-electron laser (FEL) to irradiate various types of amyloid aggregates: Aβ, calcitonin peptide fragment, insulin, lysozyme, polyglutamine peptide, and β2-microglobulin peptide. Mid- infrared FEL is an electromagnetic wave that produces an accelerator-based picosecond pulsed laser, and its oscillation wavelength is tunable within the mid-infrared region. After the irradiation experiments, several analytical results by infrared microscopy, scanning-electron microscopy, and Congo-red staining implied thatβ-sheet conformations of all peptide aggregates were substantially reduced by the FEL tuned to 6.1–6.2 μm corresponds to the amide I ($\nu_{C=O}$). The use of intense infrared laser might be potentially alternative way for dissociation of pathological peptide aggregates.

1. *Cellular and Molecular Neurobiology*, 38: 1039-1049 (**2108**).
2. *Journal of Synchrotron Radiation*, 23:152-157 (**2016**).
3. *Lasers in Medical Science*, 29: 1701-1707 (**2014**).
4. *The Protein Journal*, 31: 710-716 (**2012**).

P17 Cost Effective Peptide Purification via ZEOsphere DRP Mixed-Mode Chromatography

Jürgen Machielse[1], Alessandro Butté[2], Andrea Wild[1], Timo Nuijens[3], Marcel Schmidt[3]
[1]Zeochem AG, Rüti, Switzerland; [2]ETH-Zürich / DataHow, Zürich, Switzerland; [3]EnzyPep BV, Geleen, The Netherlands

Peptides are important API's for modern pharmaceuticals and have to be produced on preparative scale with increasing demand on separation costs. RPC and Ion Exchange are well-established chromatographic modes within the available tool-box of methods and procedures for the purification of this class of compounds in preparative scale production.

The presentation will show the beneficial use of ZEOsphere DRP (Doped Reversed Phase) Mixed-Mode stationary phases in the attractive- repulsive mode compared to non-doped RP or IEX stationary phases on real crude peptides. The orthogonal Doped Reversed Phase materials combines the dual action of strong IEX groups (acidic or basic) and Reversed Phase ligands like octyl chains on the packing surface.

Usually, Mixed-Mode stationary phases are applied under conditions which add the retention power of IEX groups by electrostatic attractive forces to the retention received from hydrophobic surface groups to the solutes. The peptides and the IEX group carry opposite charges.

It can be shown that in the majority of real life cases tested so far with ZEOsphere DRP in attractive-repulsion mode, improved selectivities and increased resolution at decreased retention time and solvent consumption can be obtained.

P18 Annotation of Protein-Coding Small Open Reading Frames in the Human Genome

Thomas Martinez, Cynthia Donaldson, Maxim Shokhirev, Alan Saghatelian
Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd, La Jolla, CA 92037

Recent work has shown that small open reading frames (smORFs) encoding peptides and small proteins of less than 150-amino acids, termed microproteins, are an overlooked source of coding potential in the genome. Several microproteins have already been characterized and implicated in a variety of critical cellular processes, including regulation of mRNA decay, DNA repair, and muscle formation. Thus, rigorous and comprehensive annotation of smORFs in the human genome is of fundamental importance to our understanding of essential cellular functions. We have developed a genomics-based workflow utilizing both RNA-Seq and Ribo-Seq to identify novel protein-coding smORFs across 4 human cell lines: HEK293, HEK293T, HeLaS3, and K562. RNA-Seq data was utilized in de novo transcriptome assembly for each cell line, and Ribo-Seq data was analyzed for translation evidence across all possible smORFs derived from *in silico* 3-frame translation of the transcriptomes. Transcription- and translation-level evidence was found for thousands of smORFs across all cell lines profiled. These novel smORFs are located within both the 5'- and 3'-untranslated regions of annotated transcripts, on ncRNAs, opposite annotated transcripts, and on unannotated transcripts, demonstrating their ubiquitousness within the genome. Over 40% of identified smORFs lack a canonical AUG start codon, suggesting widespread use of non-canonical start codons for translation initiation. The identification of these smORFs and encoded microproteins significantly increases the number of known protein-coding genes in the human genome, and radically alters the current view of the protein-coding capacity of the genome and the composition of the proteome.

P19 Rationally Designing the Most Potent Agonists and Antagonists for the Enterococcus faecalis fsr Circuit: Improving Attenuation of Quorum Sensing-Dependent Pathogenicity

Dominic McBrayer, Crissey Cameron, Brooke Gantman, Yftah Tal-Gan.
University of Nevada, Reno, Las Vegas, NV 89123

Resistance to antibiotics by bacteria remains a critical problem, particularly in cases of multi-drug resistance. *Enterococcus faecalis* is a common opportunist commensal gut bacterium, and is the leading cause of clinical enterococci infections. *E. faecalis* has exhibited both intrinsic and acquired antibiotic resistances and has been demonstrated to be capable of sharing antibiotic resistance with other bacterial species, such as Methicillin-resistant *Staphylococcus Aureus* (MRSA), increasing the prevalence of multi-drug resistant bacteria. Attenuating *E. faecalis* infections can thus not only decrease the costs associated with the initial infection, but can also help to prevent the spread of antibiotic resistance. An alternative approach to conventional antibiotics is to attenuate the pathogenicity of an organism, aiding the patient's immune system in clearing the infection while reducing the selective pressure for development of resistance to the treatment. *E. faecalis* pathogenicity has been shown to be enhanced by activation of its fsr quorum sensing (QS) circuit. The auto-inducing peptide (AIP) used by *E. faecalis* to activate this circuit is termed gelatinase biosynthesis activating pheromone (GBAP). Structure-activity relationship studies conducted by us and others has established basic understanding of components crucial for agonist and antagonist activity. In the presented study, we have rationally designed the most potent agonistic and antagonistic peptide analogs known to date while further increasing our understanding of the structural components necessary for interaction between GBAP and the fsr receptor. Our results have also suggested avenues for further improvement.

P20 BioChaperone Technology Enables the Development of Pramlintide-prandial Insulin Combinations

R. Soula, G. Meiffren, A. Geissler, Y. Meyer, A. Ranson, C. Fortier, O. Soula, B. Alluis, R. Charvet
Adocia, 115 Avenue Lacassagne, Lyon, France 69003

Pramlintide is an adjunct to mealtime insulin for improved control of post-prandial glucose (PPG). Pramlintide affects PPG by slowing gastric emptying, reducing glucagon secretion, and modulating satiety. Pramlintide use is limited as it cannot be combined in formulation with prandial insulin due to pH incompatibility, resulting in a high injection burden. BioChaperone technology enables a stable co- formulation of pramlintide and human insulin (BC PramIns) at neutral pH.

BC PramIns physical stability was evaluated by visual inspection and micro-flow imaging. For chemical stability, recovery was measured by reversed-phase HPLC while high molecular weight species were measured by size-exclusion HPLC. An in-use pump stability was performed using a commercially available pump at 37°C. Pharmacokinetics data were obtained by single subcutaneous administration at doses of 0.1875 µ/kg insulin and 1.125 µg/kg pramlintide to fasted healthy pigs.

BC PramIns is physically and chemically stable for at least 6 weeks at 30°C and 9 weeks at 25°C. Physical and chemical stability was similar to commercial Humulin® and Symlin®. Under simulated in-use pump conditions at 37°C, BC PramIns shows physical and chemical stability for at least 1 week, with insulin and pramlintide recoveries higher than 95% in a particle-free formulation. Following a single subcutaneous administration to fasted healthy pigs, BC PramIns results in slower absorption of pramlintide (LSM ratio [95%CI] $\Delta AUC_{Pram0\text{-}30min}$: 0.45 [0.20; 1.05]), and higher late exposure ($\Delta AUC_{Pram60\text{-}180min}$: 2.65 [1.44; 4.90]) compared to separate injections of insulin and pramlintide.

The *in-vitro* and preclinical pharmocodynamic properties of BC PramIns support development as a treatment for PPG.

P21 Protein Kinase C Beta Peptide Activator and Inhibitor Regulation of Phorbol 12- myristate 13-acetate (PMA) and N-formyl-L-methionyl-L-leucyl-L- phenylalanine (fMLP) Induced Superoxide Release in Polymorphonuclear Leukocytes (PMNs)

Daphne Metellus, Chinyere Ebo, Christina Lipscombe, Arjun Nair, Harsh Patel, Annam Humayun, Megan Michaels, Jennifer Dang, Matthew Finnegan, Lucy Checchio, Qian Chen, Robert Barsotti, and Lindon Young
Department of Biomedical Sciences, Philadelphia College of Osteopathic Medicine, 4170 City Avenue, Philadelphia, PA 19131

Protein kinase C beta (PKCβ) plays an important role in generating superoxide (SO) release via phosphorylation of leukocyte NADPH oxidase (NOX-2). Moreover, inhibiting tissue NOX-2 attenuates myocardial ischemia-reperfusion injury. However, comparison of PKCβ native/myristoylated (myr) peptide-activator versus peptide- inhibitor hasn't been evaluated. We hypothesize that the myr-PKCβ peptide-activator (N-myr-SVEIWD;myr-PKCβ+) would increase PMA or fMLP-induced leukocyte SO release, whereas myr-PKCβ peptide-inhibitor (N-myr-SLNPEWNET;myr-PKCβ-) would attenuate this response compared to non-drug treated controls. We further predict that unconjugated native PKCβ+/- peptide sequences wouldn't differ from
non-drug controls. Leukocytes (>90%PMNs) were isolated via peritoneal lavage from male Sprague-Dawley rats (~400g), then 5x106 leukocytes were incubated for 15min at 370C in the presence/absence of native/myr-PKCβ+/-(20 μM;n=5-7) or SO dismutase (SOD;10μg/mL;n=8) as positive control. PMA (100nM;n=17) or fMLP (1μM;n=15)-induced PMN SO release via reduction of ferricytochrome c was measured spectrophotometrically for 360 sec(PMA) or 90 sec(fMLP). Lastly, cell viability was determined by 0.2% trypan blue exclusion. PMA-induced PMN SO release increased peak absorbance to 0.41±0.039 in non-drug treated controls, whereas myr-PKCβ+ significantly increased (0.551±0.044) and myr-PKCβ- significantly decreased (0.263±0.064) PMA-induced PMN SO release. fMLP-induced PMN SO release increased peak absorbance to 0.205±0.027 in non-drug controls and was significantly inhibited by myr-PKCβ-(0.078±0.024) compared to myr-PKCβ+(0.219±0.066) and non-drug treated controls. SOD-treated samples showed >90% reduction of PMA or fMLP-induced SO release and native PKCv+/- was not different from non-drug controls. Cell viability ranged between 98±2% and 94±2% in all groups. Preliminary results suggest that myr-PKCβ+/- significantly affect PMA-induced SO release and myr-PKCβ- significantly attenuates fMLP-induced SO release.

This research was supported by the Division of Research, Department of Biomedical Sciences, and the Center for Chronic Disorders of Aging at Philadelphia College of Osteopathic Medicine. Current research license is supported by Young Therapeutics, LLC. lindonyo@pcom.edu.

P22 Design of Peptide Biomarkers for Complement System Activation

Dimitrios Morikis, Rohith Mohan, Reed Harrison
Department of Bioengineering, University of California, Riverside, Riverside, CA 92521

Within the innate immune system, complement component 3b (C3b) surveils extracellular space and covalently binds to surfaces of self and non-self. Once bound, C3b interacts with other complement proteins to illicit an innate immune response. This response may be avoided on surfaces of self by complement regulators that lead to cleavage and deactivation of C3b, leaving only the thioester containing domain, complement component 3d (C3d), covalently bound to the surface. Since C3d remains attached to the cell until membrane components are recycled, C3d accumulates on surfaces of self where C3b opsonization does not result in cell elimination. Thus, C3d is an attractive target for developing a biomarker of complement activation. Since several eye and kidney diseases result from mutations in complement species, monitoring C3d levels may also reveal mechanisms of disease. For example, in age-related macular degeneration (AMD), C3d is abundant in the drusen or cellular debris that accumulates proximal to the retinal pigment epithelium. Drusen accumulation is a hallmark of disease in AMD, though the contribution of drusen to disease is unknown. Inspired by recognition of C3d by complement regulator Factor H and Complement Receptor 2, we have developed several peptides that recognize and bind C3d through rational and computational combinatorial design. These peptides are cyclized to restrict their conformational space and improve structural stability, and are tested for binding using microscale thermorphoresis experiments. The peptides are amenable to conjugation with fluorophores suitable for *in vivo* research, and eventual clinical applications to investigate diagnosis and progression of AMD.

P23 Definition of Endocrine FGF Structure as a Means to Super-agonism

[1]Sebastian D. Parlee, [2]Archita Agrawal, [3]Diego Perez-Tilve, [1]Pengyun Li, [1]Jia Pan, [2]Piotr Mroz, [1]Ann Maria Kruse Hansen, [1]Brigitte Andersen, [1]Brian Finan, [1]Alexei Kharitonenkov, [2]Richard DiMarchi
[1]Novo Nordisk Research Center Indianapolis, Inc, Indianapolis, IN 46241; [2]Indiana University Dept. of Chemistry, Bloomington, IN 47405; [3]University of Cincinnati College of Medicine, Dept. of Internal Medicine, Cincinnati, OH 45221

FGF21 has demonstrated curative pharmacology in murine models of obesity and diabetes that include ameliorating elevated lipids, glucose and insulin while simultaneously reducing body weight. In subchronic studies in humans, FGF21-therapy has demonstrated clinically meaningful reductions in triglycerides and other markers of lipid metabolism, without any significant effect on hyperglycemia. Assessment of FGF21 in non- human obese and diabetic primates suggests this glycemic outcome requires more intensive dosing.

Accordingly, we explored the molecular elements within FGF21 responsible for receptor activity as a potential means for identifying a super-agonist. The endocrine FGFs (19 and 21) biochemically signal through a bipartite FGFR/KLB co-receptor complex, with the C-terminus believed to be of seminal importance to this interaction, and thus biological function. The 25-terminal amino acids of either FGF21 or 19 were determined to be necessary and sufficient for binding KLB and capable of antagonizing the action of each endocrine FGF. Amino acid scanning identified a common structural basis for this binding despite only partial sequence identity among FGF19 and 21. While the majority of alanine-mutations in the C-terminus peptide resulted in detrimental or neutral effects on KLB binding, a similar substitution for the terminal lysine of native FGF19 significantly improved its potency. When incorporated to full-length FGF21, the structure-activity relationship of these peptide-based antagonists translated to full length agonists, of correlative potency. A specific FGF21 analog with a C-terminal FGF19-based peptide proved pharmacologically superior to either hormone when studied in vitro, and in dietary obese mice. Collectively, these results define the structural signature for KLB-mediated signaling by endocrine FGFs and provide a directed approach to design of more efficacious FGF-based therapy for treatment of metabolic diseases.

P24 Fluorogenic Amino Acids and Their Potential as Real-Time Probes for Protein Synthesis

Jonathan T. Rittichier[1] Erkin Kuru[1], Yen-Pang Hsu[2], Garrett Coffman[2], Michael S. VanNieuwenhze[2] and George M. Church[1]
[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA; [2]Department of Chemistry, Indiana University, Bloomington, IN 47405, USA

Fluorescent proteins (FP) are among the most important imaging tools in modern biology. The amazing chemistry that transforms a string of amino acids to a fluorescent 'beacon' lies in many maturation events, including correctly folding into a βbarrel and a spontaneous cascade of cyclization, condensation, and oxidation steps. Though ubiquitous throughout the life sciences, many issues like mislocalization, photostability, brightness and long development times limit these constructs. Additionally, as our ability to track single- molecules with nanometer/microsecond spatiotemporal resolutions soars, the technologies of FPs are falling behind, primarily because of their inherent large size (multi- nanometer) and fluorophore maturation times (minutes-to-days).

The unique ability of small fluorogenic amino acids (FgAAs) to become strongly fluorescent only when incorporated into proteinaceous macromolecules promises to improve our understanding of bacterial growth and cellular organization by their selective probing proteins. The wash-free labeling of proteins with FgAAs has the potential to replace FPs in most of their current applications due to the following reasons: 1) FgAAs are up to ninety- fold smaller than typical FPs, 2) FgAAs mature instantaneously, and 3) unlike FPs, FgAAs do not require maturation or oxidation to induce fluorescence. Encoding relatively larger FgAAs into proteins is a daunting task. Here, we discuss our progress in designing, synthesizing and implementing these FgAAs into peptides and proteins to solve these aforementioned limitations.

P25 Discovery of Novel Albumin Binding Peptides Using Rational Design and Phage Display

Buyung Santoso, James Collins, Nicky Ferdyan, Ted Zee, Claudio Schteingart, Steve Qi, Max Wang, John Dwyer
Ferring Research Institute, Inc., 4245 Sorrento Valley Blvd., San Diego, CA 92121

Binding of peptides, lipids, and small molecules to albumin continues to be an attractive approach to extend the half-life of drug candidates. Since binding to albumin often affects the functional activity of these molecules, there remains a need for the discovery of unique HSA-binding motifs. Using phage display, we identified a small, cyclic peptide with submicromolar affinity to albumin. However, during affinity maturation of this lead, it was found that the binding affinity could not be significantly improved. The x-ray crystal structure of this peptide in complex with human albumin suggested that the geometry of the binding pocket offered little chance for further optimization. Furthermore, non-binding residues were observed to contribute significantly to the conformation of the peptide, suggesting that further efforts to improve the peptide were likely to be futile. In a separate effort, rational design using an existing co-crystal structure of a peptide/HSA complex was used to build affinity maturation libraries on phage. Interestingly, randomization of non-contact residues resulted in several high affinity analogs. These optimized HSA-binders were characterized for binding and species specificity and were shown to have a significantly long half-life in rodents. Both of these efforts yielded novel HSA binding peptides and could serve as starting points for the development of new half-life extension motifs.

P26 Using N-linked Glycosylation to Stabilize Bivalirudin

Marcus Van Engen, Bennett Harmelink, Jenna Veenstra, Lily Smith, Victoria Cast, and Joshua Zhu
Departments of Chemistry, Biology and Agriculture, Dordt College, Sioux Center, IA 51250

Bivalirudin is an FDA approved direct thrombin inhibitor (DTI) used to prevent blood clotting during invasive cardiovascular procedures. Two major impurities of bivalirudin are Asp-9 (α and β-Asp), caused by deamidation of the Asn-9 residue, which occurs during manufacturing, storage, and administration of the drug. N-linked glycosylation, a post-translational modification process in natural proteins, predominantly modifies Asn residues, which can stabilize them from deamidation reactions. Herein, we are chemically installing N-linked glycosylation onto the Asn-9 residue of Bivalirudin to reduce or eliminate Asp-9 impurities. Therefore, this project had three tasks: first, to demonstrate that N-linked glycosylation of the Asn-9 residue increases bivalirudin's stability; secondly, to identify α and β-Asp impurities and their ratio formed from the deamidation reaction; thirdly, to verify that the inhibitory effects of the glycosylated bivalirudin (glycobivalirudin) are equivalent to normal bivalirudin. To achieve these goals, four peptide chains have been successfully synthesized via solid phase peptide synthesis: bivalirudin and three peptides that differ at only the ninth residue, where Asp (α-Asp), isoAsp (β-Asp), and GlcNAc-Asn replaced Asn, respectively. A glycoamino acid, Fmoc-Asn(GlcNAc)-OH, was synthesized as a building-block of glycobivalirudin, and a protected glycine, Fmoc-Gly(Dmb)-OH, was synthesized and applied to synthesize the α and β-Asp bivalirudin, preventing an aspartimide side reaction. The Asp and isoAsp-containing chains will be used as standards to identify the HPLC peaks of degraded bivalirudin. Bivalirudin and the GlcNAc-Asn-containing chain, glycobivalrudin, will be placed in water-for-injection solutions to examine stability.
Glycobivalirudin will be further tested with a thrombin inhibition kit.

P27 Novel MC2R Antagonists Decrease Cortisol in Primary Human Adrenal Cortical Cells and Corticosterone in an in vivo Model of Hypercortisolism

Anastasia Velentza, Rabinovich Aleksandr, Wendy Hartsock, Michaelanne Munoz, Mark Lu, Michelle Kurano, Jolene Lau, John Kraus, Steve Qi, Melissa Roberts, Marcel van Duin
Ferring Research Institute, Inc., 4245 Sorrento Valley Blvd., San Diego, CA 92121

Adrenocorticotropic hormone (ACTH), regulates glucocorticoid (GC) production by the adrenal gland through melanocortin 2 receptor (MC2R). Chronic elevation of ACTH is associated with Cushing's disease (CD) and Congenital Adrenal Hyperplasia (CAH). CD is characterized by the hypersecretion of ACTH by pituitary adenomas, leading to chronic overproduction of cortisol, responsible for the disease morbidity and mortality.
The first line therapy, surgical removal of the pituitary tumor, suffers from high recurrence rates while pharmacotherapies are limited due to insufficient efficacy or side effects. Therefore, there is an unmet need for new treatments. In CAH, reduced negative regulation of ACTH by GC results in ACTH dependent overproduction of adrenal androgens. The standard of care for CAH is treatment with GC. However, supraphysiological levels of GC are needed to efficiently suppress androgens, leading to side effects. The MC2R is specific for ACTH and selectively expressed in the adrenal gland. MC2R antagonists should efficiently regulate ACTH driven pathophysiology, lacking the side effects of the current therapies in CD and CAH.

We report the discovery and pharmacological characterization of novel peptide MC2R antagonists. Screening of rationally designed peptide libraries in functional assays allowed the identification of potent and selective MC2R peptide antagonists. These compounds suppressed ACTH induced MC2R signaling and displayed a significant reduction of cortisol levels in primary human adrenal cortical cells. Peptide efficacy was demonstrated in an animal model of ACTH induced GC secretion. These results demonstrate the potential of MC2R antagonists to address unmet needs in the treatment of CD and CAH patients.

P28 cGMP Peptide Production from mg to kg with Automation and Microwave-Assisted Heating

Keith A. Porter, Alexandria E. Weber, Jonathan M. Collins, Michael J. Karney, Sandeep K. Singh
CEM Corporation, 3100 Smith Farm Road, Matthews, NC 28105

Peptides are ideal drug candidates due to their inherent high potency, low toxicity, and ability to effect a broad range of targets[1]. With several high revenue peptide drugs on the market and a full pipeline of potential candidates[2], the demand for a highly robust and effective synthetic method is of great importance[3].

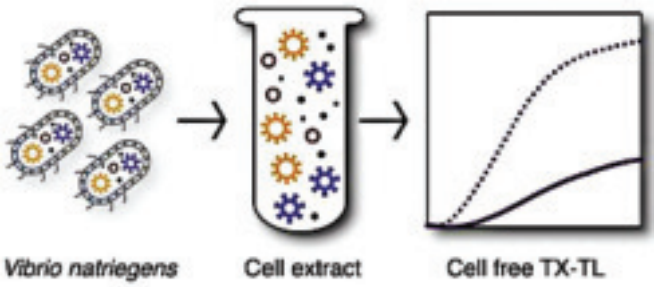
Currently, peptide synthesis research and production both face similar challenges—a sluggish and wasteful workflow in desperate need for optimization. The typical conventional optimization steps usually take a shotgun approach: screen resins, screen different reagent excesses, screen activators, etc. This synthetic process necessitates tens or hundreds of reactions, all of which can often take weeks or months to complete while requiring a great deal of time, money, and resources.

To address the needs of the market, new cGMP methodology has been developed utilizing automation and microwave-assisted heating. This work details mechanistic-based, innovative improvements to the chemical methodology of solid phase peptide synthesis, application of these improvements to high-throughput SPPS for personalized medicine via peptide vaccines[4,5], and large scale peptide production with cGMP considerations.

1. D. J. Craik, D. P. Fairlie, S. Liras, D. Price. *Chem Biol Drug Des*, **2013**, 81, 136.
2. K. Fosgerau, T. Hoffman. *Drug Discov Today*, **2015**, 20, 122.
3. J. M. Collins, K. A. Porter, S. K. Singh, G. S. Vanier. *Org. Lett.* **2014**, 16, 940.
4. P. A. Ott *et al*. *Nature*, **2017**, 547, 217.
5. R. Takahashi *et al*. *Breast Cancer Research*, **2014**,16, R70.

P29 Establishing a Cell-Free *Vibrio natriegens* Expression

Daniel J. Wiegand[1,2], Henry H. Lee[1], Nili Ostrov[1*], George M. Church[1,2*]
[1]Department of Genetics, Harvard Medical School, Boston, MA 02115; [2]Wyss Institute for Biologically Inspired Engineering, Boston, MA 02115 | *Corresponding Authors

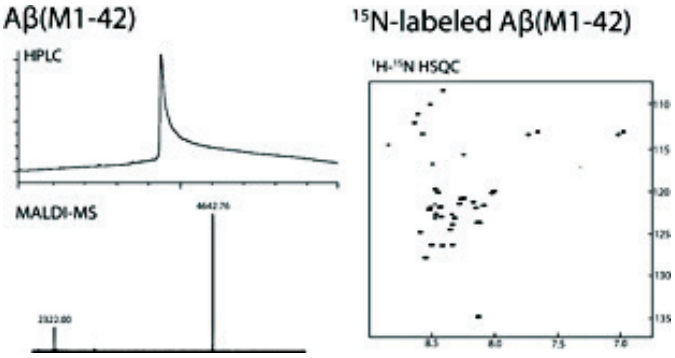


The fast-growing bacterium *Vibrio natriegens* is an emerging microbial host for biotechnology. Harnessing its productive cellular components may offer a compelling platform for rapid protein or peptide production and prototyping of metabolic pathways or genetic circuits. Here, we report the development of a *V. natriegens* cell-free expression system. We devised a simplified crude extract preparation protocol and achieved >260 µg/mL of super-folder GFP in a small-scale batch reaction after three hours. Culturing conditions, including growth media and cell density, significantly affect translation kinetics and protein yield of extracts. We observed maximal protein yield at incubation temperatures of 26°C or 30°C, and show improved yield by tuning ions crucial for ribosomal stability. Head-to-head comparisons demonstrate that our optimized system from wild-type *V. natriegens* performs as good as a highly engineered strain of *E. coli*, which is currently commonly used throughout the cell-free field. Furthermore, our approach for efficient crude extract preparation and cell-free protein synthesis is significantly faster and cheaper than similar commercially available cell-free systems due to *V. natriegens*' rapid growth and easily accessible protocols. Overall, this work establishes an initial *V. natriegens* cell-free expression system, enables probing of *V. natriegens* biology, and could serve as a high-throughput and parallelized platform to accelerate advancements in metabolic engineering in order to produce valuable natural products and therapeutic proteins or peptides.

P30 An Efficient Method for the Expression and Purification of Aβ(M1–42) and N-terminal Cysteine Aβ(1–42)

Stan Yoo, Sheng Zhang, Adam G. Kreutzer, and James S. Nowick*
Department of Chemistry, University of California, Irvine, Irvine, California 92697-2025, USA

Advances in amyloid research and in Alzheimer's Disease therapeutics rely on improved access to the β-amyloid peptide, Aβ. N-Terminal methionine-extended Aβ, Aβ(M1–42), is a readily expressed and widely used form of Aβ with properties comparable to those of the natural Aβ(1– 42) peptide. Expression of Aβ(M1–42) is simple to execute and avoids an expensive and often difficult enzymatic cleavage step associated with expression and isolation of Aβ(1–42). This presentation reports an efficient method for the expression and purification of Aβ(M1–42) and ^{15}N-labeled Aβ(M1–42). This method affords the pure peptide at ~ β19 mg/L of bacterial culture through simple and inexpensive steps in 3 days. A simple method for the construction of recombinant plasmids and the expression and purification of Aβ(M1–42) peptides containing familial mutations is developed. This presentation also reports an efficient expression of N-terminal cysteine Aβ by taking advantage of N-terminal methionine processing and a utility of N- terminal cysteine peptides through fluorescently labeling using cysteine-maleimide conjugation. We anticipate that these methods will enable experiments that would otherwise be hindered by insufficient access to Aβ.



P31 The Expression and Characterization of Disulfide-Bond Stabilized Aβ Oligomers

Sheng Zhang, Stan Yoo, Adam G. Kreutzer, and James S. Nowick
Department of Chemistry, University of California, Irvine, Irvine, CA, 92697-2025, USA

Amyloid-β peptide (Aβ) plays a central role in the pathology of Alzheimer's disease (AD). Aβ exists as three forms in equilibria: monomers, oligomers, and fibrils. Although Aβ fibrils are the most commonly found species in diseased brains, the soluble Aβ oligomers are the toxic contributors to the neurodegeneration observed in the AD. However, these oligomers are very short-lived and unstable, and will inevitably aggregate into fibrils, which makes it challenging to isolate and study their structures.

Here, I report the molecular cloning, expression, purification, and characterization of Aβ mutant peptides stabilized by a disulfide bond. Downstream biophysical studies show that, compared to the wild-type Aβ, mutant peptides have greater propensities to form higher-order Aβ oligomers. More importantly, these mutants are locked into oligomeric states, which are not able to aggregate into fibrils even after 24 hours of incubation. This provides a promising way to obtain stable and long-lived Aβ oligomers, which will facilitate studies of their structures and their roles in the pathogenesis of AD. This project may provide valuable insights into the anti-AD drug design process and the therapeutics

P32 Exploration of Mechanism of Action of Luminate®, a Therapeutic Peptide for Blinding Retinal Disease

Dan Zhou[1], Jin Mo Koo[1], Zixuan Shao[1], John Park[2], Julia A. Kornfield[1]
*1*Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125; [2]Allegro Ophthalmics, LLC, 31473 Rancho Viejo Rd., San Juan Capistrano, CA 92675.

Diabetic and age-related retinopathies, both associated with abnormal growth of blood vessels, are leading causes of blindness in the developed world. Current treatments against these retinopathies, such as Laser Photocoagulation and anti-Vascular Endothelial Growth Factor (VEGF) drugs, have limited efficacy and undesirable side effects. A recently discovered therapeutic peptide Luminate® (Allegro Ophthalmics, LLC) has proven to be effective in human clinical trials phase I and II. It has shown significantly longer lasting benefits than anti-VEGF treatments and shows synergistic effects when used with them. We believe unraveling the biological pathway affected by Luminate® will provide new perspectives in treating various retinopathies, and give new insights into the retinopathies and their managements.

The peptide and its scrambled counterparts are used to prepare fluorophore-peptide conjugates and peptide-directed coupling reagents. I*n vitro* and *ex-vivo* experimental results will be presented to visualize the distribution of bound Luminate® using fluorophore-peptide conjugates. Current progress toward the enrichment of binding using ligand-directed receptor "pull down" will also be described. In understanding retinopathies in the molecular level, our study with Luminate® could serve as a guide for studies of other therapeutic agents aiming for vision repair.

Notes

ChemPartner
Dedicated to LifeScience

FERRING
PHARMACEUTICALS

MedImmune

novo nordisk®

PolyPeptide
GROUP

ZEAL&
REVOLUTIONARY HEALTH SOLUTIONS

Zydus
dedicated to life

PEPTIDE
THERAPEUTICS
FOUNDATION