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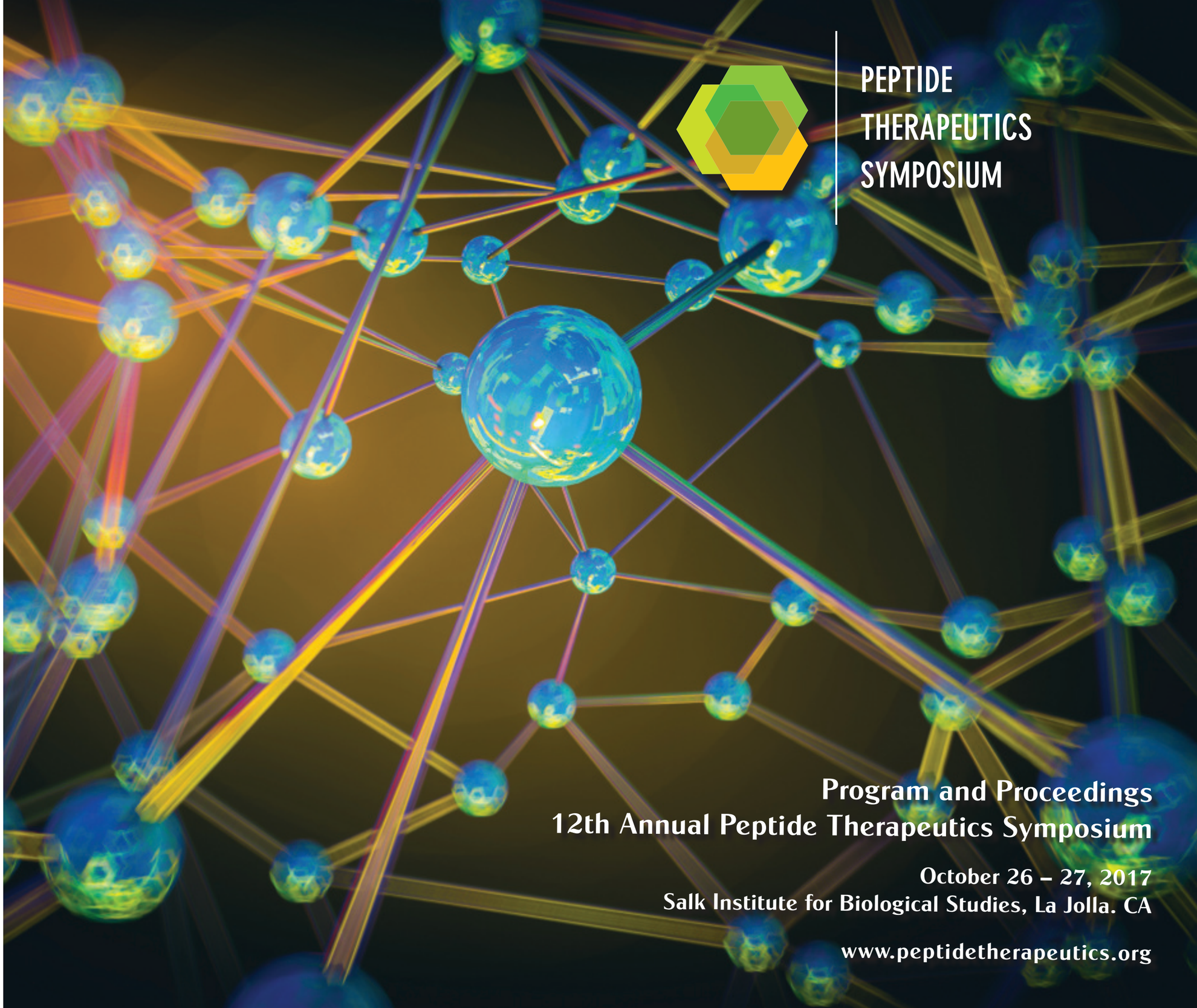

PEPTIDE THERAPEUTICS SYMPOSIUM

Program and Proceedings
12th Annual Peptide Therapeutics Symposium

October 26 – 27, 2017
Salk Institute for Biological Studies, La Jolla. CA

www.peptidetherapeutics.org

12th Annual Peptide Therapeutics Symposium

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Symposium Sponsors

12th Annual Peptide Therapeutics Symposium

Dear Colleagues,

Welcome to the 12th Annual Peptide Therapeutics Symposium at the Salk Institute for Biological Studies, La Jolla. This year's Symposium continues the tradition of showcasing novel biology and the discovery and development of peptide-based drug candidates through state-of-the-art lectures, cutting-edge poster presentations, and informal scientific networking.

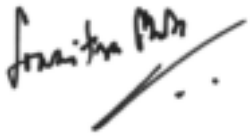
The Symposium opens on Thursday afternoon with Plenary Lectures delivered by Professor Ron Evans of the Salk Institute, and Professor David Craik of the Institute for Molecular Bioscience, The University of Queensland. The sessions that follow will present advances in peptide therapeutics. The evening concludes with a Poster Session and Opening Reception in an environment conducive to networking and non-structured personal interactions.

We reconvene on Friday morning with Keynote Lectures by Professor Jack Szostak of Harvard University and Professor Kevan Shokat of the University of California, San Francisco. The three subsequent sessions during the day will return the focus to peptide therapeutics, with lectures pertaining to enabling methodologies and advances in microbiome research.

As in previous years, the program, the meeting venue and social time have been designed to foster rich scientific exchanges and networking. We look forward to your participation in making the Symposium a memorable event.

Sincerely,

Richard DiMarchi
Chairman of the Board
Peptide Therapeutics Foundation



Soumitra Ghosh
President
Peptide Therapeutics Foundation

Sponsors, Peptide Therapeutics Foundation

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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 the global peptide/protein therapeutics research center for Ferring Pharmaceuticals. Established in 1996, FRI is located in the heart of the Southern California biopharmaceutical community. The center has attracted a diverse group of highly skilled professionals representing over twenty four countries of origin. FRI is focused on the following key therapeutic areas: endocrinology, reproductive health, urology, and gastroenterology. Our state-of-the art facility includes peptide and protein drug design, chemistry, pharmacology, biology, and preclinical ADME capabilities. FRI is committed to building a portfolio of novel, innovative peptide and protein therapeutics to address areas of high unmet medical need.

Ferring Pharmaceuticals (Ferring) is a private, research-driven specialty biopharmaceutical company active in global markets. The company identifies, develops and markets innovative products in the fields of endocrinology, gastroenterology, infertility, obstetrics, urology and osteoarthritis. In recent years Ferring has expanded beyond its traditional European base: with over 5,000 employees worldwide, it operates subsidiaries in 56 countries and makes its products available in more than 90 countries. The company has emerged as a world leader with one of the largest peptide therapeutics portfolios in the industry. As part of its commitment to developing innovative products to treat diseases with high unmet medical need, Ferring invests heavily in its research infrastructure both in terms of people and technology.

Ipsen Biosciences, Inc.

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

Peptides: long-standing expertise
Ipsen has long-standing expertise in peptides, ranging from discovery to delivery that is being leveraged to create highly differentiated drugs addressing targets that are not readily druggable by small molecules or antibodies. Ipsen Bioscience, Ipsen's new R&D center in Cambridge, develops peptide-based drugs to fulfill major unmet medical needs in endocrinology and oncology. Ipsen is a global specialty care-driven biopharmaceutical group with total sales exceeding €1.4 billion in 2015. Ipsen sells more than 20 drugs primarily across three franchises: oncology, endocrinology, and neurology. Ipsen's internal R&D focuses on its innovative and differentiated technological platforms: peptides and toxins.

MedImmune/AstraZeneca

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

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

Funds are being used exclusively for the educational portion of this program.

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.

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

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

2017 Travel Grant Awardees

Jason Allen, Texas A & M University
Dakota Brock, Texas A & M University
Carmine Pasquale Cerrato, Stockholm University
Hanna Kim, Philadelphia College of Osteopathic Medicine
Tove Kivijärvi, KTH Royal Institute of Technology, Stockholm
Bimal Koirala, University of Nevada, Reno
Helena Kondow, Texas A & M University
Ernest Y. Lee, University of California, Los Angeles
Tõnis Lehto, Stockholm University
Anahi McIntyre, Philadelphia College of Osteopathic Medicine
Kristina Najjar, Texas A & M University
Jonathan Rittichier, Harvard Medical School
Yifang Yang, University of Nevada, Reno
Hailiang Zhu, Georgia State University and Dordt College

12th Annual Peptide Therapeutics Symposium

Thursday, October 26, 2017

12:30 p.m. – 5:00 p.m.	**Registration Check-in** Fritz B. Burns Reception Center, Lower Level
1:30 p.m. – 5.30 p.m.	**12th Annual Peptide Therapeutics Symposium** Conrad T. Prebys Auditorium
1:30 p.m. – 1:45 p.m.	**Opening Remarks** Richard DiMarchi, Ph.D. *Chairman of the Board, Peptide Therapeutics Foundation* *Distinguished Professor of Chemistry, Gill Chair in Biomolecular Sciences, Department of Chemistry, Indiana University* *VP and Site Director, Novo Nordisk Research Center, Indianapolis*
1:45 p.m. – 3:15 p.m.	**Plenary Lectures** Moderator Richard DiMarchi, Ph.D.
1:45 p.m. – 2:30 p.m.	**Nuclear Receptors: Let's Make Metabolism Great Again** Ronald M. Evans, Ph.D. *Investigator, Howard Hughes Medical Institute* *Professor, The Salk Institute for Biological Studies* *March of Dimes Chair in Molecular and Developmental Biology* *The Salk Institute for Biological Studies*
2:30 p.m. – 3:15 p.m.	**Ultra-stable Cyclic Peptides from Plants and Animals as Tools in Drug Design** David Craik, Ph.D. *Professor of Chemistry, Institute for Molecular Bioscience* *The University of Queensland*
3:15 p.m. – 4:00 p.m.	**Break**
4:00 p.m. – 5:30 p.m.	**Session I:** Moderator Andrew Parker, Ph.D., MBA *Director, Peptide Therapeutics Foundation* *CSO & SVP, Head of Research & External Innovation* *Zealand Pharma*
4:00 p.m. – 4:30 p.m.	**Design of Complement-based Therapeutics and Testing in Computational Disease Models** Dimitrios Morikis, Ph.D. *Professor of Bioengineering* *University of California, Riverside*
4:30 p.m. – 5:00 p.m.	**Pharmacokinetic, Preclinical, and Clinical Updates on APL-2** Ahmad Sadr, MS *Vice-President of Technical Operations* *Apellis Pharmaceuticals*
5:00 p.m. – 5:30 p.m.	**Developing a Pipeline of Macrocyclic Peptides for Disorders of Complement Regulation** Simon Read, Ph.D. *Chief Scientific Officer* *RA Pharmaceuticals, Inc*
5:30 p.m. – 7:00 p.m.	**Poster Session & Opening Reception** Fritz B. Burns Reception Center, Lower Level

12th Annual Peptide Therapeutics Symposium

Friday, October 27, 2017

7:00 a.m. – 11:30 a.m.
Registration Check-in
Fritz B. Burns Reception Center, Lower Level

7:00 a.m. – 8:15 a.m.
Breakfast & Poster Viewing
Fritz B. Burns Reception Center, Lower Level

8:15 a.m. – 5:00 p.m.
12th Annual Peptide Therapeutics Symposium
Conrad T. Prebys Auditorium

8:15 a.m. – 8:30 a.m.
Welcoming Remarks
Soumitra Ghosh, Ph.D.
Director and President, Peptide Therapeutics Foundation
President, Doon Associates LLC

8:30 a.m. – 9:50 a.m.
Plenary Lectures
Moderator
Yvonne Angell
Director, Peptide Therapeutics Foundation
Director and Head of Peptide Chemistry
ChemPartner

8:30 a.m. – 9:10 a.m.
Directed Evolution of Highly Modified Cyclic Peptides
Jack W. Szostak, Ph.D.
Professor of Chemistry and Chemical Biology
Harvard University

9:10 a.m. – 9:50 a.m.
Chemical Tricks for Drugging the Undruggable
Kevan M. Shokat, Ph.D.
Professor, Cellular and Molecular Pharmacology
University of California, San Francisco
Department of Chemistry, University of California, Berkeley
Investigator, Howard Hughes Medical Institute

9:50 a.m. – 10:30 a.m.
Break & Poster Viewing

10:30 a.m. – 12:00 p.m.
Session I:
Moderators
Waleed Danko, Ph.D.
Distinguished Research Leader (Retired)
Hoffman-La Roche, Inc

Rajiv Sharma, Ph.D.
Head, Discovery Chemistry and Senior Vice President
Zydus Cadila

10:30 a.m. – 11:00 a.m.
Dual Peptides: The Future of Diabetes Therapy
Cristina M. Rondinone, Ph.D.
Vice President R&D, Head Cardiovascular/Metabolic Diseases
iMED
Medimmune

11:00 a.m. – 11:30 a.m.
Peptidic Innate Defense Regulators in Infectious Disease, Cancer and Cancer Supportive Care
Oreola Donini, Ph.D.
Senior Vice President and Chief Scientific Officer
Soligenix, Inc.

11:30 a.m. – 12:00 p.m.
Nanobodies as a Differentiated Drug Modality
Gerhard Niederfellner, Ph.D.
Senior Research Fellow
Ablynx

12th Annual Peptide Therapeutics Symposium

12:00 p.m. – 1:00 p.m.	**Lunch & Poster Viewing** Fritz B. Burns Reception Center, Lower Level
1:00 p.m. – 2:30 p.m.	**Session II:** Moderator Phil Dawson, Ph.D. *Professor of Chemistry; Dean of Graduate and Postdoctoral Studies* *The Scripps Research Institute*
1:00 p.m. – 1:30 p.m.	**How to Get Mass Spectrometry from a Niche Tool to Use by the General Population** Pieter Dorrestein, Ph.D. *Professor; Director, Collaborative Mass Spectrometry Innovation Center* *Co-Director, Institute for Metabolomics Medicine* *Skaggs School of Pharmacy and Pharmaceutical Sciences* *University of California, San Diego*
1:30 p.m. – 2:00 p.m.	**Boosting Endogenous and Pharmaceutical Peptide Killing of Antibiotic-Resistant Superbugs** Victor Nizet, MD *Professor and Vice Chair for Basic Research* *Chief of the Division of Host-Microbe Systems & Therapeutics* *University of California, San Diego*
2:00 p.m. – 2:30 p.m.	**Peptide-Derived Natural Products in the Design of Novel Anti-Infectives** James Balkovec, Ph.D. *Senior Vice President Research* *Cidara Therapeutics, Inc.*
2:30 p.m. – 3:15 p.m.	**Break & Poster Viewing** Fritz B. Burns Reception Center, Lower Level
3:15 p.m. – 4:45 p.m.	**Session III:** Moderator Alexksander Swietlow, Ph.D. *Director, Peptide Therapeutics Foundation* *Global Director, Quality Control* *The PolyPeptide Group*
3:15 p.m. – 3:45 p.m.	**Biodistribution of an Oxytocin Analogue After Intranasal and Central Administration to Pigs – A PET Imaging Study** Claudio D. Schteingart, Ph.D. *Director, Peptide Therapeutics Foundation* *Vice President, Science & Technology - Research* *Ferring Research Institute Inc.*
3:45 p.m. – 4:15 p.m.	**Peptide-CRM$_{197}$ Conjugate Vaccines – Considerations for Process Development** Anouk Dirksen, Ph.D. *Senior Principal Scientist* *Pfizer Inc.*
4:15 p.m. – 4:45 p.m.	**Massively Parallel Synthesis and Screening of Linear Peptides and Macrocycles Using Peptide Microarrays** Lauren Goodrich, Ph.D. *Scientist, Technology Innovation* *Roche Sequencing*
4:45 p.m. – 5:00 p.m.	**Closing Remarks** Adrienne Day, Ph.D. *Treasurer and Administrator, Peptide Therapeutics Foundation* *Senior Director, Business Development* *Ferring Research Institute Inc.*
5:00 p.m. – 6:00 p.m.	**Closing Reception**

Speaker Biographies
12th Annual
PeptideTherapeutics Symposium

12th Annual Peptide Therapeutics Symposium

James M. Balkovec, Ph.D. | Senior VP Research, Cidara Therapeutics, Inc.
Peptide-Derived Natural Products in the Design of Novel Anti-Infectives

Dr. Balkovec is Senior VP of Research at Cidara Therapeutics, a clinical-stage biotechnology company located in San Diego, CA. The company is focused on the development of new anti-infectives with the potential to transform standard of care treatment and improve patient outcomes. With over 30 years in drug discovery and development, Dr. Balkovec has experience in many aspects of Medicinal Chemistry and has led multi-disciplinary teams in the discovery of novel medicines.

Prior to joining Cidara, Dr. Balkovec had a long career at Merck Research Laboratories, where he was Senior Scientific Director and Team Leader, overseeing programs in infectious diseases, diabetes, obesity, inflammation and thrombosis disease areas. During that time, he and his teams brought forward over a dozen development candidates. Dr. Balkovec is a co-inventor of the first approved echinocandin antifungal, caspofungin acetate (CANCIDAS™), an IV-dosed β-1,3-glucan synthase (GS) inhibitor. He led the effort that identified the first orally active GS inhibitor (SCY-078) that is currently in Phase 2 development. He is an author or inventor on >60 publications and >45 patents and has received several prestigious awards including the American Chemical Society's Heroes of Chemistry Award. He also serves on the SAB of a private biotech company focused on small molecule therapeutics.

Jim received his B.S. in Chemistry at the University of Pittsburgh and a Ph.D. from the University of Wisconsin, Madison under the direction of Professor Barry Trost. He completed his postdoctoral training at Columbia University in the laboratory of Professor Gilbert Stork.

David Craik, Ph.D. | Professor of Chemistry, Institute for Molecular Bioscience, The University of Queensland
Ultra-stable Cyclic Peptides from Plants and Animals as Tools in Drug Design

David Craik is a group leader and Professor of Chemistry at the Institute for Molecular Bioscience at The University of Queensland, Brisbane, Australia. He obtained his PhD in organic chemistry from La Trobe University in Melbourne, Australia and undertook postdoctoral studies at Florida State and Syracuse Universities before taking up a lectureship at the Victorian College of Pharmacy in 1983. He was appointed Professor of Medicinal Chemistry and Head of School in 1988. He moved to University of Queensland in 1995 to set up a new biomolecular NMR laboratory and is currently an Australian Research Council Laureate Fellow. His research focuses on applications of circular proteins, toxins and NMR in drug design. He is a Fellow of the Australian Academy of Science and has received numerous awards for his research, including the Ralph F. Hirschmann Award from the American Chemical Society. He is author of 620 papers and has trained 70 PhD students.

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.

Richard DiMarchi, Ph.D. | Chairman of the Board, Peptide Therapeutics Foundation; Distinguished Professor of Chemistry, Gill Chair in Biomolecular Science, 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.

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Anouk Dirksen, Ph.D. | Senior Principal Scientist, Pfizer Inc.
Peptide-CRM_{197} Conjugate Vaccines – Considerations for Process Development

Anouk Dirksen is a Senior Principal Scientist and Group Leader in the Conjugation & Polytide Process Development group in the Bioprocess R&D organization at Pfizer, which she joined in 2013. Her group focuses on process development, support of regulatory filings, and technology & innovation for Vaccines. In addition, she is the Technology Lead for the Bioprocess R&D organization as a whole.

Career summary — **2003** Ph.D. in Chemistry from the University of Amsterdam, the Netherlands; **2003-2006** Post-doctoral fellowships in the groups of Professor E. W. Meijer (Eindhoven University of Technology) and Professor Tilman Hackeng (Maastricht University) in the Netherlands on the development of imaging agents for cardiovascular disease; **2006-2010** Research Associate in the group of Professor Phil Dawson at the Scripps Research Institute, La Jolla, CA on bioconjugation method development using aniline as a catalyst for imine ligation; **2010** Start industry career at CovX, a research unit of Pfizer, in San Diego, CA focusing on peptide-, antibody-, and protein-based bioconjugate therapeutics.

Oreola Donini, Ph.D. | Senior Vice President and Chief Scientific Officer, Soligenix, Inc.
Peptidic Innate Defense Regulators in Infectious Disease, Cancer and Cancer Supportive Care

Oreola Donini has more than 15 years of experience in drug discovery and preclinical development with start-up biotechnology companies and has been instrumental in leading early stage development of several novel therapies into the clinic. Dr. Donini has worked previously with ESSA Pharma Inc., Inimex Pharmaceuticals Inc. and Kinetek Pharmaceuticals Inc., developing novel therapies for infectious disease, cancer and cancer supportive care. Dr. Donini is a co-inventor and leader of the SGX94 innate defense regulator technology, developed by Inimex Pharmaceuticals and subsequently acquired by Soligenix. She was responsible for overseeing the manufacturing and preclinical testing of SGX94, which demonstrated efficacy in combating bacterial infections and mitigating the effects of tissue damage due to trauma, infection, radiation and/or chemotherapy treatment. These preclinical studies culminated in a successful Phase 1 clinical study and clearance of Phase 2 protocols for oral mucositis in head and neck cancer and acute bacterial skin and skin structure infections. While with ESSA Pharma as the Vice President of Research and Development, Dr. Donini led the preclinical testing of a novel N-terminal domain inhibitor of the androgen receptor for the treatment of prostate cancer. Prior to joining Inimex, she worked with Kinetek Pharmaceuticals in the discovery of novel kinase and phosphatase inhibitors for the treatment of cancer. Dr. Donini received her PhD from Queen's University in Kinston, Ontario, Canada and completed her post-doctoral work at the University of California, San Francisco. Her research has spanned drug discovery, preclinical development, manufacturing and clinical development in infectious disease, cancer and cancer supportive care.

Pieter Dorrestein, Ph.D. | Professor; Director, Collaborative Mass Spectrometry Innovation Center; Co-Director, Institute for Metabolomics Medicine, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego
How to Get Mass Spectrometry from a Niche Tool to Use by the General Population

Dorrestein is Professor at the University of California - San Diego. He is the Director of the Collaborative Mass Spectrometry Innovation Center and a Co-Director, Institute for Metabolomics Medicine in the Skaggs School of Pharmacy & Pharmaceutical Sciences, and Departments of Pharmacology and Pediatrics. Since his arrival to UCSD in 2006, Prof. Dorrestein has been pioneering the development of mass spectrometry methods to study the chemical ecological crosstalk between populations of microorganisms, including host interactions for agricultural, diagnostic and therapeutic applications. He participated in panels for the white house science and technology office of president on the launch of a national microbiome initiative and has been on panels for the National Academy of Sciences on the Chemistry of the Microbiome. He has co-authored over 220 publications and his work has been featured by the wall street journal, CNN, NYTimes, Fox, BBC and hundreds of other news outlets. He has been recognized with several awards, among them are awards from the Beckman foundation, V-foundation in cancer research, EUREKA award for unconventional and enabling research, Hearst Foundation, Pharmaceutical Research and Manufacturing Association research award and the Abel award in pharmacology. For a more detailed biography see http://www.nature.com/news/the-man-who-can-map-the-chemicals-all-over-your-body-1.20035,

Ronald M. Evans, Ph.D. | Investigator, Howard Hughes Medical Institute; Professor, The Salk Institute for Biological Studies; March of Dimes Chair in Molecular and Developmental Biology, The Salk Institute for Biological Studies
Nuclear Receptors: Let's Make Metabolism Great Again

Professor Evans is Director of the Gene Expression Laboratory and Metabolic Engineering Program and Co-Director of the Helmsley Center for Genomic Medicine. He is known for pioneering studies on hormone signaling in physiology and in disease. His discovery of the Nuclear Receptor Superfamily provided a unified signaling mechanism for steroids, vitamin A, vitamin D, thyroid hormones and bile acids. These receptors use transcription to control sugar, salt, calcium, cholesterol and fat metabolism. They are primary targets in breast, prostate and pancreatic cancers, and leukemia treatment, and have therapeutic roles in chronic inflammation, osteoporosis and asthma. His work on muscle metabolism led to the discovery of 'exercise mimetics,' which promote the benefits of fitness without training. Exercise mimetics will help battle the obesity epidemic, diabetes, heart disease, hypertension and cancer. His work on Vitamin D uncovered a hidden mechanism to reprogram pancreatic cancer and increase its response to chemotherapy. He is a Howard Hughes Medical Institute Investigator and the recipient of multiple awards, including the Albert Lasker Award (2004) and the Wolf Prize (2012), and is a Member of the National Academy of Sciences and the Institute of Medicine.

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.

12th Annual Peptide Therapeutics Symposium

Lauren Goodrich, Ph.D. | Scientist, Technology Innovation, Roche Sequencing
Massively Parallel Synthesis and Screening of Linear Peptides and Macrocycles Using Peptide Microarrays

Lauren Goodrich is a scientist at Roche Madison. Dr. Goodrich works on the Innovation Technolgy team, where she focuses on drug discovery related aplications of the peptide microarray synthesis platform. She holds a Ph.D. in chemistry from the University of Michigan.

Dimitrios Morikis, Ph.D. | Professor of Bioengineering, University of California, Riverside
Design of Complement-based Therapeutics and Testing in Computational Disease Models

Dr. Dimitrios Morikis is Professor of Bioengineering and Director of the Biomolecular Modeling and Design Lab (BioMoDeL) at the University of California, Riverside (UCR). He is also faculty member at UCR's Biomedical Sciences Graduate Program, Institute for Integrative Genome Biology, Center for Molecular and Translational Medicine, and Interdisciplinary Center for Quantitative Modeling in Biology. Dr. Morikis' work is highly cross-disciplinary using methods from biophysics, structural biology, computational chemistry, biomolecular informatics, and bioengineering. His current work focuses on immune system function and regulation, design of peptides and proteins with tailored properties, knowledge-based drug design, virtual screening for fluorescent imaging markers for disease detection, and development of structural and translational bioinformatics methods and disease models. His research is hybrid, computational and experimental, with wet and dry lab components. His computational methods are electrostatic calculations, molecular and Brownian dynamics simulations, free energy analysis, pharmacophore-based virtual screening, and systems biology approaches. His experimental methods are NMR and vibrational spectroscopy, biophysical and biochemical assays, and functional immunological assays. Dr. Morikis is AAAS Fellow and AIMBE Fellow. He has received several awards, among them the Carolyn K. McGillvray Memorial Award for Macular Degeneration Research, UCR Chancellor's Award for Excellence in Undergraduate Research, OCEC Distinguished Engineering Educator Award, UCR Non-Senate Distinguished Researcher Award, NIH National Service Fellowship Award, and Fulbright Fellowship. Dr. Morikis is author in about 140 research papers, reviews and book chapters, and has co-edited the volume "Structural Biology of the Complement System". He is also an inventor in 10 patents or patent applications. Dr. Morikis is the Editor-in-Chief of *BMC Biophysics*.

Gerhard Niederfellner, Ph.D. | Senior Research Fellow, Ablynx
Nanobodies as a Differentiated Drug Modality

Gerhard Niederfellner did his Ph.D. work in the Molecular Biology Department of Dr. Axel Ullrich at the Max-Planck-Institute for Biochemistry, Martinsried, Germany. After having worked for several years as postdoctoral fellow with HHMI Morris White at the Joslin Diabetes Center in Boston, Dr. Niederfellner joined in 2000 Lilly Research Laboratories in Hamburg as Research Scientist advancing small molecule, peptide, and siRNA programs for insulin sensitization and functional beta cell preservation. In 2007, Dr. Niederfellner moved to the Discovery Oncology group of Roche in Penzberg, Germany, where he began working on biologics drug development. Between 2013 and 2016, he led, as head of the tumour biology department, a strategic transition from cancer cell signalling inhibition approaches to tumour targeted delivery of toxic payloads and cancer immuno-therapy approaches. Among other things, Dr. Niederfellner made substantial contributions to the development of the glycoengineered anti-CD20 antibody, GAZYVA, and advanced, in collaboration with Ira Pastan, a mesothelin-targeted, deimmunized PE-immunotoxin into the clinic. He also established a state-of-the art in-vivo imaging facility that allowed non-invasive monitoring of tumour growth, drug distribution, and immune cell infiltration. Currently as Senior Research Fellow at Ablynx nv, Dr. Niederfellner uses his deep biologics development expertise to initiate new projects with clear Nanobody® advantage and high potential of delivering differentiated drug products.

Victor Nizet, MD | Professor and Vice Chair for Basic Research; Chief of the Division of Host-Microbe Systems & Therapeutics, University of California, San Diego
Boosting Endogenous and Pharmaceutical Peptide Killing of Antibiotic-Resistant Superbugs

Victor Nizet is a Professor and Vice Chair for Basic Research and Chief of the Division of Host-Microbe Systems & Therapeutics at the University of California, San Diego (UCSD), School of Medicine as well as Professor at UCSD Skaggs School of Pharmacy & Pharmaceutical Sciences. Dr. Nizet is a graduate of Reed College, received his medical training at Stanford University School of Medicine, completed a Residency and Chief Residency in Pediatrics at Harvard University's Children's Hospital in Boston, Massachusetts, and a then a Fellowship in Pediatric Infectious Diseases at the University of Washington's Children's Hospital in Seattle. Dr. Nizet leads a large basic and translational research laboratory focused on discovering virulence factors of invasive bacterial pathogens, elucidating mechanisms of host innate immunity, and novel approaches to infectious disease therapy. He is also currently leading the initiative for the UCSD Collaborative to Halt Antibiotic-Resistant Microbes (CHARM) which will debut in 2017. Dr. Nizet has authored over 370 peer-reviewed publications, including several in the most prestigious general scientific journals. He has collaborated with several biotechnology interests in developing new antibiotic and immune-based therapies against drug-resistant pathogens. Dr. Nizet's work has been recognized by an American Heart Association Established Investigator Award, the American Lung Association Career Investigator Award, the American Asthma Foundation Senior Investigator Award, the E. Mead Johnson Award for Research in Pediatrics, and the 2016-17 UCSD Chancellor's Associates Award for Faculty Excellence in Research in Science and Engineering. Dr. Nizet has been elected to the American Society for Clinical Investigation, the Association of American Physicians, and the American Academy of Microbiology. Details of his research program can be found on the laboratory website: http://nizetlab.ucsd.edu

Simon Read, Ph.D. | Chief Scientific Officer, Ra Pharmaceuticals, Inc.
Developing a Pipeline of Macrocyclic Peptides for Disorders of Complement Regulation

Simon has been Chief Scientific Officer at Ra Pharma since April 2016. Prior to joining the company, he served as Vice President and Head of the Innovative Medicines Unit (IMU) at Grünenthal GmbH, a pharmaceutical company headquartered in Aachen, Germany from 2014 to 2016. Prior to that, Simon served as Vice President and Head of Global Biomedical Sciences at Grünenthal GmbH from 2011 to 2014. Before joining Grünenthal, he was the Director, Experimental Medicine and Biomarkers, Immunology with Roche Products/Genentech Inc. from 2008 to 2011. Simon has been a member of the Medical Research Council and Association of British Pharmaceutical Industry Steering Committee for the UK's foremost Immunology Consortium since 2012. He has been a member of the Boston Children's Hospital Technology Development Fund steering group since 2016. Simon received his B.Sc. in Physiology from University of Manchester, a M.Sc. from University of Southampton and a Ph.D. from University of Hertfordshire."

12th Annual Peptide Therapeutics Symposium

Cristina M. Rondinone, Ph.D. | Vice President R&D; Head Cardiovascular/Metabolic Diseases iMED, MedImmune

Dual Peptides: The Future of Diabetes Therapy

Dr. Cristina Rondinone received her Ph.D. in Biochemistry from University of Buenos Aires. Her postdoctoral training was at the Laboratory of Cellular and Developmental Biology, NIDDK, NIH, USA as a Visiting Fellow. In 1995, she moved to Sweden where she was first Senior Scientist for the Lundberg Laboratory for Diabetes Research, Department of Internal Medicine, University of Gothenburg and then she was appointed as (Docent) Associate Professor in Molecular Medicine, University of Gothenburg. From 1998 to 2005, she worked at Abbott Laboratories, where she became first Associate Research Fellow of the Volwiler Society and then Group Leader in the metabolic disease research area. In 2005 she moved to Hoffmann-La Roche as Research Director of Metabolic Diseases and in 2007 became Senior Director and Therapeutic Area Head overseeing drug discovery programs in metabolic and vascular diseases, including target identification, lead optimization and advancement of preclinical candidates into clinical development. In 2011 she moved to MedImmune, Inc., as Vice President Research and Development and Head Cardiovascular and Metabolic Diseases, leading the portfolio of biologics in this therapeutic area. She has published more than 75 academic publications in the field of diabetes, insulin resistance and obesity, and coinventor of 5 patents. She was also Editor of the book Therapeutic Aplications of RNAi. She has also has been invited speaker in numerous national and international symposiums as well as Chairman of numerous sessions at the Keystone Symposia, American Diabetes Association and European Diabetes Association She was member of the Editorial Board of the journal Endocrinology and Associate Editor of Archives of Physiology and Biochemistry as well as reviewer for the American Diabetes Association, NAASO, National Institutes of Health (NIH), National Science Foundation (US), Institut Curie, French Ministry of the Research and Education, Czech Science Foundation, Israel Science Foundation and the Australian Science Foundation.She is also member of the Scientific Advisory Board for the Keystone Symposia and she was inducted as a member of the Real National Academy of Pharmacy in Spain.

Ahmad Sadr, MS | Vice-President of Technical Operations, Apellis Pharmaceuticals

Pharmacokinetic, Preclinical, and Clinical Updates on APL-2

Ahmad joined Apellis as the VP of Technical Operations in 2017. Ahmad has an extensive record of accomplishments in developing diverse products from early to late stage commercial assets with such companies as Portola Pharmaceuticals, Vision Medicines, Genentech, and Wyeth. At Portola, Ahmad led critical aspects of outsourced Drug Substance manufacturing for Andexanet alpha. Mr. Sadr was the VP of Technical Operations at Vision Medicines, an ophthalmic biotech start-up with both small molecule and mAb drugs in development. At Genentech and Wyeth, Ahmad led teams in manufacturing science and technology, manufacturing collaborations, and supply chain with responsibility for global manufacturing of development and commercial stage biologics and pharmaceutical drugs. Ahmad holds a Management Certificate from Wharton, MS in Chemical Engineering from Widener University, and BS in Chemical Engineering from University of Maryland.

Claudio D. Schteingart, Ph.D. | Director, Peptide Therapeutics Foundation; Vice President, Science & Technology - Research, Ferring Research Institute Inc.
Biodistribution of an Oxytocin Analogue After Intranasal and Central Administration to Pigs – A PET Imaging Study

Dr. Schteingart is Vice President, Science & Technology – Research at Ferring Research Institute Inc. His current responsibilities are the evaluation of new technologies for the discovery and development of novel peptide therapeutics and to provide guidance to drug discovery programs at the Institute as well as supporting drug candidates in Development at Ferring Pharmaceuticals. He joined the Institute in 1996 as a research chemist and participated in the discovery of the peptidic GnRH antagonist degarelix, launched in 2009, and many other peptidic drug candidates in various stages of clinical development for urology, women's health, critical care medicine, and gastroenterology. Dr. Schteingart received a Ph.D. in Chemistry at the University of Buenos Aires, Argentina. After postdoctoral studies in the Department of Chemistry at the University of California, San Diego, he moved to the Department of Medicine where he carried out research in the chemistry, physiology, metabolism, and physicochemical properties of biliary components and lipids.

Kevan M. Shokat, Ph.D. | Professor, Cellular & Molecular Pharmacology, University of California, San Francisco; Department of Chemistry, University of California, Berkeley; Investigator, Howard Hughes Medical Institute
Chemical Tricks for Drugging the Undruggable

Professor Shokat is a pioneer in the development of chemical methods for investigating cellular signal transduction pathways—with a particular focus on protein kinases and lipid kinases. Dr. Shokat uses a combination of chemical synthesis and protein engineering to create uniquely traceable and regulatable kinases, allowing the function of over 100 different kinases to be uncovered across all disease areas including oncology, metabolism, and infectious disease.

Kevan is currently an Investigator of the Howard Hughes Medical Institute, Professor in the Department of Cellular and Molecular Pharmacology, where he also served as Department Chair from 2010-2014 at the University of California at San Francisco. He is also Professor in the Department of Chemistry at the University of California at Berkeley. After receiving his Ph.D. in Organic Chemistry at UC Berkeley with Professor Peter Schultz, and post-doctoral work in immunology at Stanford University with Professor Chris Goodnow, Kevan began his independent research career at Princeton University. He has received numerous awards including being named a Fellow of several prestigious research foundations including the Pew Foundation, Searle Foundation, Sloan Foundation, Glaxo-Wellcome Foundation, and the Cotrell Foundation. He has also received the Eli Lilly Award, given to the most promising biological chemist in the country under the age of 37. He was inducted into the National Academy of Sciences (2010), the Institute of Medicine (2011), and the American Academy of Arts and Sciences (2011).

Kevan has successfully commercialized discoveries from his laboratory. His development of chemical genetic tools for tracking and validating protein kinase drug targets is licensed by Artemis-Taconic for target validation of kinases in multiple disease areas. In 2007 he co-founded Intellikine, Inc. to commercialize a series of PI3K and mTOR small molecule inhibitors for cancer and inflammatory disease. In four years Intellikine has progressed three compounds into Phase I including INK1197 (PI3Kδ)-now partnered with Infinity Pharmaceuticals, INK128 (mTOR), and INK1117 (PI3Kα). In December 2011, Intellikine was acquired by Takeda Pharmaceuticals. In 2013 he co-founded Araxes Pharmaceuticals in La Jolla, CA. Also in 2013 he co-founded eFFECTOR Pharmaceuticals in San Diego, CA.

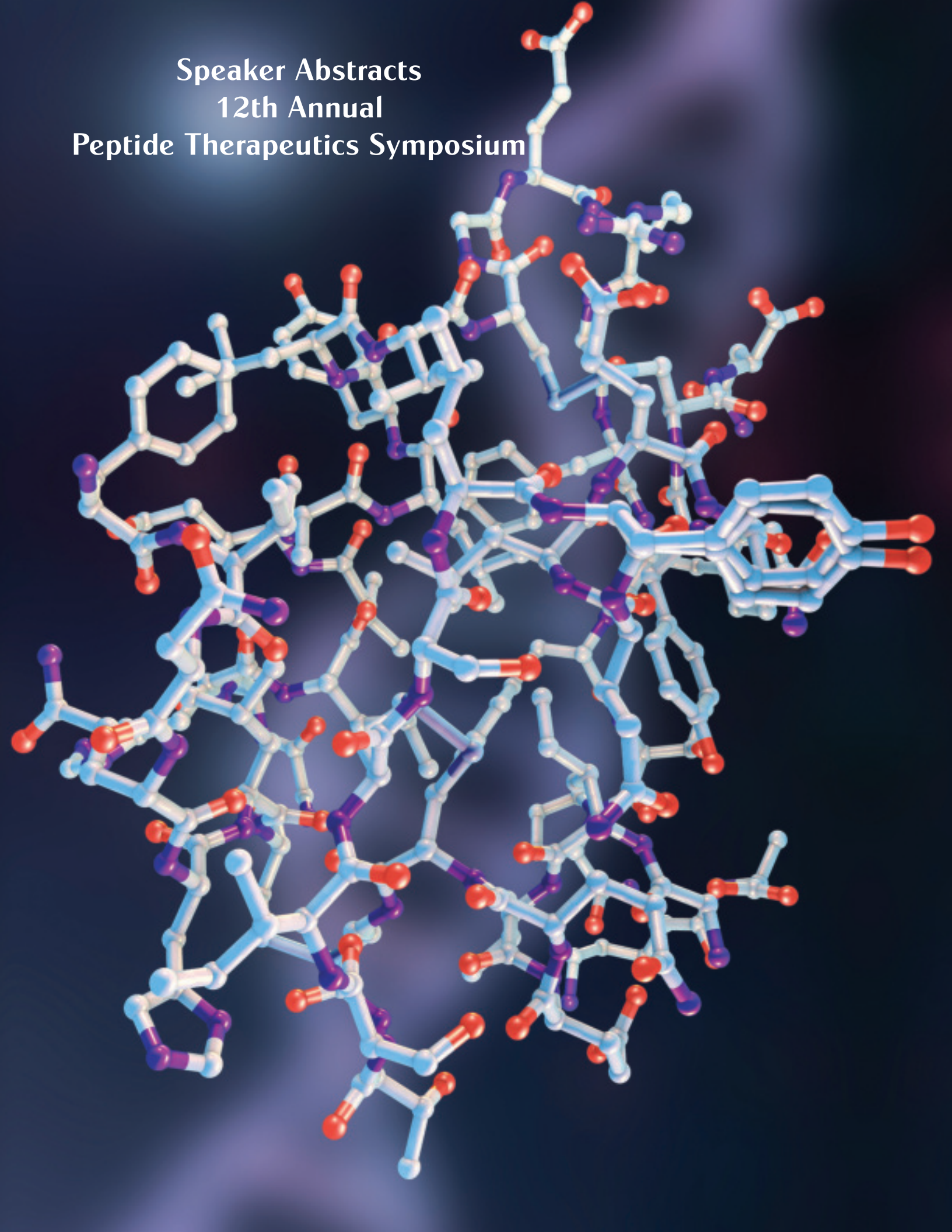
12th Annual Peptide Therapeutics Symposium

Jack W. Szostak, Ph.D. | Professor of Chemistry and Chemical Biology, Harvard University

Directed Evolution of Highly Modified Cyclic Peptides

Dr. Szostak is an Investigator of the Howard Hughes Medical Institute, Professor of Genetics at Harvard Medical School, Professor of Chemistry and Chemical Biology at Harvard University, and the Alex Rich Distinguished Investigator in the Dept. of Molecular Biology and the Center for Computational and Integrative Biology at Massachusetts General Hospital. Dr. Szostak's early research on telomere structure and function, and the role of telomere maintenance in preventing cellular senescence was recognized by the 2006 Albert Lasker Basic Medical Research Award and the 2009 Nobel Prize in Physiology or Medicine. In the 1990s Dr. Szostak and his colleagues developed in vitro selection as a tool for the isolation of functional RNA, DNA and protein molecules from large pools of random sequences. Dr. Szostak's current research interests are in the laboratory synthesis of self-replicating systems and the origin of life.

Speaker Abstracts
12th Annual
Peptide Therapeutics Symposium

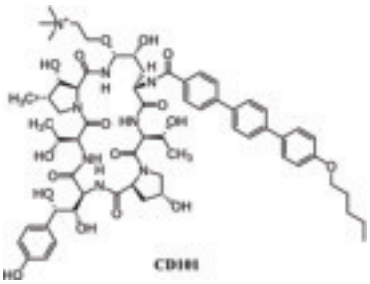


Peptide-Derived Natural Products in the Design of Novel Anti-Infectives

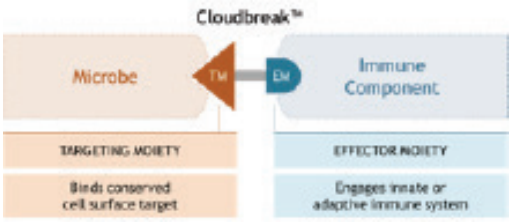
James M. Balkovec, Ph.D. | Senior Vice President Research
Cidara Therapeutics, Inc
6310 Nancy Ridge Dr., San Diego, CA 92121 | (858) 249-7383

Natural products that utilize amino acid building blocks exhibit broad structural diversity. While a variety of biological activities have been described for these compounds, antimicrobial activity is arguably their primary function. Cyclization, use of D-amino acids and post-translational modifications are "natural strategies" employed to gain function while resisting proteolysis, but additional chemical modifications can further improve potency and physical properties necessary for their utility as systemically administered therapeutics. Illustrated here are several examples of how modified peptides have been exploited to improve treatment options for infectious diseases.

The echinocandins (echinocandins, pneumocandins, FR901379) belong to a class of cyclic hexapeptides that possess potent antifungal activity through inhibition of β-1,3-glucan synthase, an enzyme that synthesizes an important fungal cell wall polysaccharide. Echinocandin B provides a natural offense for the producing organism (Aspergillus nidulans), however, the compound lacks clinical utility due to toxicity, unfavorable pharmacokinetics and limited potency and spectrum. Modification of the echinocandins provided drugs that are safe and effective once-daily treatments against serious fungal infections. We are developing a 2nd generation echinocandin CD101 with improved potency and safety coupled with a long half-life that allows once-weekly dosing. The drug displays a unique front-loaded plasma exposure profile that we believe will translate into enhanced efficacy against serious infections including those caused by resistant pathogens.



Cidara's Cloudbreak™ platform borrows concepts from cancer immunotherapy and applies them to the treatment of infectious diseases. Bispecfic small molecules employ a Targeting Moiety (TM) that binds a cell surface target on pathogens and is linked to an Effector Moiety (EM) that engages the innate immune system, physically connecting the immune system to the pathogen. By using a TM derived from a known antibiotic, Cloudbreak compounds exert traditional antibiotic activity and enjoy additional EM-driven potency using the host's immune system. We have successfully developed this platform in areas of high unmet clinical need. First generation antifungal compounds utilized an echinocandin or other antifungal as a TM linked to the potent chemoattractant formyl-Met-Leu-Phe. Using this approach, intrinsic activity was enhanced by the cidal activity of neutrophils. Second generation approaches utilize alternative EMs to bind naturally-occurring antibodies to further drive the immune-based portion of the efficacy. We have successfully applied this approach to identify novel antifungal and antibacterial compounds.



Ultra-stable Cyclic Peptides from Plants and Animals as Tools in Drug Design

David Craik, Ph.D. | Professor of Chemistry
Institute for Molecular Bioscience
The University of Queensland
Brisbane, QLD 4072, Australia | 61 413683359

Naturally occurring cyclic peptides and disulfide-rich peptides offer great potential as leads for drug design.[1-3] This talk will focus on a class of cyclic peptides known as cyclotides,[4] which are topologically unique proteins in that they have a head-to-tail cyclised peptide backbone and a cystine knotted arrangement of disulfide bonds. This makes them exceptionally stable to chemical, thermal or enzymatic treatments and, indeed, they are amongst nature's most stable proteins. They occur in plants from the Rubiaceae (coffee), Violaceae (violet), Solanaceae (potato), Fabaceae (Legume) and Cucurbitaceae (cucumber) families of plants. Their stability and compact structure makes them an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilize them. More than two dozen examples have now been published where biologically active epitopes have been grafted onto cyclic peptide frameworks to produce lead molecules with potential in the treatment of cancer, cardiovascular disease, infectious disease, autoimmune disease (multiple sclerosis) and pain. Recent computational approaches are now being used to generate de novo cyclic peptide structures with intrinsic stability that may further enhance the scope of cyclic peptides in drug design.[5]

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3. Craik D J, Fairlie D P, Liras S, Price D: *Chemical Biology & Drug Design* (2013) **81**, 136-147.
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Acknowledgments:
Work in our laboratory is supported by the Australian Research Council and the National Health & Medical Research Council.

Peptide-CRM_{197} Conjugate Vaccines – Considerations for Process Development

Anouk Dirksen, Ph.D | Senior Principal Scientist
Pfizer Inc.
700 Chesterfield Parkway West
Chesterfield, MO 63017 | (858) 414-2054

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

Peptidic Innate Defense Regulators in Infectious Disease, Cancer and Cancer Supportive Care

Oreola Donini, Ph.D. | Senior Vice President and Chief Scientific Officer
Soligenix, Inc.,
29 Emmons Drive, Suite C-10
Princeton, NJ 08540 | (604) 837-7802

Modulation of the innate immune response provides the opportunity to address a broad range of indications including infectious disease, acute inflammatory tissue injury and diseases of immune tolerance, such as cancer. In particular, immune modulation can be triggered and then sustained by the innate immune system itself, leading to compounds with a short half-life having an enduring pharmacodynamic effect. Innate Defense Regulators (IDRs) are a novel class of synthetic peptides that alter the downstream signalling and cellular recruitment after the innate immune system is activated. IDRs act downstream of the initiating messenger stimulation of the innate immune system (including binding to the Toll-like receptors and nucleotide oligomerization domain-like receptors) by binding to an intracellular adaptor protein, sequestosome-1, that is involved in the transmission of information during intracellular signal transduction, receptor trafficking, protein turnover and bacterial clearance. The lead IDR, dusquetide, has been demonstrated to be safe and efficacious in Phase 1 and 2 clinical studies. Specifically, dusquetide reduced the duration of severe oral mucositis in head and neck cancer patients receiving chemoradiation therapy, while also decreasing the incidence of bacterial infections in this patient population. Dusquetide treated groups also experienced a decreased mortality and an accelerated tumor “complete resolution”. These results demonstrate the utility of targeting the innate immune system, including: 1) indicating the translatability of innate immune studies in rodents to humans; and 2) illustrating that innate immune modulation can simultaneously increase anti-infective activity, while also modulating inflammation and potentially enhancing tumor control.

How to Get Mass Spectrometry from a Niche Tool to Use by the General Population

Pieter Dorrestein, Ph.D. | Professor; Director, Collaborative Mass Spectrometry Innovation Center;
Co-Director, Institute for Metabolomics Medicine, Skaggs School of Pharmacy and Pharmaceutical Sciences
University of California, San Diego
9500 Filman Drive #4224, La Jolla, CA 92093-0751 | (858) 534-6607

While significant advances have been made in proof-of-principle approaches in mass spectrometry, they are not yet used by the general population. Yet, and although still far away, the future potential exists that one day every person with a smart toilet, smart mirror and if size of instrumentation can be solved, a smart phone, will perform molecular analysis of any object/sample they want. This information will then be collected and we gain global insight into the molecular diversity that exists in the world and their distributions. When such a routine analysis becomes a reality, we will change what we eat, how we preserve food, how we prepare clothing and construction materials, how we exercise, and how we approach health. But what would a potential roadmap look like to achieve such amazing goal? How do we make the data that is collected more informative? How do we reuse such information to enhance our molecular understanding? How did Google, Amazon or Facebook achieve this for text searches? While it is clear that such capabilities do not yet exist for mass spectrometry, we will highlight the potential with experiments from our own laboratories. We will further demonstrate the approach to data reuse and new data analysis strategies, can be used to leverage discoveries with from one data set to another. This will be highlighted with specific example of unique microbial and plant peptides that are discovered in non-clinical settings such as fundamental biological studies but show up in human clinical samples. This will be a key requirement to make the information from mass spectrometry techniques usable for the larger community on a daily basis. This will eventually become as common as a Google search done today but instead of a text search, it will be a simple mass spectrometric scan of a sample.

Nuclear Receptors: Let's Make Metabolism Great Again

Ronald M. Evans, Ph.D. | Investigator, Howard Hughes Medical Institute; Professor, The Salk Institute for Biological Studies; March of Dimes Chair in Molecular and Developmental Biology
The Salk Institute for Biological Studies
110010 N. Torrey Pines Road, La Jolla, CA 92037 | 858-453-4100 x 1302

While FGF knockout mice seem normal, on High Fat Diet they develop an aggressive diabetic phenotype, with adipose progressively becoming fibrotic and unable to adapt to nutrient stress. FGF1 regulation in WAT is diet-induced and dependent on PPARγ activation.

The KO phenotype led us to wonder if synthetic FGF1 could restore insulin sensitization in non-mutant diabetic mice. Surprisingly, 'endocrinization' of FGF1 by simple injection rapidly lowers glucose and restores insulin sensitivity in dio, ob/ob and db/db diabetic mice. Oddly, central injection of FGF-1 also led to sustained glucose lowering in in some but not all models of murine T2 diabetes. Thus, 'endocrinized' FGF-1 is a potent metabolic regulator, achieving glucose lowering and insulin sensitization even without weight loss.

Importantly, targeted mutations in the FGF1 primary sequence show that glucose lowering and mitogenicity are completely separable effects.

But how does its rapid and sustained effects occur? We will discuss recent development on the signaling mechanisms underlying FGF-1 metabolic reprogramming.

Sihao Liu[2], Gencer Sancar[3], Sungsoon Fang[4], Jae M Suh[5], Michael Downes[2], Ronald M Evans[1]

[1]Salk Institute/HHMI, Gene Expression Laboratory, La Jolla, CA; [2]Salk Institute, Gene Expression Laboratory, La Jolla, CA; [3]Salk Institute, Gene Expression Laboratory, La Jolla, CA; [4]Sejong University, Department of Bioscience and Biotechnology, Seoul, South Korea, 5KAIST, Daejon, South Korea

Massively Parallel Synthesis and Screening of Linear Peptides and Macrocycles Using Peptide Microarrays

Lauren Goodrich, Ph.D. | Scientist, Technology Innovation
Roche Sequencing
500 S. Rosa Rd., Madison, WI 53719 | (608) 316-8010

We have developed a peptide synthesis and screening platform for the discovery of peptidic molecules that bind to protein targets of interest. This technique employs the use of digital micromirror devices to direct the maskless synthesis of millions of unique peptides in parallel on a microarray surface. Using a chemical catalog of over 300 amino acid building blocks (natural and non-natural), we can synthesize 18 million unique peptides in a 24-48 hour run. The method can be applied to synthesize both linear and cyclic peptidic molecules, enabling screens in a diverse chemical space. Here, we will describe the core technology and its application to rapidly and systematically evolve high-affinity, high-specificity binding peptides to protein targets in a reproducible and digitally controlled process.

Design of Complement-based Therapeutics and Testing in Computational Disease Models

Dimitrios Morikis, Ph.D. | Professor of Bioengineering
University of California, Riverside
Department of Bioengineering
900 University Ave.
Riverside, CA 92521 | (951) 827-2696

We will present our design of peptidic inhibitors of the complement system that have the potential to become therapeutics for complement-mediated diseases, such as age-related macular degeneration, atypical hemolytic uremic syndrome, paroxysmal nocturnal hemoglobinuria, and others. Our main target of inhibition is complement component C3. We will discuss the design progression of our newest compstatin family peptides, and how we overcame aggregation issues of previous compstatin analogs by balancing hydrophobicity and polarity in the peptide sequences. The new design is based on results from molecular dynamics simulations and past knowledge from structure-activity relations and computational optimization studies. The potencies of the new compstatin peptides are experimentally tested using C3b and C5b-9 ELISAs and hemolytic assays, and their efficacies are tested in human retinal pigmented epithelium cell-based assays that mimic the pathogenesis of age-related macular degeneration. The aggregation propensities of the new compstatin peptides are tested by performing solubility studies. Our lead peptide has higher efficacy than previous analogs, and nearly complete solubility in the tested concentration range, while it maintains the same potency as our previous most potent analog. In addition, we will present a computational model that describes the complement system activation pathways in homeostasis and in disease states, the latter generated by perturbations that are derived from clinical observations. The computational model is based on a system of differential equations, generated from the biochemical reactions that describe the activation, propagation, regulation, and termination of the complement system activation pathways, known complement component concentrations, and kinetic parameters from experimental data. The model not only describes the time dependence of all complement proteins, fragments, and complexes, but it can also be used to identify suitable biomarkers for disease-specific detection of complement activation. We will demonstrate the utility of the computational model by testing the inhibitory effects of our lead compstatin peptide and the monoclonal antibody equlizumab used in the clinic. We will compare the advantages and disadvantages of targeting C3 inhibition (compstatin) and C5 inhibition (equlizumab) on a variety of biomarkers of complement activation.

Nanobodies as a Differentiated Drug Modality

Gerhard Niederfellner, Ph.D. | Senior Research Fellow
Ablynx nv
Technologiepark 21
Zwijnaarde NA 9052, Belgium | |+32 (470) 219379

This presentation will provide an outline of the Nanobody® platform, and how inherent advantages of this platform can be used in the different contexts of disease biology to create differentiated drugs. Examples of multi-specific Nanobody® drugs that are in preclinical development and in the clinic will be used to illustrate the flexibility and versatility of Nanobodies as a drug modality.

Boosting Endogenous and Pharmaceutical Peptide Killing of Antibiotic-Resistant Superbugs

Victor Nizet, MD | Professor and Vice Chair for Basic Research; Chief of the Division of Host-Microbe Systems and Therapeutics
University of California, San Diego
9500 Gilman Drive, Mail Code 0760
La Jolla, CA 92092-0760 | (858) 534-7408

Conventional screening paradigms in antibiotic discovery are based on MIC/MBC testing in conventional bacteriologic media, and similar tests on patient isolates are used to guide physician management. Economic factors have favored development of broad spectrum agents, which exert "collateral damage" on the normal microflora, now increasingly recognized to have adverse health consequences. A single-minded focus on direct antimicrobial activities overlooks the fact that significant infections are really a disease of the host-pathogen interaction. Indeed, before the patient has even seen a doctor, their infection is already being treated by multiple antimicrobials — namely the cellular and molecular components of the innate immune system. We see value in exploring potential novel therapeutic approaches for drug-resistant bacteria that aim to tip the host-pathogen interaction back in favor of the host. This talk will illustrate three such classes of novel therapeutics: (A) Inhibitors of bacterial virulence factors that re-sensitize the pathogen to innate immune killing by endogenous antimicrobial peptides; (B) Drugs that directly boost the deployment of antimicrobial peptides by host phagocytic cells; (C) antibiotics that synergies with endogenous and pharmaceutical antimicrobial peptides to effect bacterial killing. These studies will reveal how standard MIC testing can be misleading, and overlook potent antibiotic activities that are recognized only the context of the normal innate immune system. In this new discovery and treatment framework, drugs used in medicine for other indications, or antibiotics otherwise deemed ineffective, can be "repositioned" for treatment of multi-drug resistant pathogens.

Developing a Pipeline of Macrocyclic Peptides for Disorders of Complement Regulation

Simon Read, Ph.D. | Chief Scientific Officer, Ra Pharmaceuticals, Inc.
87 Cambridge Park Drive
Cambridge, MA 02140 | (617) 209-5163

Constrained, cyclic peptides that diminish entropic penalties in protein/ligand interactions and avoid endopeptidase proteolysis, offer an attractive alternative to monoclonal antibodies. Ra Pharma is applying its mRNA display platform to create extremely large and diverse libraries of cyclic peptides comprised of natural and unnatural amino acids, with a goal of building drug-likeness into molecules at the hit identification stage. Our focus is on components of the complement cascade that may be suitable for treatment of a variety of rare and common indication, including Paroxysmal Nocturnal Hemoglobinuria (PNH), Myasthenia Gravis (MG), and dry age-related Macular Degeneration (AMD). RA101495 is a highly potent, systemically bioavailable, macrocyclic peptide inhibitor of Complement C5 that has advanced into phase 2 clinical studies for PNH and MG. Administered rapidly by subcutaneous self-injection, RA101495 may offer an attractive alternative to lifelong IV infusion of monoclonal antibodies. The discovery and development of RA101495 will be discussed, along with our efforts to develop macrocyclic peptide inhibitors of complement factor D for the treatment of dry AMD and other disorders.

Dual Peptides: The Future of Diabetes Therapy

Cristina M. Rondinone, Ph.D. | Vice President R&D; Head Cardiovascular/Metabolic Diseases iMED
One Medimmune Way
Gaithersburg, MD 20878 | (301) 366-2565

Despite a wide range of therapies for patients with type 2 diabetes and obesity, only intensive dietary and lifestyle interventions or bariatric surgery provide substantial weight loss to reduce insulin resistance and improve glycemic control. Improvements in glucose metabolism after surgery precede weight loss and are associated with alterations in circulating levels of gut hormones, including oxyntomodulin and glucagon-like peptide-1 (GLP-1). A single dual peptide with GLP-1 and glucagon (GCG) receptor agonist activity, was designed to mimic the effects of oxyntomodulin with superior metabolic effects. Results from preclinical studies and the first-time-in-humans (FTIH) study will be presented.

Pharmacokinetic, Preclinical, and Clinical Updates on APL-2

Ahmad Sadr, MS | Vice-President of Technical Operations
Apellis Pharmaceuticals
41 Grant Ave., Suite 300
San Francisco, CA 94108 | (650) 303-4543

Complement immunotherapy has the potential to provide disease-modifying benefits to patients suffering from various chronic inflammatory conditions. Along this novel therapeutic approach, Apellis Pharmaceuticals is developing APL-2, a potent inhibitor of complement component C3. This compound has is undergoing phase 1 and 2 clinical testing for age-related macular degeneration and paroxysmal nocturnal hemoglobinuria. Updated preclinical and clinical data will be presented and discussed.

Biodistribution of an Oxytocin Analogue After Intranasal and Central Administration to Pigs – A PET Imaging Study

Claudio D. Schteingart, Ph.D. | Director, Peptide Therapeutics Foundation; Vice President, Science & Technology - Research
Ferring Research Institute Inc.
4245 Sorrento Valley Boulevard
San Diego, CA 92121 | (858) 657-1400

A large body of literature reports central effects of intranasal oxytocin in humans, influencing trust, bonding, and a variety of other brain functions, for example in schizophrenia and autism,but the validity of these studies has been recently questioned. Intranasal oxytocin has been proposed to travel along perineural spaces surrounding olfactory nerve bundles leading from the nasal olfactory epithelium to the olfactory bulb, bypassing the blood-brain barrier. To investigate this largely untested hypothesis, we labeled the peptidic oxytocin analogue carbetocin with ^{11}C and used positron emission tomography (PET) to image its biodistribution in anaesthetized domestic pigs. The brain anatomy of the pig resembles that of humans, but it has a larger proportion of olfactory epithelium, the proposed site of oxytocin uptake. Intravenous administration of [^{11}C]carbetocin showed no blood to brain passage. Direct intranasal application of [^{11}C]carbetocin to the olfactory epithelium via a catheter clearly labeled the ethmoid turbinates of the animals, but no uptake of the radiolabel in the olfactory bulb or any part of the brain was observed up to 2 h post dosing. Introduction of [^{11}C]carbetocin in the cisterna magna followed by infusion of artificial CSF showed rapid passage of the radiolabel from the subarachnoid space to the ethmoid turbinates, but without spilling into the nasal cavity. Bulk fluid flow along the perineural spaces surrounding olfactory nerve bundles, if it occurs under normal physiological conditions, may take place only in the direction from brain to the space behind the olfactory epithelium as described in other species. Our results question the likelihood that meaningful quantities of oxytocin and related peptides can reach the brain after intranasal administration in humans. We suggest that future studies of CNS effects by the intranasal route of administration should consider the possibility of alternative sites of action outside the brain.

Chemical Tricks for Drugging the Undruggable

Kevan M. Shokat, Ph.D. | Professor, Cellular & Molecular Pharmacology; Professor, Department of Chemistry, University of California, Berkeley; Investigator, Howard Hughes Medical Institute
University of California, San Francisco
600 16th Street Genentech Hall, Room N514
San Francisco, CA 94158 | (415) 514-0575

Somatic mutations in the small GTPase K-Ras are the most common activating lesions found in human cancer, and are generally associated with poor response to standard therapies. Efforts to directly target this oncogene have faced difficulties due to its picomolar affinity for GTP/GDP and the absence of known allosteric regulatory sites. Oncogenic mutations result in functional activation of Ras family proteins by impairing GTP hydrolysis. With diminished regulation by GTPase activity, the nucleotide state of Ras becomes more dependent upon relative nucleotide affinity and concentration. This gives GTP an advantage over GDP and increases the proportion of active GTP-bound Ras. I will discuss the development of small molecules that irreversibly bind to a common oncogenic mutant, K-RasG12C. These compounds rely on the mutant cysteine for binding and therefore do not affect the wild type protein (WT). Crystallographic studies reveal the formation of a new pocket that is not apparent in previous structures of Ras, beneath the effector binding switch-II region. These data provide structure based validation of a novel allosteric regulatory site on Ras that is targetable in a mutant-specific manner.

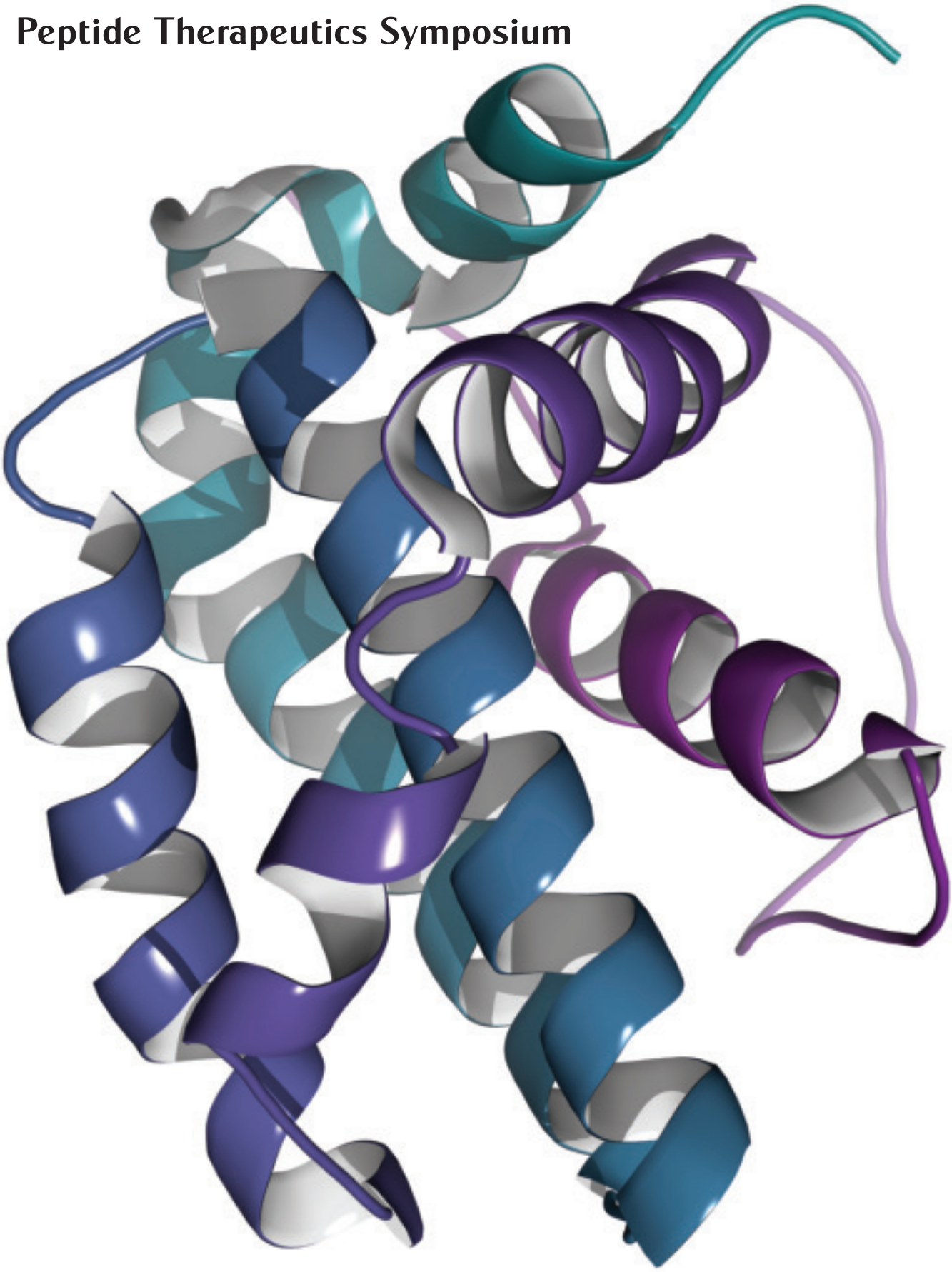
Directed Evolution of Highly Modified Cyclic Peptides

Jack W. Szostak, Ph.D. | Professor of Chemistry and Chemical Biology
Harvard University
185 Cambridge Street
Boston, MA 02114 | (617) 643-7581

I will review recent advances in the use of mRNA-display to select for highly modified cyclic peptides. Continued advances in the preparation of mRNA-displayed libraries have made the process faster and more efficient, and have enabled the incorporation of a growing list of unnatural amino acids into the translated peptide. One such selected peptide, from Ra Pharmaceuticals, is a complement factor C5 inhibitor, and has entered phase II clinical trials as a potential treatment for PNH.

Notes

Poster Abstracts
12th Annual
Peptide Therapeutics Symposium



P01 Cellular Manipulation through Cell Penetrating Peptide-Loaded Exosome Transfer

Jason K. Allen and Jean-Philippe Pellois
Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

The ability to introduce material inside of cells is critical for many areas of research and therapy. Among the many techniques developed to achieve this, exosomes have become a popular alternative due to their biological compatibility and stability. Typically the exosomes must be loaded with the desired cargo, however this process has limitations. Loading of exosomes through intracellular mechanisms is limited to endogenous protein expression and/or transfection, while external loading requires exosome purification, and is often inefficient and damaging to the exosomal integrity. To circumvent these issues a donor-to-recipient cell configuration can be utilized. Specifically, a donor cell line can be loaded with the desired cargo wherein the exocytosed vesicles (exosomes) derived from those cells are also loaded. Loading of donor cells can be accomplished using the highly efficient cell penetrating peptide dfTAT. dfTAT is ideal for the tracking of exosomes through the donor-to-recipient transfer due to its fluorescence, however in vivo applications would require a non-fluorescent variant. With in vivoapplications in mind, additional structural characterization has been done on the dfTAT prototype. This has resulted in a series of dfTAT variants, both fluorescent and non-fluorescent, spanning a range of efficiencies and toxicities. Altogether this platform enables flexibility for exosome loading and a streamlined protocol for studying the processes associated with exosome biogenesis and recipient cell uptake.

P02 A Novel One-Pot Synthesis Strategy for Bicyclic Peptide Assembly

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

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

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

This novel strategy is applicable to facilitate the synthesis of a broad range of bicyclic peptides when the preparation of a disulfide bridge and triazole bridge within a single sequence is required.

P03 ORBIT Peptide Display—High throughput Selection of Peptides with Biological Function

Alex Batchelor, Graham Ogg
Orbit Discovery Ltd, Brookes Innovation Hub, Gipsy Lane, Oxford, OX3 0BP

Peptides are highly active biological molecules i*n vivo* and as their stability and bioavailability is improved they offer greater potential as therapeutic drugs. Peptide library screening incorporating enhancements and non-linear structures is increasingly important to identify molecules with activity on a diverse range of therapeutic targets.

Existing screening approaches include phage display, yeast display, ribosomal and mRNA displays. The *in vitro* display methods struggle to express peptides at the numbers required for detection of low affinity interactions while the in vivo methods cannot incorporate non-natural amino acids or avoid interference from the autologous proteins of the host.

The ORBIT display technology multiplexes a library of translated peptides and their encoding DNA on the surface of beads. It has the positive attributes of in vitro display combined with the sensitivity of *in vivo* methods. It can be adapted for cell surface screening, enabling measurement of cell surface binding and functional response. We will show data from three target screens in three therapeutic areas: GP120 binding protein (infectious disease), mutant RAS (oncology) and T-cell epitopes (inflammation). Peptides that inhibit virus infection, bind mutants and have specific sequence to T cell receptor were identified.

The tolerance of beads to chemical and physical treatment expands the application of ORBIT to cyclic peptides and the incorporation of non-natural amino acids. The bead display approach has many potential applications for probing biological systems and for drug lead development.

P04 Efficient Cell Aelivery and Lipid-specific Endosomal Escape by Aupercharged Peptides

Dakota Brock, Lauren Kustigian, Menqgiu Jian, Junjie Zhang, Hays Rye, Jean-Philippe Pellois
Texas A&M University

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. The derivatives with two and three TAT branches, 2TAT and 3TAT, induce the leakage of lipid bilayers specifically containing BMP. Furthermore, the compounds lead to liposomal flocculation, fusion and an increase in lamellarity. In contrast, the monomeric counterpart, 1TAT, causes none of these effects. Overall, these results indicate that an increase in peptide density in these branched structures leads to the emergence of membrane-disrupting and cell

P05 Effect of a Fusion Peptide by Covalent Conjugation of a Mitochondrial Cell-penetrating Peptide and a Glutathione Analog Peptide

Carmine Pasquale Cerrato*[1], and Ülo Langel[1,2]
[1]Department of Neurochemistry, the Arrhenius Laboratories for Natural Sciences, Stockholm University, Svante Arrhenius väg 16B, SE-10691 Stockholm, Sweden; [2]Laboratory of Molecular Biotechnology, Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
*Correspondence: carmine@neurochem.su.se

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

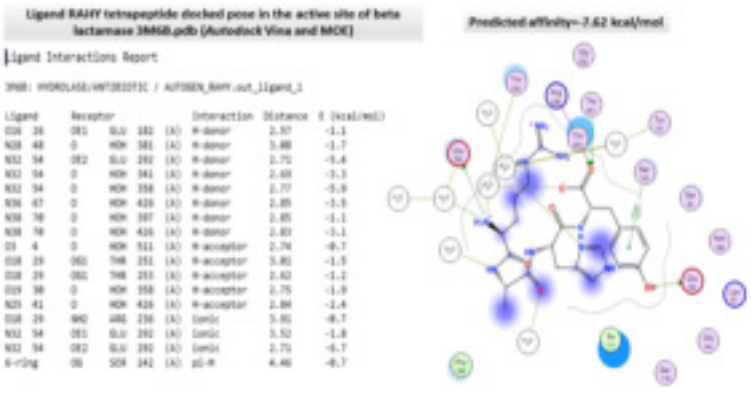
Here, a follow-up study is described, investigating the effects of the most promising members from both libraries on reactive oxidative species scavenging ability. None of the peptides influenced cell viability at the concentrations used. Fluorescence microscopy studies showed that the fluorescein-mtCPP1-UPF25 (mtgCPP) internalized into cells, and spectrofluorometric analysis determined the presence and extent of peptide into different cell compartments. mtgCPP has superior antioxidative activity compared with mtCPP1 and UPF25 against H_2O_2 insult, preventing ROS formation by 2- and 3-fold, respectively. Moreover, we neither observed effects on mitochondrial membrane potential nor production of ATP.

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

Cerrato CP., Langel Ü. Effect of a Fusion Peptide by Covalent Conjugation of a Mitochondrial Cell-Penetrating Peptide and a Glutathione Analog Peptide. Molecular Therapy – Methods & Clinical Development. 2017, vol5:221-231.

P06 MOE Coupled with AutoDock Vina Molecular Docking and Virtual Screening Empowered Discovery of Tetrapeptides Inhibitors of Y-49 β-Lactamase

Cristina Clement[2,3], Janet Gonzalez[1], Manfred Philipp[2]
[1]Natural Sciences, LaGuardia Community College, Long Island City, New York, United States; [2]Chemistry Department, Lehman College, Bronx, New York; [3]Pathology Chemistry, Albert Einstein Coll Med CUNY, Bronx, New York, 10461, United States;

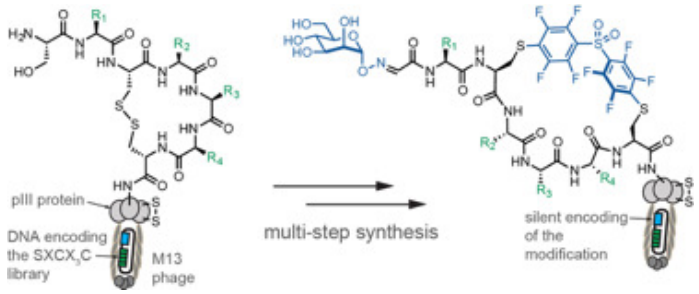


One of the most effective resistance mechanisms to β-lactam antibiotics involves the production of β-lactamases that hydrolyze β-lactam antibiotics. The most effective approach developed to overcome resistance to these drugs involves the discovery of new *non β-lactam scaffolds*, that are inhibitors of β-lactamases. Herein we report a structure-based drug design (SBDD) approach for the discovery of potential tetrapeptides inhibitors of Y-49 enzyme—a class A beta-lactamase—from *Mycobacterium tuberculosis*. The tetrapeptide scaffold was derived from the original sequence RRGHYY that was found to inhibit class A *Bacillus anthracis* Bla1 (Ki = 42 μM), and class A TEM-1 β-lactamase, (Ki = 136 μM) (Protein Eng Des Sel 16:853-860). *In silico* docking experiments were performed with Autodock Vina (from Scripps Institute) coupled with MOE (from Chemical Computing Group, Canada), and StarDrop-ADMET module (from Optibrium, UK). The beta-lactamase 3M6B.pdb was used as a target protein, while the tetrapeptides 2HN-R-X-H-Y-CONH2 were tested as potential competitive inhibitors of beta-lactamase. X was varied with all 20 natural L- aminoacids. New lead tetrapeptides were discovered, including RLHY, which has Ki of 0.81 uM. The tetrapeptides dRGHY and dRVHY (where "d" defines the D-isomer) have Ki of 0.76 uM (i.e., 760 nM) and 0.77 uM (i.e. 770 nM), respectively. In all cases the replacement of L-isomer of Arg at the N-terminus with the D-isomer (dR) resulted in at least two-fold enhanced inhibitory activity. The SBDD and SAR presented herein will enable further discovery of novel pharmacophores of linear and cyclic tetrapeptides with D- and unnatural amino acids, with improved selectivity and anti-Y 49 β lactamase activity.

P07 Genetically Encoded Discovery of Chemically-Modified Peptides

Ratmir Derda
Department of Chemistry, Alberta Glycomics Centre, University of Alberta, Edmonton, T6G2G2, Canada
ratmir@ualberta.ca

Genetically-encoded (GE) libraries of proteins and peptides are one of the major sources of discovery of biological drugs and development of ligands. These techniques, however, are often limited to handling of structures made of 20 natural amino acids. Our group uses GE-libraries of peptides as a starting material for multi-step organic synthesis to produce GE-libraries of peptide derivatives with specific emphasis on production of chemically-glycosylated peptide and macrocyclic peptide libraries. Examples are N-terminal conjugation[1] and cyclization of linear peptides[2] with simultaneous introduction of glycan entities. These chemical modifications allowed us to develop Genetically-Encoded Fragment-Based Discovery (GE-FBD) platform,[3] which combines >108 peptide fragments with variable, silently-encoded modifications.[4] I will describe progress and challenges in application of GE-FBS platform to several carbohydrate binding proteins, such as ConA,3 Galectins, DC-SIGN and anti-LAM antibodies. I will also share our vision and technologies we develop to maximize the reproducibility of discovery within a genetically-encoded library framework.



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P08 Scaffold-based Citrullinated Peptides for Inhibition of Autoantibodies in Rheumatoid Arthritis

Camilla Eriksson[1], Sunithi Gunasekera[1], Cátia Fernandes-Cerqueira[2], Per-Johan Jakobsson[2] and Ulf Göransson[1]
[1]Uppsala University, Department of Medicinal Chemistry, Division of Pharmacognosy, Biomedical Centre, Uppsala University, Husargatan 3, 751 23, Uppsala, Sweden; [2]Karolinska University Hospital, Department of Medicine, Rheumatology Unit, Rheumatology Clinic, 171 76, Stockholm, Sweden.

INTRODUCTION

Rheumatoid arthritis (RA) is a progressive autoimmune disease primarily affecting the synovial joints. A new class of rheumatoid arthritis-specific autoantibodies has recently been discovered that targets citrullinated proteins: the Anti-Citrullinated Protein Antibodies (ACPAs)[1]. Besides being widely used as a diagnostic tool, several lines of evidence support ACPAs importance for the disease pathogenicity[2]. We hypothesize that inhibition of ACPAs may serve as a strategy for development of novel RA-therapies[3]. Using known target sequences and stable cyclic peptide scaffolds with epitope-stabilizing properties[4,5], we developed a set of citrullinated peptide analogues with i*n vitro* ACPA-neutralizing activity.

METHODS

A set of linear and scaffold-based citrullinated peptides was synthesized using Fmoc-SPPS. Scaffold peptides were designed by inserting the sequence of the citrullinated target epitope into the sequence of the scaffold-peptide Sunflower Trypsin Inhibitor 1 and were synthesized, cyclized and folded. To determine degree of autoantibody inhibition, a competitive CCP2-ELISA kit was used. ACPAs isolated from patient sera were incubated with our peptides at different concentrations prior to addition to the plate. The remaining ACPA reactivity to surrogate peptide targets on the plate were used for calculating ACPA inhibition by our peptides. Peptides containing arginine instead of citrulline where used as controls.

RESULTS

Our citrullinated peptides showed ACPA inhibitory activity when compared to arginine- control peptides. In addition, improved inhibition was observed for the scaffold peptide analogues in comparison to corresponding linear peptides.

CONCLUSIONS

These results further support the potential of scaffold-based citrullinated peptides as inhibitors of anti-citrullinated protein autoantibodies in rheumatoid arthritis.

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P09 Towards Mild and Selective Incorporation of Freidinger Lactams into Expressed Peptides

Dillon Flood and Philip E. Dawson
The Scripps Research Institute

In solution, peptides exist in an equilibrium of conformational states.[1] Backbone conformational constraints have long been utilized to control peptide topology.[1] Amino-γ- lactam bridged dipeptides, commonly known as Freidinger Lactams, have been shown to stabilize Type II' β–turns, a common motif in peptide secondary structure.[1,2] While amino-γ- lactams have been incorporated into peptides before, they must either be synthesized as bridged dipeptide units for use in chemical peptide synthesis or cyclized on resin through various methods requiring harsh conditions and long reaction times.[1,2,3] The utility of these links as peptide constraints has inspired new approaches to their incorporation into complex peptides and peptoids.[4,5] Here, we employ a mild and selective alkylation of Selenomethionine in aqueous solution at acidic, followed by lactamization of the alkylated peptide while adsorbed to Reverse Phase C_{18} Silica resin in DMSO.[7] The utilization of Selenomethionine, which is readily expressed by auxotrophic systems, and mild conditions could enable selective access to Freidinger lactams in expressed peptide and protein systems.

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P10 GUB06-046, a Novel Secretin/GLP-1 Co-Agonist, Decreases Food Intake, Improves Glycemic Control and Preserves Beta Cell Mass in db/db Mice

Keld Fosgerau[a], Søren B. van Witteloostuijn[a,b], Louise S. Dalbøge[a], Gitte Hansen[a], Knud J. Jensen[b], Niels Vrang[a], Jacob Jelsing[a] and Søren L. Pedersen[a]
[a]Gubra ApS, Agern Allé 1, 2970 Hørsholm, Denmark; [b]Department of Chemistry, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Bariatric surgery is currently the most effective treatment option for obesity, which has spurred an interest in developing a pharmaceutical mimetic (a 'medical bypass'). In a rat model of Roux-en-Y gastric bypass (RYGB) we identified the gut hormone secretin (Sct) to be markedly upregulated in the alimentary limb, indicating a plausible role of secretin in the beneficial effects of RYGB. Consequently, a library of novel Sct-based peptide co-agonists was developed and a lead compound (named GUB06-046) exhibiting potent agonism of both the Sct receptor and the GLP-1 receptor was identified. Acute single-dose administration of GUB06-046 (0.3 mg/kg, SC) to lean mice decreased food intake by 25% ($p<0.001$ vs. vehicle) and improved glucose tolerance by 38% assessed as total AUC during an oral glucose tolerance test ($p<0.001$ vs. vehicle). Chronic administration of GUB06-046 (0.3 mg/kg, SC, BID) to *db/db* mice for eight weeks decreased cumulative food intake by 20% ($p<0.001$ vs. vehicle) and improved glycemic control as indicated by a decrease in fasting blood glucose levels (28.6 mmol/L vs. 15.6 mmol/L, $p<0.001$ vs. vehicle) and a 1.6% reduction of HbA1c levels (7.2% vs. 5.6%, $p<0.001$ vs. vehicle). Subsequent stereological analysis revealed a marked increase in beta cell mass (81%, $p<0.01$ vs. vehicle), with no impact on exocrine pancreas mass or pancreatic duct epithelial mass. The data suggest that Sct/GLP-1 co-agonism has beneficial effects on appetite regulation, glucose homeostasis and beta cell mass, without exhibiting proliferative effects on the exocrine pancreas and the pancreatic duct epithelium.

P11 Machine Learning Techniques for Peptide Optimization from Sequence Information

Mark R. Hansen, Evgenia Razumovskaya, Jason Hodges, Hugo O Villar
[1]Altoris, Inc. San Diego, CA; [2]UC San Diego

Traditionally peptides have been optimized in a series of sequential steps where activity is maximized, followed by selectivity and subsequently other pharmacokinetic and toxicological properties are adjusted. Gains in efficiency could be realized if multiple properties are optimized simultaneously via the use of machine learning techniques. Unfortunately, the most appropriate methods for each property can be different and require some expertise in their use. We have created a computer system that automatically examines the utility of different machine learning techniques for a dataset and selects the most predictive methods for different properties of clinical interest for peptides. The combination of these best in class algorithms provides an avenue to solve the multifactorial problem of peptide optimization. A wide range of machine learning techniques are needed to make accurate prediction for the full range of preclinical properties of interest. To that end, we analyzed large datasets that revealed that while properties such as half-life or affinity towards a target can be modeled using Bayesian regression techniques, in other cases techniques such as support vector machines are needed to predict amyloid aggregation, when using positional physicochemical descriptors. The models can be used to predict peptides most likely to have the desired activity from within the combinatorial expansion of amino acids, natural, unnatural including modified peptides. Our ultimate goal is to develop a decision support system that guides the optimization of peptides towards the definition of a clinical candidate minimizing the number of peptides that need to be evaluated and useful to non-experts.

P12 Fast Synthesis of 84-mer Human Parathyroid Hormone for the Study of Osteoporosis and Hypoparathyroidism

Katya Karankevich, Daniel Martinez, Cyf Ramos-Colón and James P. Cain
Gyros Protein Technologies, Tucson, AZ 85714

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

H-SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAP
RDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ-NH2

Figure 1. PTH structure.

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P13 A Novel Tri-Peptide Elicits Cardioprotective Effects in Myocardial Ischemia/Reperfusion Injury by an Opioid Receptor Mechanism

Hanna Kim, Anahi McIntyre, John Woodley, Alexandra Lopez, Tejaswi Dittakavi, Matt Finnegan, Kevin Amuquandoh, Maxwell Ambrosino, Kiana Walker, Harsh Patel, Qian Chen, Robert Barsotti, Lindon H. Young
Bio-Medical Sciences, Philadelphia College of Osteopathic Medicine, 4170 City Avenue, Philadelphia, PA 19131

Preliminary studies have shown that a novel tri-peptide (Phe-D-Arg-Phe-Amide, MW=468 g/mol) attenuates ventilation induced diaphragm dysfunction when given as pretreatment in mice. Tri-peptide structure is similar to the SS-20 peptide (Phe-D-Arg-Phe-Lys-Amide) and some cardioprotective opioid receptor (δ, κ, μ) agonists. Our study aims to determine whether pre- or post-treatment with tri-peptide is more efficacious in mitigating the deleterious effects of myocardial ischemia/reperfusion (I/R) injury. Moreover, we aim to determine if the cardioprotective mechanisms involve the opioid receptor pathway using naloxone, a nonselective opioid receptor antagonist. Tri-peptide (50μM) was administered prior to I (pretreatment, n=8) or given during R (posttreatment, n=8) in isolated perfused rat hearts subjected to I(30min)/R(45min) and compared to untreated control hearts (n=7) and pre-treated tri-peptide (50 μM) + naloxone (Nlx, 10μM, n=8) hearts. The tri-peptide pretreatment group had significantly recovered post-reperfused left ventricular developed pressure by 55±6% of baseline compared to untreated control, posttreatment tri-peptide, and pretreatment tri-peptide + Nlx hearts which only recovered to 30±7%, 31±8%, and 26±4% of baseline respectively ($p<0.01$). Furthermore, pre-treated tri-peptide hearts had significantly reduced infarct sizes of 26±1% compared to untreated control and posttreatment tri-peptide, which had infarct sizes of 38±4% and 37±2% respectively ($p<0.05$) using 1%triphenyltetrazolium chloride staining. Pretreated tripeptide + Nlx hearts had an infarct size of 32±3% and was statistically similar to untreated controls. These data suggest that pretreatment with this novel tri-peptide may attenuate heart tissue injury in cardiac transplant recipients or to coronary bypass patients. The putative mechanism of this tri-peptide may involve opioid receptor activation.

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

P14 Designing Cell-material Interactions by Controlling the Chemistry of the Surface of Degradable Porous Scaffolds

Tove Kivijärvi[*,†], Daniela Pappalardo[†,‡], Anna Finne-Wistrand[†]
[†]Department of Fibre and Polymer Technology, KTH Royal Institute of Technology, Teknikringen 56-58, 100 44 Stockholm, Sweden; [‡]Department of Science and Technology, University of Sannio, via dei Mulini 59/A, 82100 Benevento, Italy
*Correspondence: toveki@kth.se

Peptide/protein-polymer conjugates have gained interest in the development of biomedical materials, as they provide engineered milieus for directing the cellular behavior.[1] In this project, we are developing three-dimensional scaffolds, aiming to mimic the characteristics of natural extracellular matrix by chemical conjugation of peptides and proteins to functionalized poly(ester)s. The overall aim is to fabricate an implantable and degradable device that serves as a microenvironment for soft tissue regeneration, requiring defined architecture, pore-size, and cell response. By ring-opening polymerization, we enable control over molecular weight and microstructure of the aliphatic poly(ester)s, while compromising a platform of polymers and copolymers with varying physico-chemical properties and degradation rate.[2] Utilizing 3D-printing techniques, we are envisioning a well-defined and interconnected porous structure to act as a stabilizing framework for cell attachment. For increased biological recognition of the final material, we are tailoring the nature of the polymer backbone by incorporation of amino acid-mimicking motifs, while extending our post-modification strategies[3] to covalently anchor peptides which can serve as a bridge for further covalent immobilization of proteins.

This project is financially supported by the Swedish Foundation for Strategic Research (RMA15- 0010).

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P15 Modulation of Quorum Sensing in *Streptococcus pneumoniae*; An Alternative Approach to Antimicrobial Therapy

Bimal Koirala, Naiya R. Phillips, Sally R. Hamry, Yftah Tal-Gan
Department of Chemistry, University of Nevada, Reno, 1664 North Virginia Street, Reno, NV 89557, USA

Streptococcus pneumoniae is a gram-positive, alpha-hemolytic, facultative anaerobic bacterial pathogen that inhabits the nasopharynx of humans, and is the cause of numerous diseases.[1] The increasing prevalence of antibiotic resistance in *S. pneumoniae* represents a great threat to successful treatment of its associated infections. *S. pneumoniae* acquires antibiotic resistant genes through genetic transformation, which occurs when the bacteria enters the competent state.[2] Competence in *S. pneumoniae* is initiated by the competence stimulating peptide (CSP)-mediated quorum sensing (QS) circuit.[2] The same QS circuitry is also responsible for the formation of biofilms and production of virulence factors. *S. pneumoniae* strains can be divided into two main specificity groups, Group 1 and Group 2, based on the CSP signal (CSP1 or CSP2)and associated receptor (ComD1 or ComD2) they produce. In this study we systematically altered the sequence of CSP2 to find analogs that can modulate the QS response and attenuate the pathogenicity of *S. pneumoniae*. We identified several key residues that are critical for receptor binding, activation, and specificity, based on which we designed next generation analogs with desired activity profiles.[3] We then evaluated the overall 3D structures of CSP2 and its analogs using circular dichroism (CD) to correlate between the structure and function of these peptides, suggesting that α-helix is required for effective binding to the ComD2 receptor.

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P16 Characterizing the Impact of the Highly Endosomolytic Cell-Penetrating Peptide, dfTAT, on Human Cells

Helena M. Kondow and Jean-Philippe Pellois
Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

One of the challenges of delivering molecules inside live cells is maintaining cellular homeostasis after delivery. Our lab has generated a highly endosomolytic cell-penetrating peptide, dfTAT, that aids in the delivery of macromolecules into live cells without impacting cell viability or transcriptome regulation. With its highly efficient endosomal release, dfTAT would be expected to have a negative impact on cell physiology. Therefore, it is of interest to investigate what is happening to the cell post endosomal release. An initial step in doing so is to determine whether membrane damage can be detected. Using galectin-3 as a marker of vesicle damage, we observe galectin-3 recruitment to leaked endosomes. The galectin-3 response is augmented by a light-induced endosomal lysis that is observed within seconds. Furthermore, dfTAT-leaked endosomes exhibit a loss of intraluminal acidic pH, suggesting compromised membrane integrity. Despite this evidence of membrane damage, the overall cellular distribution, as well as characteristic sedimentation behavior in fractionation assays, of dfTAT-leaked vesicles remains unaffected. Another approach to probe the absence of cytotoxicity post-release is to determine the threshold at which cytotoxicity is triggered. We have determined that as many as four consecutive one hour dfTAT incubations do not confer a cytotoxic response. This lack of cytotoxicity can be explained by both a reduction in the amount of dfTAT uptake, as well as the intracellular and extracellular degradation of dfTAT over time. Overall, the highly endosomolytic efficiency of dfTAT can be exploited to learn about non-toxic membrane damage that occurs in other biological systems.

P17 Investigation of Novel Cyclic Peptides for Preferential Binding to Melanocortin 4 Receptors

Maj Krumberger, C.A. Page, J. L. Rios-Monterrosa, Y. Zhou, S. Mowlazadeh Haghighi, M. Cai, V.J. Hruby
University of Arizona, Department of Chemistry and Biochemistry, Tucson, AZ 85721

Melanocortin Receptors (MCR's) are a family of G-Coupled Protein Receptors (GPCR's) and extensive research has thus far shown 5 functionally different MCR's. The GPCR family is one of the major targets for the pharmaceutical industry, hence developing potent ligands for MCR's can be very advantageous[1]. In this study, we focus on the Melanocortin 4 Receptor (MC4R). These receptors are found in virtually all brain regions including the thalamus, hypothalamus, hippocampus, brainstem and the spinal cord[2]. Our study focuses on the design of cyclic ligands that will act as agonists to MC4R, promoting synaptic plasticity and reducing the cognitive impairment that is associated with Alzheimer Disease. Cyclization of the potential ligands both reduces the number of possible configurations that the peptide can adopt, increasing the binding probability to the receptor, as well as exposes the necessary pharmacophore HFRW residues, a sequence that is present in all endogenous ligands that bind to MCR's, and has been shown to be required for binding to these receptors[4]. We introduced Beta amino acids to the pharmacophore for increased flexibility of the side chain due to the Carbon chain elongation. Additionally, the structures contain highly electronegative Chlorine or Fluorine atoms on the HFRW moiety for increased binding capability to the receptors. All the peptides are cyclic structures, bonded via lactam bridge between Aspartate and Lysine residues.

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P18 Mapping Membrane Activity in Undiscovered Peptide Sequence Space Using Machine Learning

Ernest Y. Lee[1], Benjamin M. Fulan[2], Gerard. C. L. Wong[1*], Andrew L. Ferguson[3,4*]
[1]Department of Bioengineering, University of California, Los Angeles; [2]Department of Mathematics; [3]Materials Science and Engineering; and [4]Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign
Contact: *gclwong@seas.ucla.edu or alf@illinois.edu

There are some ~1,100 known antimicrobial peptides (AMPs), which permeabilize microbial membranes but have diverse sequences. Here, we develop a support vector machine (SVM)-based classifier to investigate α- helical AMPs and the interrelated nature of their functional commonality and sequence homology (Lee EY *et al.* PNAS 2016). SVM is used to search the undiscovered peptide sequence space and identify Pareto-optimal candidates that simultaneously maximize the distance σ from the SVM hyperplane (thus maximize its "antimicrobialness") and its α-helicity, but minimize mutational distance to known AMPs. By calibrating SVM machine learning results with killing assays and small-angle X-ray scattering (SAXS), we find that the SVM metric σ correlates not with a peptide's minimum inhibitory concentration (MIC), but rather its ability to generate negative Gaussian membrane curvature. This surprising result provides a topological basis for membrane activity common to AMPs. Moreover, we highlight an important distinction between the maximal recognizability of a sequence to a trained AMP classifier (its ability to generate membrane curvature) and its maximal antimicrobial efficacy. As mutational distances are increased from known AMPs, we find AMP-like sequences that are increasingly difficult for nature to discover via simple mutation. Using the sequence map as a discovery tool, we find an unexpectedly diverse taxonomy of sequences that are just as membrane-active as known AMPs, but with a broad range of primary functions distinct from AMP functions, including endogenous neuropeptides, viral fusion proteins, topogenic peptides, and amyloids. The SVM classifier is useful as a general detector of membrane activity in peptide sequences.

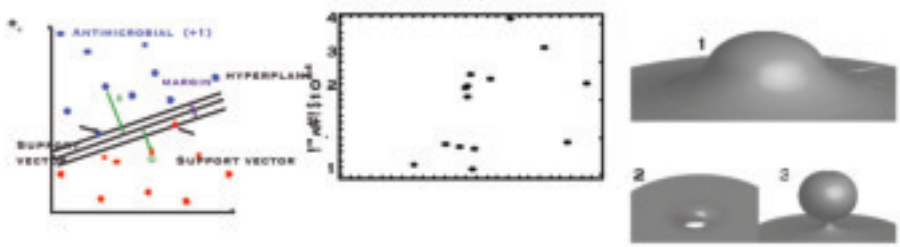

Figure 1: Machine learning discovery of membrane-active peptides. The SVM classifier (left) differentiates between antimicrobial and non-antimicrobial peptides. The SVM metric distance to hyperplane (σ) correlates with the ability to generate negative Gaussian curvature (<*K*>) (middle), the type of membrane curvature topologically required for membrane permeation events pore-formation (right).

P19 On the Effects of Acylation of Cell-penetrating Peptides in Nucleic Acid Delivery *In Vitro* and *In Vivo*

Tõnis Lehto,[1] Kadi-Liis Veiman,[3] Helerin Margus,[2] Kaido Kurrikoff,[3] Margus Pooga,[2,3] Mattias Hällbrink,[1] Ülo Langel[1,3]
[1]Department of Neurochemistry, The Svante Arrhenius Laboratories for Natural Sciences, Stockholm University, Svante Arrhenius väg 16B, 10691 Stockholm, Sweden; [2]Institute of Molecular and Cell Biology, University of Tartu, Riia 23a, 51010 Tartu, Estonia; and [3]Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
Tonis.lehto@neurochem.su.se

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

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

P20 Effective Prediction of Long-term Stability and Aggregation Propensity of Proteins via Differential Scanning Calorimetry (DSC)

Yingkai Liang[1], Grace Okoh[2], Erika Walsh[1], Andrew Leithead[1], Nicole Buist[1]
[1]Discovery Pharmaceutical Sciences, Merck & Co., Inc., Kenilworth, NJ; [2]Oral Formulation Science & Technology, Formulation Sciences, Merck & Co., Inc., Rahway, NJ

Owing to the delicate nature of proteins, the conformational integrity and long-term stability of these molecules are important considerations for the discovery and development of peptide/protein therapeutics. The stability of the protein under storage/process conditions, reversibility of conformational changes, and tendency to aggregate are usually dependent on inherent structures, pH and buffer/excipient compositions. Differential scanning calorimetry (DSC) can be a powerful tool in understanding these effects via characterization of protein thermal stability and domain folding integrity. In this work, using insulin peptide analogues as model compounds, thermal stability data obtained from DSC was utilized to predict the folding related aggregation propensity of proteins. Specifically, through analysis of the relative thermal stability of related peptide compounds and different buffer/excipient formulations, the correlation between thermal stability and actual long-term physical stability determined by Size Exclusion Chromatography was studied. The results demonstrate that DSC allows for early identification of long-term stability issues of proteins within hours, providing an effective screening tool in addition to the conventional storage stability assessment. The unique application of DSC technique in protein characterization suggests great potential in the efficient screening of therapeutic candidates as well as facile selection of buffers and excipients for process condition and formulation development.

P21 Evaluation of Apelin-13 Analogues as Antidiabetic Therapeutics

Charlotte S. Madsen[a], Esben M. Bech[a], Rikke V. Grønlund[a], Gitte Hansen[a], Søren L. Pedersen[a], Keld Fosgerau[a]
[a]Gubra ApS, Hørsholm Kongevej 11B, 2970 Hørsholm, Denmark

Apelin is a bioactive peptide identified as the endogenous ligand of the G-protein-coupled receptor APJ. The main active forms are apelin-13, -17, -36, and the pyroglutamated form of apelin-13 (pGlu-apelin-13). The peptide is widely expressed in both the central nervous system and peripheral tissues. Various physiological functions have been associated with the apelinergic system over the past decade, such as regulation of fluid homeostasis, modulation of the cardiovascular system, and regulation of energy metabolism.[1] Interestingly, apelin exhibits strong inotropic effects, and have furthermore been reported to stimulate glucose utilization in normal and obese insulin-resistant mice.[2] Additionally, apelin analogues have been shown to reduce the body weight of DIO NIH Swiss mice with similar efficacy as Liraglutide (28 days) as well as reduce blood glucose levels extensively.[3]

In this study, we aimed to confirm the effect of apelin-13 and lipidated analogues on glucose homeostasis. For this purpose, DIO C56Bl/6J mice were administered with apelin-13 and analogues thereof and the effect evaluated in an acute oral glucose tolerance test (OGTT) and a 16h delayed OGTT. Contrary to the hypothesis, administration of apelin-13 and analogues acutely increased blood glucose levels in DIO mice. Furthermore, apelin-13 and lipidated variants did not influence the blood glucose levels 16h after administration. In conclusion, these results show no effect of the selected apelin-13 analogues on glycemic control, and based on current results, apelin is not an attractive target for the treatment of diabetes.

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P22 Myristoylated Protein Kinase C Epsilon Peptide Inhibitor Reduces Infarct Size and Improves Cardiac Function Following Myocardial Ischemia/Reperfusion (I/R)

Anahi McIntyre, Christine Adekayode, Hanna Kim, John Woodley, Tejaswi Dittakavi, Matt Finnegan, Joseph Heron, Ifeanyi James, Israel Benjamin, Stephanie Liu, Kiana Walker, Qian Chen, Robert Barsotti, Lindon H. Young
Bio-Medical Sciences, Philadelphia College of Osteopathic Medicine, 4170 City Avenue, Philadelphia, PA 19131

The increase of myocardial reactive oxygen species (ROS) release during reperfusion (R) following prolonged ischemia (I) exacerbates tissue injury. A key source of ROS during reperfusion is uncoupled endothelial nitric oxide synthase (eNOS). Protein kinase C epsilon (PKC ε) activation during reperfusion stimulates ROS release, in part via increased uncoupled eNOS activity. We hypothesize that isolated perfused rat hearts from male SD rats (~300g) subjected to I(30 min)/R(90 min) would exhibit a reduction in infarct size and improved post-reperfused cardiac function when treated with a myristic acid conjugated PKC ε peptide inhibitor (PKC ε-) (N-myr-EAVSLKPT, MW=1054 g/mol, 5 μM, 10 μM or 20 μM) compared to untreated hearts. PKC ε- was infused into hearts during the first 10 min of reperfusion. All PKC ε- hearts (5-20 μM; n=8) showed significant reduction in infarct size from 38±3% to 28±2% untreated (n=9) vs treated hearts ($p<0.05$) assessed by 1% triphenyltetrazolium chloride staining of heart tissue after cardiac function measurements. Interestingly, PKC ε- significantly restored the maximal rise in left ventricular developed pressure at 90 min R to 56±3% (10 μM) and 50±3% (20 μM) of baseline values compared to untreated controls and low-dose PKC ε- hearts (5 μM) which recovered to 30±4% and 33±5% of baseline values ($p<0.01$). The results show that PKC ε- effectively reduces infarct size and improves cardiac function presumably by inhibition of uncoupled eNOS induced ROS release during reperfusion. PKC ε- treatment could reduce patient morbidity and mortality following I/R injury and organ transplantation.

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

P23 CPP and Small Molecule Synergy Enhances Endosomal Escape and Cytosolic Delivery in Live Cells

Kristina Najjar[1], Alfredo Erazo-Oliveras[2], Rudolph L. Juliano[3], and Jean-Philippe Pellois[1,4]
[1]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843; [2]Department of Nutrition and Food Science, Texas A&M University, College Station, TX 77843; [3]UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599; and [4]Department of Chemistry, Texas A&M University, College Station, TX 77843

The delivery of macromolecules into live cells is important for areas in biomedical research and therapeutic applications. Cell-penetrating peptides (CPPs) have been exploited as promising tools to internalize cell impermeable macromolecules. CPPs enter cells by utilizing the endocytic pathway, however endosomal entrapment remains a severe bottleneck. Our lab has generated a CPP, dfTAT, which penetrates live cells by escaping from late endosomes with an unprecedented efficiency. We have established that dfTAT can deliver proteins into live cells through a simple co-incubation protocol with no negative impact on cell physiology. Despite its high efficiency, dfTAT suffers from limitations that ultimately reduce its activity. Due to its peptidic nature, dfTAT is readily degraded by proteases found along the endocytic pathway. Moreover, cells that exhibit low levels of endocytic uptake are negatively impacted by this delivery method. Furthermore, negatively charged macromolecules cannot simply be delivered, as they electrostatically interact with dfTAT, which affects its activity. Recently, a proprietary small molecule was shown to successfully induce the escape of macromolecules from late endosomes. Therefore, we hypothesized that dfTAT and this molecule may act in synergy and provide a cocktail with enhanced endosomal escape activity. Results show that this cocktail leads to robust efficiencies and permits delivery in cells that have previously proven resistant to penetration. Furthermore, this cocktail allows the delivery of DNA, a negatively charged molecule that could not previously be delivered using dfTAT. This work lays the foundation for the future design of CPP and small molecule cocktails with improved therapeutic significance.

P24 Optimizing Peptide Manufacturing on the Small Scale and its Application in the Rapid Development of New Personalized Medicines

Keith Porter, Jonathan Collins, S. Sub Singh, Michael Karney
CEM Corporation

Peptides make idea drug candidates due to their inherent high potency, low toxicity (compared their small molecule drug counterparts), and their ability to effect a broad range of targets[1]. More recently, peptides have gained great acceptance for applications in personalized medicine in the form of personalized peptide vaccine[2,3]. This approach requires a robust synthetic protocol to ensure the peptide combination is assembled and administered in the least amount of time. Rapid synthesis of peptides is achieved through high efficiency solid phase peptide synthesis (HE-SPPS). This method provides benefits for the synthesis of peptides due to its high purity, rapid speed, and low chemical usage characteristics [4].This has been advanced further through the development of a one pot coupling and deprotection process and an improved carbodiimide coupling that enhances both O-acylisourea formation and the subsequent acylation[5].Together these features maximized achievable synthesis purity, reduced the entire cycle time to < 3 minutes, and required only a single washing step per cycle. Unlike conventional SPPS protocols, this process is readily scalable to generate up to 200 mg purified peptide in each run. As an example, a 20mer at 0.3 mmol scale, can be synthesized in little as an hour, allowing up to 24 peptides of similar length to be synthesized autonomously in a full day. This unique chemistry, which is ideal and readily applicable for developing peptide vaccines for personalized medicine, will be discussed.

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P25 Novel Therapeutic Peptides Targeting Matricellular Signaling in Currently Untreatable Fibrotic Disease and Cancer

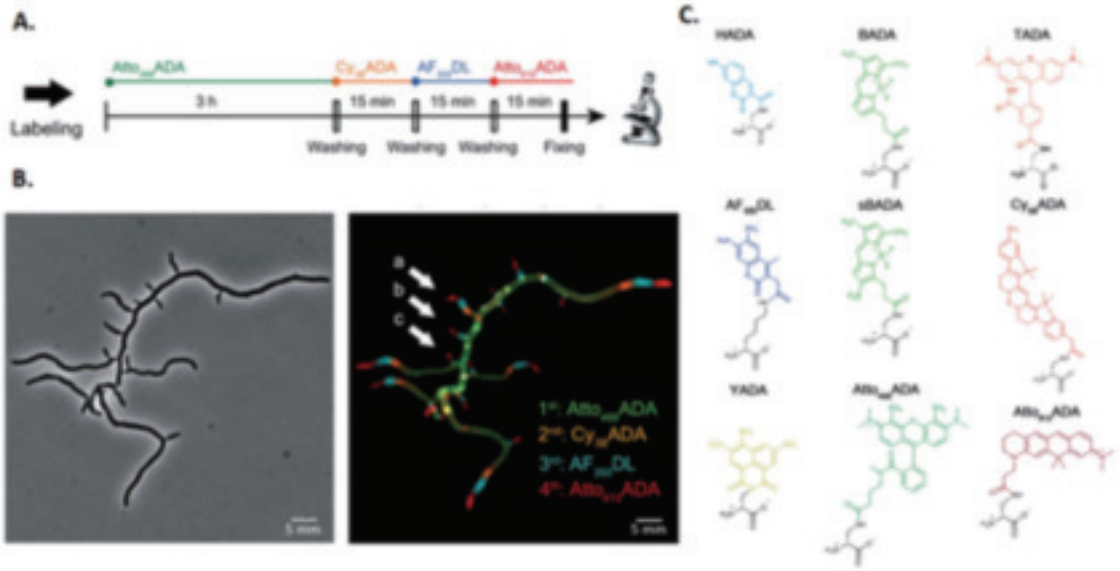
Bruce L. Riser[1,2], Dorina Belotti[3], and Giulia Taraboletti[3]
[1]BLR Bio LLC, WI, USA; [2]The Department of Physiology and Biophysics, and Department of Medicine, Rosalind Franklin University of Medicine and Science, North Chicago, Illinois, USA; and [3]Mario Negri Institute of Pharmaceutical Research, Bergamo, Italy

Half the deaths in the developed world are associated with progressive fibrosis, and cancer associated stroma production, both ultimately leading to organ failure. In spite of this knowledge, there is no single FDA approved drug specifically designed to totally arrest or reverse fibrosis, and no approved cancer drug targeting the fibrotic stroma. Over the last decade, targeting the CCN family of the matricellular signaling proteins (particularly CCN2) has emerged as a highly promising and attractive approach to the treatment of fibrotic diseases and cancer, and has been our focus. We first showed that another CCN family member, CCN3, downregulated by TGF-β, is a natural antagonist of CCN2 and fibrosis development in kidney cells. Then, in an established mouse model of human type 2 diabetic nephropathy with obesity we showed that treatment with rhCCN3 both halted and reversed key hallmarks of established fibrosis and the loss of kidney function (Riser et al., Amer. J. Path, 2014). This led us to create and screen small peptides specific for anti-fibrotic and cancer function, based on regions we identified as critical for the interaction between CCN3 and other CCN proteins. These proprietary peptides are part of the pipeline at BLR Bio, and are capable of not only halting fibrosis initiation and stromal support of cancer progression, but also of re-establishing tissue homeostasis. In pancreatic cancer, they shrink tumors and augment the efficacy of conventional chemo- and immuno-therapeutic drugs. We are preparing to enter clinical trials targeted at both multi-organ fibrosis as well as cancer.

P26 Improved Florescent Amino Acids For *In Situ* Labeling of Bacterial Cell Walls

Jonathan Rittichier[1], Erkin Kuru[1], Yen-Pang Hsu[2], Yves V. Brun[2], Michael S. VanNieuwenhze[2], and George M. Church[1]
[1]Harvard Medical School-Boston, MA; [2]Indiana University-Bloomington, IN; [4]Cambridge, MA

Fluorescent molecules and their fascinating properties have played a key role in advancing human understanding. They are especially useful as trackers and probes in biological applications. We have previously shown that the enzymes involved in peptidoglycan biosynthesis incorporate certain fluorescent D-amino acids (FDAAs).[1] This has provided an efficient method for labeling nascent peptidoglycan *in situ* for a variety of diverse bacterial species. The method has become broadly applicable and has revealed important information about bacteria growth, cell morphology and remodeling activity.[2,3] However, this method has suffered from several drawbacks such as few orthogonal colors and a lack of information on the physiochemical properties and stabilities of the probes. Described herein is the design, synthesis, and photochemical and physical properties of a set of new and improved fluorescent D-amino acids (FDAAs).[4] These probes have the advantage of brightness and solubility, and make up a full spectrum of fluorescent colors. Also presented is the effectiveness of FDAAs for visualizing peptidoglycan synthesis for Gram-negative and Gram-positive bacterial species. We believe that these new FDAAs provide a complete toolkit that will enable numerous applications for the study of peptidoglycan biosynthesis and dynamics.



Abstract Figure[4] | **A.** Virtual time-lapse FDAA labeling. Streptomyces venezuelae cells successively labeled with FDAAs indicated. Cells are then fixed and imaged. **B.** Left: resultant phase-contrast image of cell. Right: fluorescence image of cell. White arrows point out new branches formed at different time points during the experiment (oldest to newest: (a) to (c)). **C.** Some structures of FDAAs reported in this study.

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P27 Development of a New Method on Npys-based Disulfide Bond Formation for the Preparation of Bioactive Cyclic Disulfide Peptides

Akihiro Taguchi[1], Kiyotaka Kobayashi[1], Akira Kotani[2], Kyohei Muguruma[1], Misaki Kobayashi[1], Kentarou Fukumoto[1,3], Kentaro Takayama[1], Atsuhiko Taniguchi[1], Hideki Hakamata[2], Yoshio Hayashi[1]
[1]Department of Medicinal Chemistry and [2]Department of Analytical Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan; [3]Kokusan Chemical Co., Ltd., Tokyo, Japan

Disulfide bonds play an important role in maintaining the folded functional conformations of proteins and peptides. Therefore, disulfide bond formation is an important step in the chemical synthesis of cystine-containing peptides and proteins.

3-Nitro-2-pyridinesulfenyl (Npys) group is a well-known protecting group in peptide chemistry. It functions as an active disulfide in the protection of Cys side chain, so that selective disulfide exchange reaction occurs under coexistence of an unprotected thiol group. Based on this unique property of Npys, we recently developed a solid-phase disulfide ligation (DSL) method[1], in which Npys-Cl resin is used to form a disulfide bond between two different fragment peptides. In combination with the subsequent intra-molecular amide bond formation, this system provides a new synthetic method so-called "disulfide-led cyclic peptide synthesis". Based on this strategy, oxytocin was efficiently synthesized as a fundamental model.

Furthermore, Npy sulfenate (**1**) was also developed to efficiently form a disulfide bond from SH-free peptides. Through the model study of disulfide bond formation using reduced oxytocin, we found that **1** acts as a mild oxidation reagent for the preparation of disulfide peptide. The polymer formation was limited under the condition with high peptide concentration (1 mM). In order to apply this method to other bioactive peptides with more complex structures, we synthesized human ANP and α-conotoxin. The results indicated that **1** was a suitable agent to form disulfide bonds in these peptides. In the presentation, the synthesis will be discussed in details.

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P28 Discovery of Selective Hexapeptide Agonists to Human NMUR1 and NMUR2

Kentaro Takayama[1], Kenji Mori[2], Akiko Tanaka[3], Akihiro Taguchi[1], Atsuhiko Taniguchi[1], Toshiyasu Sakane[4], Akira Yamamoto[3], Naoto Minamino[5], Mikiya Miyazato$_2$, Kenji Kangawa[2], and Yoshio Hayashi[1]
[1]Department of Medicinal Chemistry, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan; [2]Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka, Japan; [3]Department of Biopharmaceutics, Kyoto Pharmaceutical University, Yamashina, Kyoto, Japan; [4]Laboratory of Pharmaceutical Technology, Kobe Pharmaceutical University, Kobe, Hyogo, Japan; and [5]Omics Research Center, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan.

Neuromedin U (NMU), a ligand for two NMU receptors (NMUR1 and NMUR2), is a noteworthy target for development of an anti-obesity drug. However, selective agonists toward respective receptors had not been yet disclosed. We focused on the C-terminal amidated heptapeptide structure **1** (H-Phe^0-Leu^1-Phe^2-Arg^3-Pro^4-Arg^5-Asn^6-NH_2), and carried out a structure-activity relationship study using in vitro calcium mobilization assay to obtain receptor selective agonists. First, we found a human NMUR2 selective hexapeptidic full agonist **2** with an EC_{50} value of 6.4 nM [1]. The NMUR2 agonist **2** successfully suppressed body weight gain after intranasal administration in mice. On the other hand, we discovered a potent NMUR1 agonist **3** (2-thienylacetyl-Trp^1-Phe(4-F)^2-Arg^3-Pro^4-Arg^5-Asn^6-NH_2), and identified two major biodegradation sites (Phe(4-F)^2-Arg^3 and Arg^5-Asn^6) in serum on stability evaluation[2]. The latter site is preferentially cleaved by serum thrombin[3]. In particular, the agonist **3** is rapidly degraded in serum. Recently, we discovered another NMUR1 selective hexapeptidic full agonist **4** bearing α-methyl-Trp^2 with an EC_{50} value of 0.25 nM, which displayed significantly improved NMUR1 selectivity, serum stability and pharmacokinetics compared to **3** bearing Phe(4-F)^2 [4]. The agonist **4** subcutaneously injected significantly suppressed body weight gain in mice[4]. The agonists **2** and **4** would be potential lead compounds for treating obesity, and useful tools for investigating NMU receptor-directed endocrinology.

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P29 Highly Efficient Synthesis of Therapeutic Macrocyles by Ligases

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

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

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P30 Computational Design and Experimental Characterization of Peptides Intended for pH-Dependent Membrane Insertion and Pore Formation

David Tellers
Merck and Co., Inc.

There are many opportunities to use macromolecules, such as peptides and oligonucleotides, for intracellular applications. Despite this, general methods for delivering these molecules to the cytosol in a safe and efficient manner are not available. Efforts to develop a variety of intracellular drug delivery systems such as viral vectors, lipoplexes, nanoparticles, and amphiphilic peptides have been made, but various challenges such as delivery efficiency, toxicity, and controllability remain. A central challenge is the ability to selectively perturb, not destroy, the membrane to facilitate cargo introduction. Herein, we describe our efforts to design and characterize peptides that form pores inside membranes at acidic pH, so-called pH-switchable pore formation (PSPF) peptides, as a potential means for facilitating cargo translocation through membranes. Consistent with pore formation, these peptides exhibit low- pH-triggered selective release of ATP and miRNA, but not hemoglobin, from red blood cells.

Consistent with these observations, biophysical studies (tryptophan fluorescence, circular dichroism, size-exclusion chromatography, analytical ultracentrifugation, and attenuated total reflectance Fourier transformed infrared spectroscopy) show that decreased pH destabilizes the PSPF peptides in aqueous systems while promoting their membrane insertion. Together, these results suggest that reduced pH drives insertion of PSPF peptides into membranes, leading to target-specific escape through a proposed pore formation mechanism.

P31 Plasma Protein Binding Analysis of Peptides with a Novel Single-compartment Mass Spectrometry Assay

Jan Ungewiss[1], Hinnerk Boriss[2]
[1]3B Pharmaceuticals GmbH; [2]Sovicell GmbH

Since peptides undergo rapid renal clearance, peptide-based drugs usually require a half-life extension to ensure optimal exposure. A popular way of extending the circulation half-life of peptides is to increase their plasma protein binding. However, the plasma protein binding of peptides and other medium-sized organic molecules has been notoriously difficult to determine *in vitro*, since commonly used methods such as rapid equilibrium dialysis and ultrafiltration frequently over- or underestimate their plasma protein binding.

We have developed a novel bead-based equilibrium shift assay that allows the accurate quantification of the plasma protein binding of peptides. To determine the free fraction (f_u) of a compound in plasma, its HSA-binding is measured in the presence of a range of plasma concentrations. The change in the compound's apparent K_D for HSA is then plotted against the plasma concentrations and fitted to calculate the free fraction f_u in plasma. Due to this indirect determination, the assay allows the accurate determination of very small free fractions and is thus well suited to analyze very strong plasma protein binders. The assay was validated using a peptide strongly binding to plasma proteins, Liraglutide, and a set of well-studied small molecules to demonstrate is reliability and accuracy.

The equilibrium shift assay allows for the first time the accurate determination of the plasma protein binding of peptides, thus supporting development decisions such as the choice of an optimal technology for half-life extension and the selection of appropriate species for toxicity studies.

P32 Targeting Quorum Sensing in *Streptococcus Pneumoniae*: An Alternative Antibacterial Approach

Yifang Yang,[†] Bimal Koirala,[†] Lucia A. Sanchez,[†] Gabriel Cornilescu,[‡] and Yftah Tal-Gan[†]
[†]Department of Chemistry, University of Nevada, Reno; [‡]National Magnetic Resonance Facility at Madison, University of Wisconsin-Madison

Quorum sensing (QS) is a mechanism through which bacteria coordinate gene expression in response to cell density. QS is considered as an alternative antibacterial approach due to the potential to treat bacterial infections with minimal development of resistance. *Streptococcus pneumoniae* is an important pathogen that utilizes QS to regulate genetic transformation, virulence and biofilm formation. The competence stimulating peptide (CSP) is a 17-amino acid peptide signal that is used by *S. pneumoniae* to trigger QS. *S. pneumoniae* strains can be divided into two main specificity groups based on the CSP signal they produce (CSP1 or CSP2) and their compatible transmembrane histidine kinase receptor (ComD1 or ComD2 respectively). In our lab we aim to develop synthetic CSP-based analogs capable of modulating pneumococcal QS by intercepting the CSP:ComD interaction. Therefore, we performed full alanine and D-amino acid scans of CSP1 and tested the ability of the analogs to modulate QS in the two *S. pneumoniae* QS specificity groups. We identified several key residues that are critical to receptor binding, activation, and specificity, based on which we designed second-generation analogs. Additionally, we used CD spectroscopy to assess the global structural features of CSP1 and found that it adopts an α-helix conformation in membrane mimicking condition. We then evaluated the secondary structure of all the CSP1 analogs and identified a strong correlation between helicity and bioactivity. Moreover, our recent 2D-NMR studies on CSP1 and several analogues revealed an interesting amphiphilic feature of the helical structure that is likely required for effective ComD binding.

P33 N-linked Glycosylation Prevents Deamidation of Glycopeptide and Glycoprotein

Hailiang Zhu[†,"], Ding Liu[†], Vy P Tran[‡], Zhigang Wu[†], Kuan Jiang[†,§], He Zhu[†], Xu Li[†], Xifang Liu[†], Jingyao Qu[†], Bingzhong Xue[‡,*], Hang Shi[‡,*] and Peng George Wang[†,*]

[†]Department of Chemistry, Georgia State University, Atlanta, GA 30303, United States; [‡]Department of Biology, Georgia State University, Atlanta, GA, 30303, United States; "Department of Chemistry, Dordt College, Sioux Center, IA 51250, United States

Preventing deamidation of glycopeptide and glycoprotein by N-linked glycosylation was described in this work (**Figure 1**). Deamidation has been recognized as a common spontaneous pathway of protein degradation and its rate is sequence-dependent.

Deamidation is a prevalent concern in pharmaceutical industry, causing the reduction of protein/peptide drug efficacy and shelf-life in some cases. Therefore it is critical to discover means of controlling the deamidation rate of protein and peptide drugs.

Deamidation of physiological proteins is also related to several human diseases. In this paper, it was demonstrated that deamidation is prevented by the naturally occurring glycosylation of Asn. Glycopeptides and corresponding non-glycosylated peptides were synthesized through solid phase peptide synthesizer (SPPS). All of the non- glycosylated peptides have different half-lives ranging from one to twenty days through incubation. Deamidation reaction was significantly reduced by the introduction of N- linked glycosylation. RNase B was used to demonstrate that glycoprotein has elongated deamidation half-life than non-glycosylated protein RNase A. In this work, N- linked glycosylation was also applied on a therapeutic peptide and it was found that the N-linked glycosylation not only prevented its deamidation but also increased its potency.

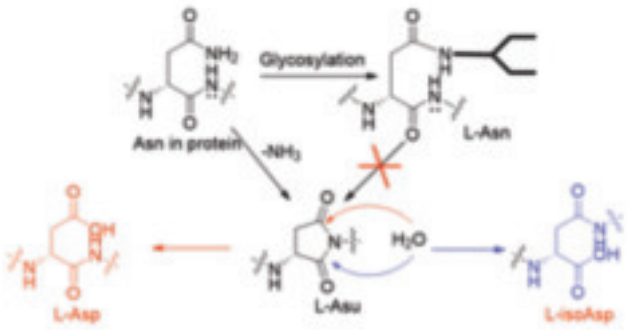

Figure 1.

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