PEPTIDE THERAPEUTICS SYMPOSIUM

Program and Proceedings
14th Annual Peptide Therapeutics Symposium

October 24 – 25, 2019

Salk Institute for Biological Studies, La Jolla. CA

www.peptidetherapeutics.org

14th Annual Peptide Therapeutics Symposium

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PEPTIDE THERAPEUTICS FOUNDATION

14th Annual Peptide Therapeutics Symposium

Dear Colleagues,

We are delighted to welcome you to the 14th Annual Peptide Therapeutics Symposium at the Salk Institute for Biological Studies, La Jolla. The Symposium, as in past years, will feature presentations by prominent scientists, and highlight the discovery and development of novel peptide-based drug candidates.

The conference will open on Thursday afternoon with Plenary Lectures from Professors Beili Wu of the Shanghai Institute, and Lotte Knudsen of Novo Nordisk. This will be followed by a session in celebration of Professor Victor Hruby's 80th birthday, and to recognize the numerous contributions he and his associates have made to peptide sciences. Tomi Sawyer formerly of Merck Research Labs, Henry Mosberg of the University of Michigan, and Guigen Li of Texas Tech University will deliver lectures in this session. Please join us in congratulating Dr. Hruby at the end of day reception with socialization at Thursday's concluding poster session.

The Friday morning session will feature Keynote Lectures by Peter Senter of Seattle Genetics and Professor Dale Boger of Scripps Research. The sessions that follow will pertain to lectures presenting clinical advances in peptide therapeutics and cutting-edge technology promoting the discovery of next generation drug candidates.

We are excited to provide what we believe is another superb program, along with opportunity to promote scientific exchange and informed peer-review. As always, we are grateful for your participation in the symposium. Your attendance has been key to making this annual scientific event a notable success.

Sincerely,

Richard DiMarchi
Chairman of the Board
Peptide Therapeutics Foundation

Soumitra Ghosh
President
Peptide Therapeutics Foundation

Sponsors, Peptide Therapeutics Foundation

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14th Annual Peptide Therapeutics Symposium

AstraZeneca

AstraZeneca is a global, science-led biopharmaceutical company that focuses on the discovery, development and commercialisation of prescription medicines, primarily for the treatment of diseases in three therapy areas – Oncology, Cardiovascular, Renal & Metabolism and Respiratory. AstraZeneca operates in over 100 countries and its innovative medicines are used by millions of patients worldwide. AstraZeneca has three global R&D centers, in Gaithersburg, MD, South San Francisco, CA and Cambridge' UK. For more information, please visit www.astrazeneca.com.

Ferring Research Institute, Inc.

Headquartered in San Diego, California, Ferring Research Institute, Inc., (FRI) is a critical component of Ferring Pharmaceutical's global therapeutics research and discovery engine. Established in 1996, FRI is located in the heart of the Southern California biopharmaceutical community. The center has attracted a diverse group of highly skilled professionals representing over twenty four countries of origin. FRI is focused on the following key therapeutic areas: reproductive medicine & women's health, urology and gastroenterology/hepatology. Our state-of-the art facility includes peptide and protein drug design, chemistry, pharmacology, biology, and preclinical ADME capabilities. Historically FRI has focused on the discovery of amino acid-based therapeutics utilizing the body's signaling hormones. Today FRI is committed to building a portfolio of novel, innovative therapeutics using a wide array of modalities in order to address areas of high unmet medical need in our core therapeutic areas. Driving value through personalized medicine.

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

Novo Nordisk

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

The PolyPeptide Group

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

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

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

Zealand Pharma

Zealand Pharma A/S (NASDAQ OMX Copenhagen: ZEAL) ("Zealand") is a biotechnology company specialized in the discovery, design and development of peptide-based therapeutics. The company has a portfolio of medicines and product candidates under license collaborations with Sanofi and Boehringer Ingelheim and a proprietary pipeline of product candidates, which primarily target specialty diseases with significant unmet needs.

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

Zealand's proprietary pipeline includes; glepaglutide*, a GLP-2 analog for the treatment of short bowel syndrome which will initiate Phase III studies in 1H18; dasiglucagon*, a glucagon analog in Phase III as a single-dose rescue therapy for severe hypoglycemia and in Phase II as a multiple-dose component in a dual-hormone artificial pancreas system; and other earlier stage clinical and preclinical peptide therapeutics. The company has approximately 130 employees and is based in Copenhagen, Denmark.

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

Zydus Cadila

Zydus Cadila is an innovative, global pharmaceutical company that discovers, develops, manufactures and markets a broad range of healthcare therapies, including small molecule drugs, biologic therapeutics and vaccines. The group employs over 20,000 people worldwide, including 1200 scientists engaged in R&D, and is dedicated to creating healthier communities globally. www.zyduscadila.com

PEPTIDE THERAPEUTICS FOUNDATION

Peptide Therapeutics Foundation

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

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

2019 Travel Grant Awardees

Tameka Dean, Philadelphia College of Osteopathic Medicine
Mikhail Kolonin, University of Texas, Houston
Xingyue Li, University of California, Irvine
Tuan Samdin, University of California, Irvine
Marcus Van Engen, Dordt University
Weiliang Xu, University of Utah

14th Annual Peptide Therapeutics Symposium

Thursday, October 24, 2019

11:30 a.m. – 5:30 p.m.
Registration Check-in
Fritz B. Burns Reception Center, Lower Level

1:00 p.m. – 5:30 p.m.
14th Annual Peptide Therapeutics Symposium
Conrad T. Prebys Auditorium

1:00 p.m. – 1:15 p.m.
Opening Remarks
Adrienne Day, Ph.D.
Director of the Board, Peptide Therapeutics Foundation
Senior Director, Business Development,
Ferring Research Institute, Inc.

1:15 p.m. – 2:45 p.m.
Plenary Lectures
Moderator
Richard DiMarchi, Ph.D.
Chairman of the Board, Peptide Therapeutics Foundation
Distinguished Professor of Chemistry, Gill Chair in Biomolecular Sciences,
Department of Chemistry, Indiana University
VP and Site Director, Novo Nordisk Research Center, Indianapolis

1:15 p.m. – 2:00 p.m.
Structural Basis of Signal Recognition and Regulation at the Full-length Glucagon Receptor
Beili Wu, Ph.D.
Professor, Shanghai Institute of Materia Medica, Chinese Academy of Sciences

2:00 p.m. – 2:45 p.m.
GLP-1@Novo Nordisk: Innovating with Fatty Acid Acylation
Lotte Bjerre Knudsen, DMSc
Professor, Scientific Corporate Vice President, Global Drug Discovery,
Novo Nordisk A/S

2:45 p.m. – 3:30 p.m.
Beverage Break & Poster Viewing
Fritz B. Burns Reception Center, Lower Level

3:30 p.m. – 5:30 p.m.
Session I: In Honor of Victor Hruby
Moderator
James P. Tam, Ph.D.
Director, Synzymes and Natural Products Center
School of Biological Sciences, Nanyang Technological University

3:30 p.m. – 4:00 p.m.
Peptide Drug Hunter: Innovation, Integration and Inspiration
Tomi K. Sawyer, Ph.D.
Entrepreneurial Drug Hunter, Maestro Therapeutics

4:00 p.m. – 4:30 p.m.
GAP Chemistry and Multi-Layer 3D Chirality for the Synthesis of Amines, Amino Acids and Peptides
Guigen Li, Ph.D.
Paul Whitfield Horn Professor,
Department of Chemistry & Biochemistry, Texas Tech University

4:30 p.m. – 5:00 p.m.
Development of Bifunctional Mu Opioid Receptor (MOR) Agonists/ Delta Opioid Receptor (DOR) Antagonists: Opioid Analgesics with Improved Side Effect Profiles
Henry I. Mosberg, Ph.D.
Tom D. Rowe Professor of Medicinal Chemistry,
College of Pharmacy, University of Michigan

5:00 p.m. – 5:30 p.m.
Peptide Science 1968-2019, A Retrospective
Victor J. Hruby, Ph.D.
Regents Professor, Department of Chemistry and Biochemistry,
University of Arizona

5:30 p.m. – 7:00 p.m.
Poster Session & Reception
Fritz B. Burns Reception Center, Lower Level

14th Annual Peptide Therapeutics Symposium

Friday, October 25, 2019

7:00 a.m. – 11:30 a.m.	**Registration Check-in** Fritz B. Burns Reception Center, Lower Level
7:00 a.m. – 8:15 a.m.	**Breakfast & Poster Viewing** Fritz B. Burns Reception Center, Lower Level
8:15 a.m. – 5:15 p.m.	**14th 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. – 10:00 a.m.	**Plenary Lectures** **Moderator:** Lutz Jermutus, Ph.D. *Director of Board, Peptide Therapeutics Foundation* *Senior Director R&D, AstraZeneca*
8:30 a.m. – 9:15 a.m.	**Potent Antibody-Based Conjugates for Cancer Therapy: From Early Stage Research to a Clinically Approved Drug** Peter Senter, Ph.D. *Vice President for Chemistry and Distinguished Research Fellow,* *Seattle Genetics*
9:15 a.m. – 10:00 a.m.	**Redesign of Vancomycin for Resistant Bacteria** Dale L. Boger, Ph.D. *Richard and Alice Cramer Professor of Chemistry,* *Department of Chemistry, Scripps Research*
10:00 a.m. – 10:45 a.m.	**Beverage Break & Poster Viewing** Fritz B. Burns Reception Center, Lower Level
10:45 a.m. – 12:15 p.m.	**Session II** **Moderator:** Rajiv Sharma, Ph.D. *Head, Discovery Chemistry and Senior Vice President, Zydus Cadila*
10:45 a.m. – 11:15 a.m.	**Evidence of Central Signaling after Administration of Oxytocin at Olfactory and Peripheral Sites** Mary R. Lee, M.D. *Associate Research Physician, Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, National Institute on Alcohol Abuse and Alcoholism, National Institute on Drug Abuse Intramural Research Programs*
11:15 a.m. – 11:45 a.m.	**Discovery and Characterization of Viral Insulin-like Peptides** Emrah Altindis, Ph.D. *Assistant Professor, Biology Department, Boston College*
11:45 a.m. – 12:15 p.m.	**A Dual ApoC-II Mimetic-apoC-III Antagonist Peptide for Lowering Plasma Triglycerides** Alan T. Remaley, M.D., Ph.D. *Senior Investigator, NHLBI, National Institutes of Health*
12:15 p.m. – 1:30 p.m.	**Lunch & Poster Viewing** Fritz B. Burns Reception Center, Lower Level

14th Annual Peptide Therapeutics Symposium

1:30 p.m. – 3:00 p.m.	**Session III** **Moderator:** Fa Liu, Ph.D. *Director of Board, Peptide Therapeutics Foundation* *Director Chemistry, Novo Nordisk*
1:30 p.m. – 2:00 p.m.	**Molecular Mechanisms of Transcriptional Control by Intrinsically Disordered Proteins (aka Peptides)** Rebecca B. Berlow, Ph.D. *Staff Scientist, Department of Integrative Structural and Computational Biology, Scripps Research* H. Jane Dyson, Ph.D. *Professor, Department of Integrative Structural and Computational Biology, Scripps Research*
2:00 p.m. – 2:30 p.m.	**Targeting Intracellular Pathogenic Bacteria with Unnatural Proline-Rich Peptides** Jean Chmielewski, Ph.D. *AW Kramer Distinguished Professor, Department of Chemistry, Purdue University*
2:30 p.m. – 3:00 p.m.	**Mini-Ins: A Minimized, Bioactive Insulin Analog with Fast-Acting Properties Inspired by A Cone Snail Peptide** Danny Chou, Ph.D. *Assistant Professor of Biochemistry, University of Utah*
3:00 p.m. – 3:30 p.m.	**Beverage Break & Poster Viewing** Fritz B. Burns Reception Center, Lower Level
3:30 p.m. – 5:00 p.m.	**Session IV** **Moderator:** Yvonne Angell, Ph.D. *Director and Head of Peptide Chemistry* *ChemPartner – San Francisco*
3:30 p.m. – 4:00 p.m.	**Elastin-Like Polypeptide Biopolymers Enhance the Pharmacology of Therapeutic Peptides** Jim Ballance, Ph.D. *VP Research & Scientific Affairs,* *PhaseBio Pharmaceuticals, Inc.*
4:00 p.m. – 4:30 p.m.	**Oral Delivery of Peptides: Present and Future** Stephen T. Buckley, Ph.D. *Head of Discovery ADME,* *Novo Nordisk A/S*
4:30 p.m. – 5:00 p.m.	**Discovery of FE 201836, a Short Acting V2R Agonist for the Treatment of Nocturia** Kazimierz Wisniewski, Ph.D. *Senior Scientist II,* *Ferring Research Institute, Inc.*
5:00 p.m. – 5:15 p.m.	**Closing Remarks** Richard DiMarchi, Ph.D.
5:15 p.m. – 6:15 p.m.	**Closing Reception** Fritz B. Burns Reception Center, Lower Level

Speaker Biographies

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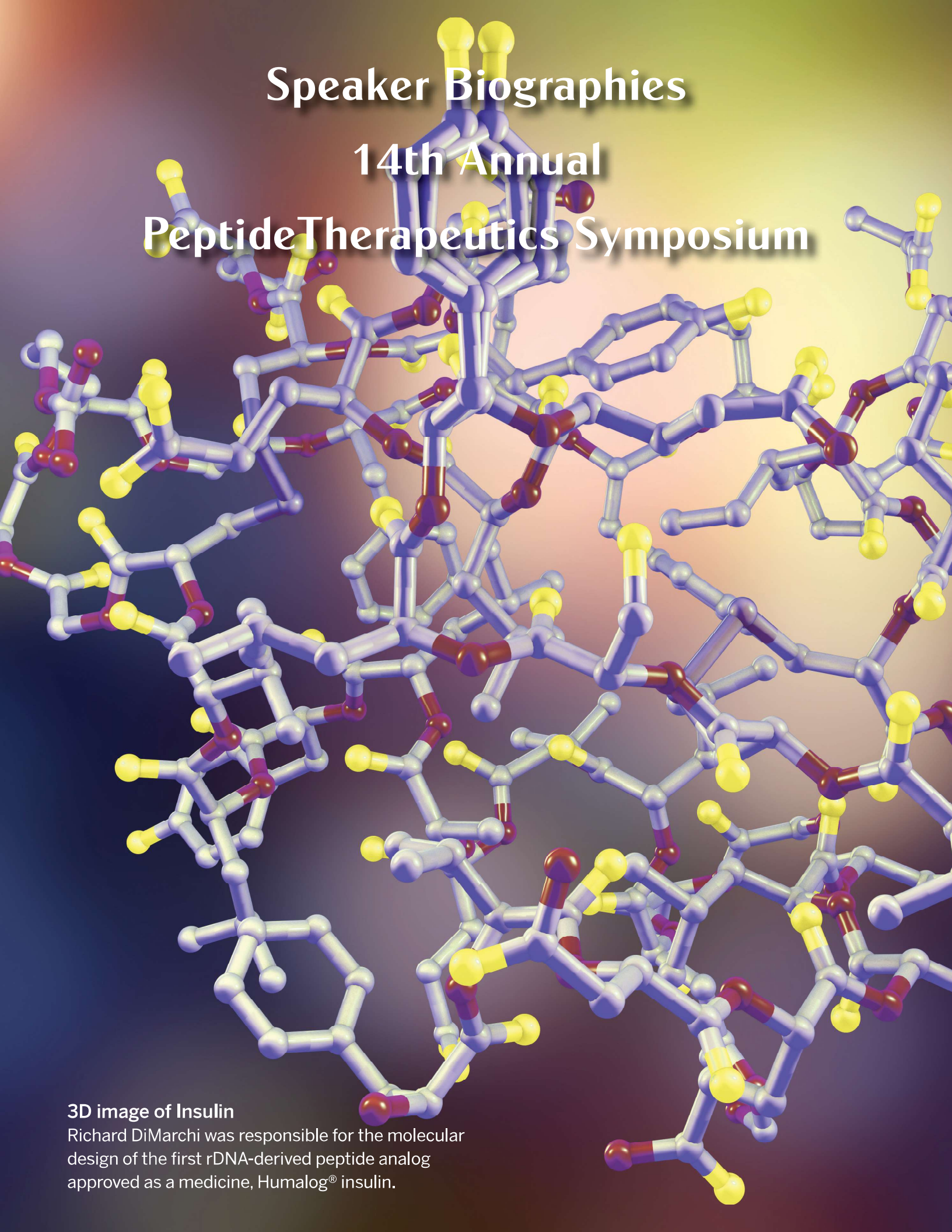

3D image of Insulin
Richard DiMarchi was responsible for the molecular design of the first rDNA-derived peptide analog approved as a medicine, Humalog® insulin.

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Emrah Altindis, Ph.D. | Assistant Professor, Biology Department, Boston College
Discovery and Characterization of Viral Insulin-like Peptides

Emrah Altindis received his B.S. degree from Ege University (Izmir, Turkey) and completed an M.Sc. at the Middle East Technical University (Ankara, Turkey). He then moved to Italy to work at Novartis Vaccines and to start his Ph.D. at Bologna University (Bologna, Italy). During his Ph.D., he developed a novel vaccine discovery tool (Protectome) and identified new immunogenic proteins for two important human pathogens. In 2011, he joined the Mekalanos Lab at Harvard Medical School (HMS) for his first postdoctoral training position to identify new virulence factors in Vibrio cholera (2011-2014). Because he became more interested in the microbiome field, he started a second training in the C. Ronald Kahn's laboratory at Joslin Diabetes Center (HMS). In Kahn lab, he discovered the presence of bioactive viral hormones and characterized viral insulins for the first time. He also worked on two additional projects to explore the role of gut microbiome on metabolic syndrome and Type 1 Diabetes (T1D). During his training, he was awarded the Mary K. Iacocca Postdoctoral Fellowship and received an NIDDK-KO1 Mentored Research Scientist Career Development Award. He is an Assistant Professor at Boston College Biology Department since September 2018 and an Associate Faculty at Harvard Medical School and Joslin Diabetes Center. His laboratory investigates multiple facets of viral insulins, with an emphasis on (i) exploring the new characteristics of these novel insulin/IGF-1 receptor ligands to design better insulin analogs, (ii) understanding their role in Type 1 Diabetes (T1D) autoimmunity and (iii) characterizing the role of viral insulins in host-pathogen interactions.

Jim Ballance, Ph.D. | VP Research & Scientific Affairs, PhaseBio Pharmaceuticals, Inc.
Elastin-Like Polypeptide Biopolymers Enhance the Pharmacology of Therapeutic Peptides

Jim has more than 25 years of experience in the biopharmaceutical industry, in both small biotechnology and large pharmaceutical companies, specializing in early stage research, product development, cGMP manufacturing, technology transfer and the management of intellectual property. He has led the development of drug products from conception through process development and scale-up to manufacturing, and has extensive business development experience with large pharmaceutical and biotechnology companies. Prior to PhaseBio, Jim was Vice President, Technology Development at BioRexis Pharmaceutical and Director of Business Development at Aventis Behring. Jim invented albumin fusion proteins while at Delta Biotechnology in the United Kingdom, where he was head of research and development. Jim received a BSc in applied biology from the University of Wales and a Ph.D. in fungal molecular genetics from the University of Bristol.

Rebecca B. Berlow, Ph.D. | Staff Scientist, Department of Integrative Structural and Computational Biology, Scripps Research
Molecular Mechanisms of Transcriptional Control by Intrinsically Disordered Proteins (aka Peptides)

Rebecca Berlow received her B.A. in Chemistry from the Johns Hopkins University in 2005 and her Ph.D. in Molecular Biophysics and Biochemistry from Yale University in 2011. She was a postdoctoral research associate in the laboratory of Dr. Peter Wright and Dr. Jane Dyson at The Scripps Research Institute from 2011-2018 and was awarded a fellowship from the American Cancer Society for her studies of intrinsically disordered proteins involved in regulation of the cellular response to hypoxia. Rebecca is now a Staff Scientist in the Department of Integrative Structural and Computational Biology at Scripps Research, where she is working with Dr. Wright and Dr. Dyson to further characterize the roles of intrinsically disordered proteins in cellular signaling processes. Her research utilizes a wide range of experimental approaches to integrate biophysical studies of intrinsically disordered proteins with functional studies of proteins and peptides *in vivo*.

Dale L. Boger, Ph.D. | Richard and Alice Cramer Professor of Chemistry, Scripps Research

Redesign of Vancomycin for Resistant Bacteria

Dale Boger received his B.Sc. in chemistry from the University of Kansas (1975, with highest distinction and honors in chemistry) and Ph.D. in chemistry from Harvard University (1980) under the direction of E. J. Corey and supported by an NSF fellowship. He returned to the University of Kansas as a member of the faculty in the Department of Medicinal Chemistry (1979-1985), moved to the Department of Chemistry at Purdue University (1985-1991), and joined the faculty in the newly created Department of Chemistry at The Scripps Research Institute (1991-present) as the Richard and Alice Cramer Professor of Chemistry. From 2012-2018, he served as the Chairman for the Department of Chemistry. Professor Boger is internationally recognized for his work in organic synthesis, heterocyclic chemistry, medicinal chemistry, natural products total synthesis and their biological characterization, synthetic methodology development, and chemical biology, and has made seminal contributions to discovering new therapeutic targets (eg. FAAH, serine hydrolases), improving the glycopeptide antibiotics

Stephen T. Buckley, Ph.D. | Head of Discovery ADME, Novo Nordisk A/S

Oral Delivery of Peptides: Present and Future

Dr. Stephen T. Buckley is Head of Department in the Discovery ADME department at Novo Nordisk A/S, Denmark. He holds a degree in Pharmacy from Trinity College Dublin (Ireland), and a Ph.D. in Biopharmaceutics and Cell Physiology from the same university. During this time, he also worked as a Visiting Fellow at the University of Southern California (USA). Prior to his current position at Novo Nordisk A/S, Dr. Buckley was a Postdoctoral Research Fellow at the University of Southern Denmark (Denmark).

He is the recipient of honors and awards from the American Association of Pharmaceutical Scientists (AAPS) and the Scandinavian and German Physiological Societies. Dr. Buckley's work is focused on understanding the various processes underlying the absorption and distribution of proteins and peptides *via* employment of *in vitro* cellular permeability models, *ex vivo* tissue models and binding assays. In addition to his leadership responsibilities, he is responsible at Novo Nordisk for a team tasked with identifying and evaluating novel drug delivery technologies.

He is (co-) author of 25+ articles in peer-reviewed journals (including *Science and Science Translational Medicine*), 1 book chapter and 30+ abstracts, and has been invited to give numerous presentations at research institutions, international conferences and workshops.

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Jean Chmielewski, Ph.D. | AW Kramer Distinguished Professor, Department of Chemistry, Purdue University

Targeting Intracellular Pathogenic Bacteria with Unnatural Proline-Rich Peptides

Jean Chmielewski completed her Ph.D. in Bioorganic Chemistry with Ronald Breslow at Columbia University. She joined the labs of E. T. Kaiser of Rockefeller University and, subsequently, Peter Schultz of the University of California, Berkeley for NIH postdoctoral fellowships in Chemical Biology. After postdoctoral appointments, she was recruited to the faculty in the Chemistry Department of Purdue University in 1990 where she is now the AW Kramer Distinguished Professor of Chemistry and a faculty member in the Weldon School of Biomedical Engineering. Chmielewski has won numerous awards for her research, including the Arthur C. Cope Scholar Award and the Edward Leete Award, both from the American Chemical Society, the Agnes Fay Morgan Research Award from Iota Sigma Pi, and the Vincent du Vigneaud Award from the American Peptide Society. She has also been honored many times for her teaching, including the Charles R. Murphy Award the highest teaching award of Purdue University, and for her efforts in diversity, including the Stanley C. Israel Award for Advancing Diversity in the Chemical Sciences from the American Chemical Society. She is a fellow of the American Association for the Advancement of Science and a member of the Teaching Academy of Purdue University. Her research interests include the design of novel biomaterials for regenerative medicine, the design of antibiotics that target intracellular pathogenic bacteria, and the development of agents that modulate drug efflux transporters.

Danny Chou, Ph.D. | Assistant Professor of Biochemistry, University of Utah

Mini-Ins: A Minimized, Bioactive Insulin Analog with Fast-Acting Properties Inspired by a Cone Snail Peptide

Danny Chou received his bachelor degree in Chemistry from NTU in 2006. After a year in Army, he went to Harvard for Ph.D. in Stuart Schreiber lab. At Harvard, he developed small-molecule chemical probes to study pancreatic beta-cell apoptosis and received his Ph.D. in 2011. He then moved to MIT as a JDRF Postdoctoral Fellow working with Profs. Robert Langer and Daniel Anderson. At MIT, Danny started the journey of insulin protein engineering. In 2014, he began his independent career in Department of Biochemistry at U of Utah. His lab focuses on developing new insulin therapeutics using a combination of structurally guided designs and library screening. Furthermore, his lab develops chemical probes to study insulin signaling pathways and their implications in human diseases. He has received research supports from federal and private institutes including NIH, DoD, JDRF etc. He is also a recipient of American Diabetes Association Junior Faculty Award and JDRF Career Development Award.

Adrienne Day, Ph.D. | Director, Peptide Therapeutics Foundation; Senior Director, Business Development, Ferring Research Institute, Inc.
Opening Remarks

Dr. Adrienne Day is the Director of the Board of the Peptide Therapeutics Foundation. 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 Sciences, Department of Chemistry, Indiana University; VP and Site Director, Novo Nordisk Research Center, Indianapolis
Closing 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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H. Jane Dyson, Ph.D. | Professor, Department of Integrative Structural and Computational Biology, Scripps Research
Molecular Mechanisms of Transcriptional Control by Intrinsically Disordered Proteins (aka Peptides)

Jane Dyson received the degree of B.Sc.(hons) from the University of Sydney in 1973 and a Ph.D. from the University of Sydney in 1977. She was a postdoctoral fellow at Massachusetts Institute of Technology from 1977-78, and held a Damon Runyon-Walter Winchell postdoctoral award. She was appointed as a Lecturer in Chemistry at the University of New South Wales in 1979, and joined the Scripps Research Institute in 1984, where she is presently a Professor. She received the degree of D.Sc. from the University of Sydney in 2009 and has been awarded the 2019 ISMAR Prize from the International Society of Magnetic Resonance. Her research interests are in the conformation of peptides, protein folding and dynamics, and structure and functional studies of proteins, both folded and intrinsically disordered, using NMR and other spectroscopic techniques. She is currently serving as Editor-in-Chief of the *Biophysical Journal*.

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.

Victor J. Hruby, Ph.D. | Regents Professor, Department of Chemistry and Biochemistry, University of Arizona
Peptide Science 1968-2019, A Retrospective

Professor Victor J. Hruby is a Regents Professor in the Department of Chemistry and Biochemistry at the University of Arizona since 1989. Professor Hruby's research has made significant contributions to chemistry, conformation-biological activity relationships, molecular mechanisms of information transduction and of molecular diseases associated with peptide hormones and neurotransmitters and their receptors. Specific methods and approaches used in this research include: *de novo* design of biologically active peptides and peptidomimetics, peptide and peptidomimetic synthesis, asymmetric synthesis, design and asymmetric synthesis of novel amino acids, computational chemistry, conformational analysis using NMR, X-ray crystallography and other biophysical tools, combinatorial chemistry, conformation-biological activity relationships, the design, synthesis and biological evaluation of peptide and peptide mimetic ligands that affect pain, addictions, feeding behaviors, pigmentation, sexual behavior and motivation, energy homeostasis, CNS diseases, cancer and others, peptide mimetic design, and the structure-function of G-protein coupled receptors. His group also has developed new methodologies for the assembly of multivalent ligands for the detection and treatment of pain, cancer and other diseases, new approaches to design of ligands for disease states involving the concept of overlapping pharmacophores to address several receptors simultaneously in a single ligand. He has over 1200 publications, reviews and editorials, and over 25 patents.

Victor Hruby has received numerous awards and honors, including a Guggenheim Fellowship (1984), the Alan E. Pierce Award (now the Merrifield Award) (1993), a Senior Humboldt Fellowship (1999-2000), the American Chemical Society Ralph F. Hirschmann Award (2002), the ACS Arthur C. Cope Scholar Award (2009), the Murray Goodman Award (2011), the ACS Medicinal Chemistry Hall of Fame (2012) and the Roche Meienhofer Award (2012).

Lotte Bjerre Knudsen, DMSc | Professor, Scientific Corporate Vice President, Global Drug Discovery, Novo Nordisk A/S
GLP-1@Novo Nordisk: Innovating with Fatty Acid Acylation

Lotte Bjerre Knudsen is a Scientific Corporate Vice President in the research organisation of Novo Nordisk in Denmark. Originally trained in biotechnology, she also is a Doctor of Medical Sciences, and an Adjunct Professor in translational medicine at Aarhus University. She has worked for Novo Nordisk for 30 years, and has been involved in the GLP-1 area for more than 20 years as a project manager, working primarily with molecular and in vivo pharmacology. Lotte was responsible for inventing liraglutide, and has published numerous papers on GLP-1, liraglutide, semaglutide, mode(s) of action, toxicology, and receptor expression.

14th Annual Peptide Therapeutics Symposium

Mary R. Lee, M.D. | Associate Research Physician, Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, National Institute on Alcohol Abuse and Alcoholism, National Institute on Drug Abuse Intramural Research Programs

Evidence of Central Signaling after Administration of Oxytocin at Olfactory and Peripheral Sites

I am a physician trained in internal medicine, psychiatry and addiction medicine; I have over 20 years of experience in translational, clinical, and neuroimaging research studies in psychiatry and addiction. At the Intramural Research Program of the National Institute on Alcoholism and Alcohol Abuse as well as the National Institute on Drug Abuse, my research has focused on investigating new therapeutic peptide targets for alcohol and drug addiction and the effect of psychiatric illness and addiction on the expression of endogenous peptides and their receptors in the brain. My nonhuman primate research involves examining central nervous system penetrance of systemically delivered peptides as well positron emission tomography studies to investigate central signaling of systemically delivered peptides. In phase II trials I use laboratory paradigms and neuroimaging (fMRI) to assess the effect of peptides such as oxytocin and ghrelin on response to stress, decision making as wells as appetitive (alcohol, food, social) cues.

Guigen Li, Ph.D. | Paul Whitfield Horn Professor, Department of Chemistry & Biochemistry, Texas Tech University

GAP Chemistry and Multi-Layer 3D Chirality for the Synthesis of Amines, Amino Acids and Peptides

Guigen Li is Paul Whitfield Horn Professor which is the most prestigious honor at Texas Tech University. He hold a distinguished adjunct Professor position at Nanjing University in China where he worked for three years before he came to the US in 1990. He obtained his MS degree at Nankai University in 1987 with the later Professor Zhenheng Gao, Ph.D.in 1995 at University of Arizona with Prof Victor J. Hruby and conducted postdoc research during 1995-1997 at Scripps with K. Barry Sharpless. He was the key member of the Sharpless team who achieved the Sharpless Asymmetric Aminohydroxylation reaction (Sharpless AA) in 1995. Before that 34 coworkers of the Sharpless team had been rendering this reaction for 25 years (at MIT, Stanford and Scripps). At Texas Tech University, Prof Li invented new GAP imines (chiral and achiral N-phosphonyl imines), discovered new diamination reaction of alkenes and multi-layer 3D chirality (organic sandwich chirality). So far, Prof Li achieved over 300 publications, h-index = 53, with 7 paper as highly cited (1%) and one hot paper (<1%). His recent discovery on multi-layer 3D chirality will have a great impact on chemistry, pharmaceutical and material sciences. This novel chirality will provide new attract materials for organic textbooks in future.

Henry I. Mosberg, Ph.D. | Tom D. Rowe Professor of Medicinal Chemistry, College of Pharmacy, University of Michigan

Development of Bifunctional Mu Opioid Receptor (MOR) Agonists/ Delta Opioid Receptor (DOR) Antagonists: Opioid Analgesics with Improved Side Effect Profiles

After receiving his Ph.D. in physical chemistry from the University of Illinois, Dr. Mosberg joined Victor Hruby's group, first as a postdoctoral associate and, later, as an Assistant Research Professor. Inspired by the large number of projects yielding exciting results in Victor's group Mosberg's Interests evolved from spectroscopic studies on peptide conformation to peptide synthesis and medicinal chemistry. Mosberg's first, and still major, interest was in the design and synthesis of novel opioid peptides, which resulted in the development the highly delta opioid receptor selective ligand DPDPE, which remains a standard in the opioid field. Mosberg joined the faculty in the Department of Medicinal Chemistry at the University of Michigan in 1983 and continued work on the development of novel receptor-selective opioid peptides and the elucidation of opioid ligand-receptor interactions. Over the years interest in receptor selective opioids gave way (for reasons that will be discussed) to a focus on bifunctional opioids and the emphasis on peptides was supplanted by an emphasis on peptidomimetics.

Alan T. Remaley, M.D., Ph.D. | Senior Investigator, NHLBI, National Institutes of Health

A Dual ApoC-II Mimetic-apoC-III Antagonist Peptide for Lowering Plasma Triglycerides

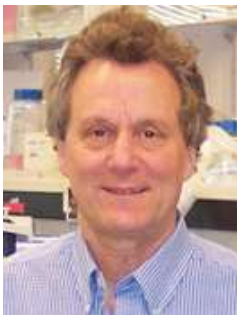
Alan T. Remaley, M.D., Ph.D., is a Senior Investigator in NHLBI at the National Institutes of Health. Dr. Remaley received his B.S. in Biochemistry and Chemistry from the University of Pittsburgh in 1981. He received in 1987 a M.D. and Ph.D. (Biochemistry) degree from the University of Pittsburgh. He completed in 1990 a residency in Clinical Pathology at the University of Pennsylvania and became board certified in Clinical Pathology in 1992. He joined the National Institutes of Health in 1990, as a medical staff fellow, and did a postdoctoral fellowship on lipoprotein metabolism at the Molecular Disease Branch of the National Heart, Lung, and Blood Institute. He became in 1995 a senior staff member of the Department of Laboratory Medicine at the National Institutes of Health, where he directs the Immunoassay and HPLC/Mass Spectrometry laboratories. As of 2005, he became the section chief of the Lipoprotein Metabolism Laboratory at NHLBI. His current research is focused on the mechanism of action of the ABCA1 transporter and Lecithin:Cholesterol Acyltransferase and their role in HDL metabolism. In addition, he is involved in developing new diagnostic immunoassays and lipoprotein assays. He is the author of over 300 publications, mostly in the fields of lipoprotein metabolism and clinical pathology and has 8 patents. He is a member of the editorial board for Clinical Chemistry, Journal of Lipid Research, and Atherosclerosis and an associate editor for Journal of Clinical Lipidology.

Tomi K. Sawyer, Ph.D. | Entrepreneurial Drug Hunter, Maestro Therapeutics

Peptide Drug Hunter: Innovation, Integration and Inspiration

Tomi is the founding Chief Drug Hunter and President of Maestro Therapeutics, an emerging multidisciplinary and innovative enterprise dedicated to transforming and accelerating peptide modality therapeutics. Most recently, Tomi was a Distinguished Scientist, Global Chemistry at Merck & Company, where he led a Peptide Drug Hunter Network of more than 100+ scientists actively engaged in peptide drug discovery, core capabilities and a knowledge engine. Noteworthy, this effort advanced new cell permeability screening tools (NanoClick and TC-PAMPA) and design rules (bRo5-wise) for macrocyclic peptides, including those generated by super-diverse mRNA-display libraries in a strategic R&D collaboration with PeptiDream. Prior to joining Merck & Company in 2014, Tomi was the Founding Chief Scientific Officer at Aileron Therapeutics from 2007 to 2013 and Senior Vice-President of Drug Discovery at Ariad Pharmaceuticals (recently acquired by Takeda) from 1997 to 2006. He is an entrepreneurial drug hunter with about 40-years of industrial experience in both large pharma and biotech. He is well known for his contributions to GPCR, kinase, protease and protein-protein interaction drug discovery. Tomi has three marketed drugs, including Scenesse® (a peptide superagonist of MC1R for the treatment of the orphan skin disease known as erythropoietic protophyria and related indications), Iclusig® (a small-molecule inhibitor of Bcr-Abl kinase for the treatment of clinically-resistance of chronic myelogenous leukemia) and Ridaforolimus (a natural product macrocycle mTOR antagonist for the treatment of coronary artery disease via an EluNIR® stent system). Some other examples of his drug discovery campaigns include peptidomimetic renin inhibitors (Ditekiren and U-84700), the first reported peptidomimetic HIV protease and nonpeptide inhibitors (U-81749 and PD-107067), the first reported peptidomimetic and nonpeptide Src SH2 antagonists (AP21774 and AP22408), the first reported small-molecule dual Src/Bcr-Abl kinase inhibitor (AP23464), and the first stapled peptide dual MDM2/MDMX antagonist (ALRN-6924) to advance into clinical trials. Tomi is credited with ~600 scientific publications, patents, and presentations. His scholarly and scientific awards include a DuVigneaud Award (American Peptide Society), a Distinguished Alumni Award (Minnesota State University Moorhead) and a Professional Award (University of Arizona). He is a member of numerous editorial boards (e.g., Peptide Science), and he was the Founding Editor-in-Chief of Chemical Biology and Drug Design. Tomi is past-President of the American Peptide Society and co-Chair of the Eighteenth American Peptide Symposium. Lastly, Tomi is engaged in the founding of a Peptide Drug Hunter Consortium to establish a global network to empower the advancement of peptide preclinical research and clinical development at the interface of

academia, biotech/pharma and investors as well as contract organizations and vendors engaged in peptide science.Pasquale's laboratory has been to elucidate key signal transduction pathways involved in Eph receptor-dependent regulation of neural function and cancer cell malignancy. Translational aspects of Dr. Pasquale's research include the development of peptides that target Eph receptors and modulate their function, and have resulted in 4 patents and 3 patent applications. Dr. Pasquale has co-authored nearly 200 publications, including highly cited reviews on the Eph/ephrin system as an important regulator of physiological and pathological processes and as a promising therapeutic target.



Peter Senter, Ph.D. | Vice President for Chemistry and Distinguished Research Fellow, Seattle Genetics

Potent Antibody-Based Conjugates for Cancer Therapy: From Early Stage Research to a Clinically Approved Drug

Peter Senter joined Seattle Genetics in August 1998 and has served as Vice President, Chemistry since September 2002. He leads Seattle Genetics' chemistry department, which carries out research in cancer drug design, antibody-drug conjugate technologies for cancer therapy, and protein glycoengineering. Several of the molecules generated in Dr. Senter's lab have entered clinical trials, and two of them have been approved: Adcetris and Etopophos. Dr. Senter has authored more than 140 scientific publications and holds more than 40 issued patents. He received an A.B. in Biochemistry from the University of California, Berkeley, a Ph.D. in Chemistry from the University of Illinois, and did postdoctoral research at the Max Planck Institute of Experimental Medicine in Göttingen, Germany. He is the Senior Editor of Molecular Cancer Therapeutics (published by the American Association of Cancer Research) and serves as an Affiliate Professor of Bioengineering at the University of Washington.

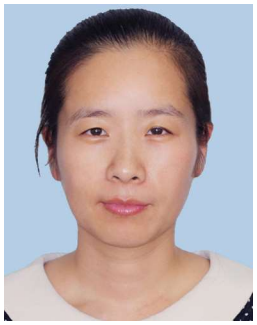
Kazimierz Wisniewski, Ph.D. | Senior Scientist II, Ferring Research Institute, Inc.

Discovery of FE 201836, a Short Acting V2R Agonist for the Treatment of Nocturia

Kazimierz Wisniewski received a Ph.D. in Chemistry at the University of Gdansk, Poland. After postdoctoral studies at Ferring Research Institute, Malmoe, Sweden he became an assistant professor at the University of Gdansk, Department of Chemistry where he carried out research in chemistry of amino acids and peptides.

In 1996 he joined Ferring Research Institute Inc., where he is now Senior Scientist II. Ferring Research Institute Inc., in San Diego, California, is the Research arm of Ferring Pharmaceuticals, headquartered in St. Prex, Switzerland, with development activities in Copenhagen, Denmark and Parsippany, NJ.

As a chemistry lead, Dr. Wisniewski has participated in the discovery of seven peptidic drug candidates currently in various stages of clinical development for women's health, critical care medicine, gastroenterology and urology



Beili Wu, Ph.D. I Professor, Shanghai Institute of Materia Medica, Chinese Academy of Sciences

Structural Basis of Signal Recognition and Regulation at the Full-length Glucagon Receptor

Beili Wu got her Ph.D. degree at Tsinghua University, Beijing in 2006, and worked as a postdoc fellow at Scripps Research Institute in La Jolla, California from 2007 to 2011. She is currently a Professor of Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences. Her current research is focused on a deep understanding of the structural basis of G protein-coupled receptor signaling transduction, leading to the development of new therapeutics for severe human diseases. Dr. Wu and her group have solved the structures of human chemokine receptors CXCR4 and CCR5, purirnergic receptors P2Y1R and P2Y12R, full-length glucagon receptor, and neuropeptide Y receptor Y1R. These structures provide insights about the molecular mechanisms of the interactions between the receptors and their ligands, as well as the inhibition mechanisms of several marketed drugs, which would enable structure-based drug discovery.

Notes

Speaker Abstracts

14th Annual PeptideTherapeutics Symposium

Discovery and Characterization of Viral Insulin-like Peptides

Emrah Altindis, Ph.D. | Assistant Professor
Biology Department, Boston College
Higgins Hall 515, 140 Commonwealth Avenue, Chestnut Hill, MA 02467

Martina Chrudinova[1], Francois Moreau[2], HyeLim Noh[3], Jason Kim[3], Fa Zhang[4], Richard DiMarchi[4], C. Ronald Kahn[2], Emrah Altindis[1]* * Corresponding author
[1]Boston College Biology Department; [2]Joslin Diabetes Center; [3]University of Massachusetts Medical School; [4]Indiana University, Chemistry Department

Viruses are the most abundant biological entities and carry a wide variety of genetic material, including the ability to encode host-like proteins. Using a bioinformatics analysis, we showed that four different viruses belonging to the Iridoviridae family carry sequences with significant homology to human insulin and insulin-like growth factor-1 (IGF-1). Insulin and IGF-1 are structurally related peptide hormones that are involved in the control of cell growth and metabolism and their misbalance in the organism is connected to many pathological states such as diabetes, metabolic syndrome, growth problems, and cancer. The newly discovered viral insulin-like peptides (VILPs) show up to 50% homology to human insulin/IGF-1. We previously showed that chemically synthesized single-chain VILPs can bind to human IGF-1/insulin receptors and stimulate downstream signaling. VILPs can also stimulate the proliferation of human fibroblasts and stimulate glucose uptake in vitro and in vivo. In this study, we used a euglycemic hyperinsulinemic clamp in mice model to test the in vivo properties of two double chain VILPs. While Lymphocystis virus-1 (LCDV-1) VILP could not stimulate the in vivo glucose uptake, Grouper Iridovirus (GIV) VILP stimulates in vivo insulin signaling in liver, skeletal muscle, and white adipose tissue. Interestingly, the potency of GIV-VILP on white adipose tissue is significantly higher than human insulin and stimulates significantly more glucose uptake. These results support the hypothesis that VILPs might have an important role in human disease. Moreover, the information regarding the specificity of GIV VILP to white adipose tissue has the potential to help us design better insulins for obese-diabetes patients

Elastin-Like Polypeptide Biopolymers Enhance the Pharmacology of Therapeutic Peptides

Jim Ballance, Ph.D. | VP Research & Scientific Affairs
PhaseBio Pharmaceuticals, Inc.
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Elastin-like polypeptide (ELP) biopolymers are based on a five-amino acid repeat motif found in the human protein elastin. Recombinant ELP biopolymers or fusion proteins comprising ELP and an active peptide moiety undergo a fully reversible, temperature-dependent phase transition. ELP fusion proteins can be engineered such that the transition temperature is slightly below body temperature, leading to formation of a coacervate or depot at the subcutaneous injection site from which the fusion protein is slowly released. This unique sustained release mechanism leads to prolonged exposure, enabling injection once a week or less. ELP fusions to active peptides are generally active as fusions proteins, but can also be engineered to enable release of the active moiety.

PhaseBio's clinical pipeline includes PB1046, a long-acting vasoactive intestinal peptide ELP fusion for treatment of pulmonary arterial hypertension, a progressive and life-threatening orphan disease with no known cure. Native VIP is rapidly degraded, and, when injected into the body, is eliminated within minutes, limiting its therapeutic effect. On the other hand, high levels of native VIP can result in severe gastrointestinal problems due to activation of the VPAC1 receptor. We have used our ELP technology to extend the half-life of VIP in PB1046 following subcutaneous injection to approximately 60 hours. In addition, we designed PB1046 to be active predominantly on the VPAC2 receptor rather than VPAC1 in order to preferentially affect the lung and cardiac tissue and reduce the potential for gastrointestinal side effects associated with VPAC1 activation.

Molecular Mechanisms of Transcriptional Control by Intrinsically Disordered Proteins (aka Peptides)

Rebecca B. Berlow, Ph.D. | Staff Scientist
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H. Jane Dyson, Ph.D. | Professor
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Rebecca B. Berlow, Peter E. Wright and H. Jane Dyson

Disordered proteins and disordered segments of larger proteins play important roles in protein-protein interactions in many different metabolic processes. Cellular signaling is vitally dependent on the presence of disorder in the interacting proteins (1) – the same polypeptide can make interactions with many partners, leading to pathway cross-talk, and combinatorial post-translational modifications turn signals on and off. We study disordered proteins and their interactions in solution, primarily using NMR spectroscopy. The transcriptional consequences of hypoxia signaling are largely mediated by the interactions of disordered protein segments of two proteins: the C-terminal transactivation domain of the hypoxia-inducible factor HIF-1α and the transactivation domain of its negative feedback regulator CITED2. Both the HIF-1α and CITED2 peptides bind tightly to the TAZ1 domain of the transcriptional coactivator CREB-binding protein (CBP), in partially-overlapping binding sites. CITED2 rapidly displaces HIF-1α from TAZ1 through a unidirectional molecular switch that involves a short-lived ternary complex of TAZ1, HIF-1α and CITED2 (2). NMR studies of the dynamics of TAZ1 in the two binary complexes further show that the mechanism of negative feedback by CITED2 is strongly dependent on the allosteric response of TAZ1 to peptide binding (3). The mechanistic insights afforded by these fundamental studies into the molecular mechanism of these interactions have allowed us to re-think possible therapeutic strategies targeting hypoxia signaling.

References:
(1) Intrinsically disordered proteins in cellular signalling and regulation. P.E. Wright and H.J. Dyson (2015) *Nat. Rev. Mol. Cell Biol.* **16**, 18-29.
(2) Hypersensitive termination of the hypoxic response by a disordered protein switch. R.B. Berlow, H.J. Dyson and P.E. Wright (2017) *Nature* **543**, 447-451.
(3) Role of backbone dynamics in modulating the interactions of disordered ligands with the TAZ1 domain of the CREB-binding protein. R.B. Berlow, M.A. Martinez-Yamout, H.J. Dyson and P.E. Wright (2019) *Biochemistry* **58**, 1354-1362.

Redesign of Vancomycin for Resistant Bacteria

Dale L. Boger, Ph.D. | Richard and Alice Cramer Professor of Chemistry
Department of Chemistry, Scripps Research
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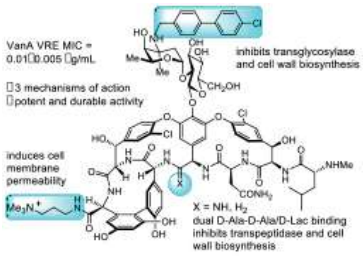
A summary of studies on the total synthesis and evaluation of the vancomycin family of glycopeptide antibiotics, their ligand binding pocket redesign to address the underlying molecular basis of resistance, and their subsequent peripheral tailoring to address the emerging public health problem of vancomycin resistance will be presented.



Oral Delivery of Peptides: Present and Future

Stephen T. Buckley, Ph.D. | Head of Discovery ADME
Novo Nordisk A/S
Novo Nordisk Park, 2760 Maaloev, Denmark

Peptides such as insulin and glucagon-like peptide-1 (GLP-1) receptor agonists are used in the treatment of type 2 diabetes. The inherent physicochemical properties of these peptides (high molecular weight, enzymatically labile, hydrophilicity, and low permeability) have hampered attempts to deliver peptides via the oral route and necessitated that they be administered by injection. Recently, oral semaglutide, a GLP-1 analog, coformulated with the absorption enhancer sodium N-[8-(2-hydroxybenzoyl) aminocaprylate] (SNAC) in a tablet has completed Phase III clinical trials in more than 9000 patients. This coformulation provides unique, site-directed release and absorption in the stomach and effectively surmounts inherent challenges relating to solubility, molecular size, and proteolytic lability to achieve therapeutically relevant plasma exposure of semaglutide. Even more recently, another breakthrough technology exploiting the stomach as a delivery site has emerged – an ingestible self-orienting millimeter-scale applicator (SOMA) that autonomously positions itself to engage with gastric tissue and deploys milliposts fabricated from peptides or proteins directly through the stomach mucosa, which result in plasma levels comparable to those achieved with subcutaneous millipost administration in pre-clinical animal models. This presentation will provide a review of the aforementioned scientific advancements within oral peptide delivery and reflect upon the current and future prospects in this field.

Targeting Intracellular Pathogenic Bacteria with Unnatural Proline-Rich Peptides

Jean Chmielewski, Ph.D. | AW Kramer Distinguished Professor
Department of Chemistry, Purdue University
560 Oval Drive, West Lafayette, IN 47907

A significant challenge in the development of effective antibacterial agents arises from bacterial pathogens that have evolved to inhabit mammalian cells, such as phagocytic macrophages. Within these intracellular safe havens the bacteria reproduce and form a repository, and are able to evade the host immune response as well as a number of antibiotic drugs. Therefore, there is a great need to develop antibiotics with the ability to enter mammalian cells and target intracellular pathogens at their specific sub-cellular site. We have developed a class of molecules, cationic amphiphilic polyproline helices (CAPHs), that enter mammalian cells through both direct transport and endocytosis. We have determined that CAPHs also have potent antibacterial activity *in vitro*, with additional activity against a range of preformed biofilms. This dual mode of action, antibacterial activity with the ability to localize within mammalian cells, provided us with agents with a pronounced ability to target and kill pathogenic intracellular bacteria. Applications, including wound healing, will be discussed.

Mini-Ins: A Minimized, Bioactive Insulin Analog with Fast-Acting Properties Inspired by A Cone Snail Peptide

Danny Chou, Ph.D. | Assistant Professor of Biochemistry
University of Utah, School of Medicine, Department of Biochemistry
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Human insulin and its current therapeutic analogs self-associate into dimers and hexamers, which delays their onset of action and makes blood glucose management difficult for people with diabetes. Recently, we described a monomeric, insulin-like peptide in cone-snail venom with moderate human insulin bioactivity. Here, with insights from structural biology studies, we report the development of mini-Ins — a human des-octapeptide insulin analog — as a structurally minimal, bioactive insulin. Mini-Ins is monomeric, has similar receptor binding affinity to native insulin, having four distinct mutations that increase its affinity to human insulin receptor. It also has similar in vitro insulin signaling and *in vivo* bioactivities, and demonstrates faster onset action in a diabetic pig model than currently available insulins. The full bioactivity of mini-Ins demonstrates the dispensability of the B24-B26 aromatic triplet and opens a previously unexplored analog direction. Mini-Ins is a promising therapeutic candidate for a prandial insulin and a foundation for the development of further insulin analogs.

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

Peptide Science 1968-2019, A Retrospective

Victor J. Hruby, Ph.D. | Regents Professor
Department of Chemistry and Biochemistry, University of Arizona
1306 East University Boulevard, Tucson, AZ 85721

A Perspective on Fifty-plus Years as a Professor in Peptide Science

Peptide Science was in its relative infancy when I became an Assistant Professor in 1968. Little was known about the conformation and dynamics of peptides, solid phase synthesis was in its infancy, the relationships between structure and biological activities were largely unknown, and peptides did not "behave" as most organic compounds. Our initial goals were therefore to develop solid phase synthesis and new purification methods, establish NMR and biophysical tools to elucidate peptide structures and conformations, and discover the targets for peptide hormones and neurotransmitters. These studies naturally led to the robust development of NMR, CD, ORD and other biophysical tools, including computational methods and force fields for the study of peptide conformations and their relationships to biological activities. This is turn led to the design, asymmetric syntheses and structures of novel amino acids designed in Ramachandran and chi space, and to the design of novel cyclic peptides and peptidomimetics with novel conformations and structure-activity relationships. Novel biologically active peptides and their receptors/acceptors were being discovered which led to many novel opportunities for the design, syntheses and biological evaluation of peptides and peptidomimetics. Close collaborations with biologists and medical doctors provided the opportunities for many new discoveries and developments in peptide science. The molecular biology revolution has transformed peptide science into a multidisciplinary science, involving chemistry, physics, biology, medicine and mathematics. Our exciting journey will be briefly discussed.

Supported by grants from NIH, NSF and several companies

GLP-1@Novo Nordisk: Innovating with Fatty Acid Acylation

Lotte Bjerre Knudsen, DMSc | Professor, Scientific Corporate Vice President
Novo Nordisk A/S, Global Drug Discovery
Novo Nordisk Park, 2760 Maaloev, Denmark

The receptor for the incretin hormone Glucagon-Like Peptide-1, the GLP-1R, is a family B GPCR characterized by having no subtypes or RAMP associations, and with a very broadly applicable biology leading to receptor agonist drug approvals for treatment of both type 2 diabetes and obesity, documented cardiovascular risk reduction, and several other potential drug indications under testing. Liraglutide is the first GPCR agonist discovered and marketed by Novo Nordisk, designed as a fatty acid acylated long-acting GLP-1R agonist (GLP-1RA). The protraction is obtained by binding to serum albumin non-covalently via the fatty acid binding sites. Semaglutide is the 2nd generation GLP-1RA from NN, designed basically by using the same fatty acid protraction technology as liraglutide but substantially optimized to have a higher affinity specific albumin binding, better potency and brain uptake, the latter thought to be the mechanism behind the superior clinical efficacy now reported in several large randomized controlled clinical trials. Understanding the GLP-1R structure has been a strong scientific foundation leading to design of better agonist compounds, and has provided structural input to engineering specific antibodies against the GLP-1R. These antibodies have made it possible to identify highly specific localizations of the GLP-1R in both endocrine and neuronal sites of actions. GLP-1Rs in the pancreas and brain have been shown to account for the respective improvements in glycemic control and body weight that are evident with liraglutide and semaglutide. Both liraglutide and semaglutide also positively affect cardiovascular (CV) outcomes in individuals with T2D. Although the precise mechanism is still being explored, it is hypothesized that long-acting GLP-1RAs reduce systemic inflammation, leading to reduced atherosclerotic burden. Significant weight loss, through an effect to reduce energy intake, led to the approval of liraglutide (3.0 mg) for the treatment of obesity, an indication currently under investigation with semaglutide. The mechanism for weight loss is reduced energy intake, mediated by brain GLP-1Rs. More specifically the mechanism leads to reduced overall appetite (increased satiety and reduced hunger) as well as effects on the reward system resulting in reduced craving for food, and improved food choices. Other ongoing investigations with semaglutide include the treatment of nonalcoholic fatty liver disease (NASH), this programme is in phase 2 clinical testing, and its use in an oral formulation for the treatment of T2D, this programme has completed phase 3 clinical testing. There may also be a potential for GLP-1 in neurodegenerative diseases like Alzheimer's, Parkinson's and traumatic brain injury.

Evidence of Central Signaling after Administration of Oxytocin at Olfactory and Peripheral Sites

Mary R. Lee, M.D. | Associate Research Physician
Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology
National Institute on Alcohol Abuse and Alcoholism and National Institute on Drug Abuse Intramural Research Programs
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Preclinical studies suggest that central endogenous signaling of the nine amino acid peptide, oxytocin (OT) is altered in drug and alcohol addiction. There are preliminary preclinical and clinical studies indicating that administration of OT reduces addiction related behaviors such as self-administration, conditioned place preference and withdrawal symptoms. As such, OT may represent a novel treatment for alcohol and drug dependence. However, there are still many unanswered questions that limit further clinical development of this promising treatment, such as the brain penetrance of OT when given systemically via intranasal or intravenous routes. Another question relates to its mechanism of action to mediate processes related to addiction such as its modulation of DA signaling in mesocorticolimbic pathways. We have conducted a series of nonhuman primate studies to investigate the brain tissue penetrance of labelled OT administered intravenously and intranasally, measuring labelled and endogenous OT by mass spectrometry. Labelled OT was detected in several brain regions and regional differences in endogenous brain OT were detected. We have also measured endogenous and administered labelled OT in the CSF over a prolonged time course (2 hours). We conducted an [11C] raclopride positron emission tomography (PET) study in nonhuman primates (N= 6 male rhesus macaques) to investigate the effect of intravenous OT (80 IU) on [11C] raclopride binding potential in the striatum after an IV methylphenidate challenge. In the caudate and ventral striatum, there was a significant main effect of methylphenidate ($p<0.001$), a main effect of OT ($p=0.01$) and a significant OT x methylphenidate interaction ($p=0.04$) where OT reduced the raclopride binding potential and attenuated the methylphenidate induced reduction in raclopride binding potential. These results indicate a possible mechanism underlying the effect of OT to reduce the rewarding effect of psychostimulants as reported in previous preclinical studies. The demonstration of this effect in nonhuman primates has translational relevance particularly as the brain receptor distributions of OT vary considerably between rodents and primate species. Finally, we have conducted human brain post-mortem studies in patients with AUD as well as individuals with other psychiatric disorders (with and without substance and alcohol use disorder comorbidity) that indicate changes in the oxytocin system (peptide or receptor) in the patient cohorts, compared to matched controls.

GAP Chemistry and Multi-Layer 3D Chirality for the Synthesis of Amines, Amino Acids and Peptides

Guigen Li, Ph.D. | Paul Whitfield Horn Professor
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Group-Assisted Purification (GAP) chemistry is the first concept consisting of both chemical and physical aspects: reagent, reaction, separation, and purification. By taking advantage of GAP chemistry, the synthesis of organic products including amino acids and peptides can be conducted without the need of column chromatography and recrystallization. GAP functional groups can convert oily and sticky products into their solid forms, and often enable single crystals to be formed more easily than traditional methods. Essentially, pure GAP products can be obtained simply by washing crude mixtures with common solvents and/or co-solvents. Therefore, GAP chemistry has the advantages of both solution-phase and solid-phase, but can avoid their disadvantages. This talk will cover the GAP synthesis of amino acids and peptides; GASyn chemistry (Group-Assisted Synthesis chemistry) to maximize outcomes of reactions; auto solution-phase peptide synthesizer developed by GAPetides, LLC. (www.GAPPeptides.com). Finally, a conceptually new concept, multi-layer 3D chirality and its applications for the design, synthesis and structural control for amino acids and peptidomimetics will be discussed.

Development of Bifunctional Mu Opioid Receptor (MOR) Agonists/ Delta Opioid Receptor (DOR) Antagonists: Opioid Analgesics with Improved Side Effect Profiles

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Opioids have long been the gold standard for treatment of chronic and severe pain, however serious side effects vitiate the use of these potent analgesics. The development of tolerance and dependence, as well as constipation, limit the value of long-term opioid use, while the euphoria elicited by opioids, coupled with physical dependence lead to addiction. This addiction drives the trade in illicit opioids of variable and uncertain potency which contribute to the epidemic of opioid deaths due to respiratory depression.

Several approaches have shown promise for the development of opioid analgesics with reduced side effects, among which is simultaneous agonism at MOR and antagonism at DOR. We have developed several series of bifunctional MOR agonist/DOR antagonist peptidomimetics that show promise as leads for potential opioid analgesics with sharply attenuated side effect profiles. This presentation will focus on one of these, AAH8. AAH8 displays antinociception in several murine pain models and is approximately equipotent with and has a similar duration of action as morphine. In contrast to morphine, chronic administration of escalating doses of AAH8 does not result in tolerance or dependence. Further, AAH8 does not exhibit rewarding properties, does not substitute for morphine in animals trained to the latter, and is not self-administered. These and other features of AAH8 will be described.

A Dual ApoC-II Mimetic-apoC-III Antagonist Peptide for Lowering Plasma Triglycerides

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Recent genetic studies have established that hypertriglyceridemia (HTG) is causally related to cardiovascular disease, making it an active area for drug development. We describe a new strategy for lowering triglycerides (TG) with an apolipoprotein C-II (apoC-II) mimetic peptide called D6PV that not only activates lipoprotein lipase (LPL), the main plasma TG-hydrolyzing enzyme, but also antagonizes the TG-raising effect of apoC-III. The design of D6PV was motivated by a combination of all-atom molecular dynamics simulation of apoC-II on the Anton 2 supercomputer, structural prediction programs, and assorted biophysical techniques. The efficacy of D6PV, a bi-helical peptide containing 40 amino acid resisues, was first assessed ex vivo in human HTG plasma and was found to be more potent than full-length apoC-II in activating LPL. D6PV markedly lowered TG by more than 80% within a few hours in both apoC-II-deficient mice and hAPOC3-transgenic (Tg) mice. The peptide displaced apoC-III from TG-rich lipoproteins, leading to increased renal clearance of apoC-III. Low-density lipoprotein (LDL) cholesterol did not accumulate, and in fact decreased by 10%, when hAPOC3-Tg mice lacking the LDL-receptor (hAPOC3-Tg x Ldlr-/-) were treated with the peptide. D6PV lowered TG by 50% in whole-body inducible Lpl knockout (iLpl-/-) mice, confirming that it can also act independently of LPL. Furthermore, D6PV displayed good subcutaneous bioavailability. Because it binds to high-density lipoproteins, which serve as a long-term reservoir, it has an extended half-life (42-50 h) in non-human primates. In summary, D6PV decreases plasma TG by acting as a dual apoC-II mimetic and apoC-III antagonist, thereby demonstrating its potential as a treatment for HTG.

Peptide Drug Hunter: Innovation, Integration and Inspiration

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Since the beginning of the new millennium, there has been a re-emergence of peptide drug discovery tackling compelling extracellular/receptor and intracellular targets. Significantly, this effort will impact the global market in terms of expanding the molecular armamentarium of therapeutic modalities yet needed to conquer debilitating and/or deadly diseases. This lecture will share a perspective of peptide drug discovery with respect to innovation, integration and inspiration, and such will reflect upon my 40-year career that began under the mentorship of Victor J. Hruby at the University of Arizona. In particular, I will highlight progress toward the design and development of peptide modalities to modulate intracellular target functions, including work on stapled α-helical peptides. This has leveraged new tools to understand cellular permeability and some key biophysical property relationships.

Potent Antibody-Based Conjugates for Cancer Therapy: From Early Stage Research to a Clinically Approved Drug

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Monoclonal antibodies (mAbs) have played a major role in cancer medicine, with active drugs such as trastuzumab (Herceptin), pembrolizumab (Keytruda), bevacizumab (Avastin) and rituximab (Rituxan) in a wide range of therapeutic applications. The mechanisms of these agents involve such activities as direct signaling, interactions with Fcγ receptors on effector cells, complement fixation, and stimulation of cells in the tumor micro-environment. Several approaches have been explored to improve antibody-based therapies for cancer treatment by optimizing such activities and by conscripting their selectivity profiles for the delivery of high potency cytotoxic drugs. The field has advanced significantly, with the approval of Adcetris (brentuximab vedotin) for the treatment of relapsed Hodgkin and anaplastic large cell lymphomas. The drug is comprised of a potent antimitotic agent, monomethyl auristatin E (MMAE), conjugated to an anti-CD30 mAb through a lysosomally cleavable dipeptide linker. Since the approval of this drug, several others have also become approved, and many are in late stage clinical development. Advancements have been made in delivery, control of toxicity, biodistribution, pharmacokinetics, and several other critical parameters. This presentation will describe the discovery and development of Adcetris, and will cover several of the new developments in the area of antibody-drug conjugates for cancer therapy.

Discovery of FE 201836, a Short Acting V2R Agonist for the Treatment of Nocturia

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The vasopressin analogue desmopressin, dDAVP, is a potent and moderately selective V2 receptor agonist that also activates the related V1b receptor. Desmopressin is approved in many countries for the treatment of diabetes insipidus, primary nocturnal enuresis, nocturia, and coagulation disorders including hemophilia A and von Willebrand's disease. Hyponatremia is the most frequently encountered V2R agonist treatment-related plasma electrolyte abnormality in humans and is a known dose-limiting adverse effect of dDAVP. Since the compound is primarily excreted via the kidneys, an age-related decline in kidney function leads to slower elimination and at times undesirable prolonging of antidiuresis effect. A drug discovery program to identify novel, potent, selective and pharmacologically useful peptidic V2R agonists was initiated at Ferring. We designed and synthesized a series of C-terminally truncated analogues of [Val4]dDAVP, modified in positions 2, 3, 7 and/or at the disulfide bridge and evaluated the peptides for in vitro potency at the hV2 receptor, selectivity versus the related receptors (hV1aR, hV1bR, hOTR) and pharmacokinetic profiles in rats and other higher species. The truncated analogs show excellent potency at the V2R, increased CL mediated by proteolytic mechanisms in addition to renal clearance and resulting shorter half-life in rats. High potency and short duration of action for selected peptides was confirmed in a rat antidiuresis model. Two compounds FE 201836 (c(Bua-Cpa-Thi-Val-Asn-Cys)-Pro-Agm) and FE 202217 (c(Bua-Cpa-Thi-Val-Asn-Cys)-Pro-D-Arg-NEt2) have been selected for clinical development for nocturia and other water retention diseases.1 The details of the drug discovery program including the pharmacological profiles of the two aforementioned molecules that led to their nomination as clinical candidates as well as their current development status will be presented.

[1] Discovery of Potent, Selectiv125 Croston, E. La, H. Wisniewska, C. Ortiz, R. Laporte, P. J-M. Rivière, G. Neyer, D. M. Hargrove, C. D. Schteingart. J. Med. Chem. 2019, 62, 4991–5005. DOI: 10.1021/acs.jmedchem.9b00132

Structural Basis of Signal Recognition and Regulation at the Full-length Glucagon Receptor

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The human glucagon receptor (GCGR) belongs to the class B G protein-coupled receptor (GPCR) family and plays a key role in glucose homeostasis and the pathophysiology of type 2 diabetes. Here we report two crystal structures of full-length GCGR containing both extracellular domain (ECD) and transmembrane domain (TMD) at different coformational states. Notably, the stalk region, which connets the ECD and TMD, and the first extracellular loop (ECL1) undergo major conformational changes in secondary structure during peptide ligand binding, forming key interactions with the peptide. Hydrogen/deuterium exchange, disulfide cross-linking and molecular dynamics studies suggest that the stalk and ECL1 play critical roles in modulating peptide ligand binding and receptor activation. We further propose a dual-binding-site trigger model for GCGR activation, which requires conformational changes of the stalk, ECL1 and TMD. These insights into the full-length GCGR structure deepen our understanding about the signaling mechanisms of class B GPCRs.

Notes

Poster Abstracts

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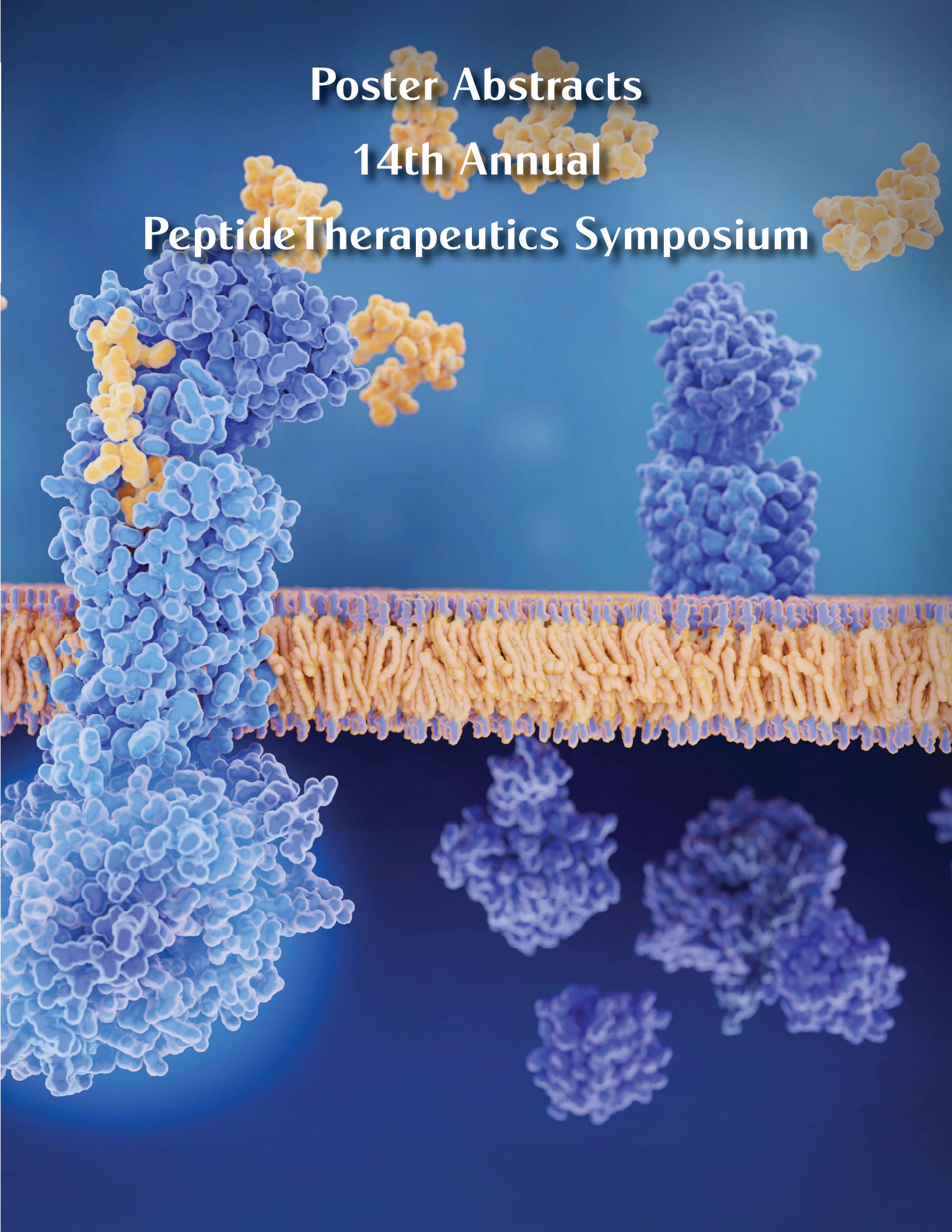

P01 High Throughput Discovery of Novel Peptides with Biological Function

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Peptides are highly active biological molecules that, with advances in stability and delivery, present huge potential as drug candidates. With an increased interest in the use of peptides as therapeutics comes the need for strategies to allow for the discovery of novel hit candidates, in a high throughput manner, from highly complex peptide libraries. Current technologies for the discovery of novel peptide therapeutics include phage, yeast, ribosomal and mRNA display but these all hold their own limitations. Phage and yeast display are sensitive but a reliance upon a biological host can result in replication bias and interference from native host proteins. These systems are also restricted to the use of natural amino acids. In vitro technologies based on mRNA and ribosomal display have the ability to incorporate unnatural amino acids but are limited by small peptide copy number leading to a reduced detection of low affinity binders. Moreover, none of the current peptide display technologies can effectively address cell surface targets for the discovery of functional peptides. The ORBIT in vitro display technology uses beads to present randomized peptide sequences and the DNA which encodes them, thereby linking genotype to phenotype, and combines the advantages of in vitro display, such as highly diverse libraries and a cell-free environment, with the high sensitivity of in vivo technologies. The ORBIT display platform presents thousands of identical peptide copies per bead allowing for the discovery of low affinity binders and allows for the incorporation of unnatural amino acids and the generation of cyclic peptides which can increase chemical diversity and improve stability, specificity and affinity. In addition to screening for binding peptides, the ORBIT platform can be adapted to screen whole cell surfaces for peptides which elicit functional cellular responses. In summary, the ORBIT peptide display platform offers peptide drug screening with high diversity, sensitivity and plasticity, and has the potential to discover novel, selective and functional peptide therapeutic leads with a broad target coverage, including cell surface targets, for a wide range of diseases.

P02 High-Efficiency Solid Phase Synthesis of Peptides and Peptidomimetics with the Liberty Blue

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The development of peptide-based therapeutics and drug delivery systems is continually increasing, due in part to the high selectivity of peptide-receptor interactions.[1] In addition, peptides typically exhibit low tissue accumulation and, therefore, reduced toxicity.[2] While these traits are advantageous, they are not without shortcomings; peptides can exhibit low oral bioavailability and are subject to ready proteolytic degradation.

To combat these limitations, new peptide modifications and peptidomimetic systems are being developed and tested in order to increase bioavailability and resist proteolytic degradation.[3] Highly efficient synthetic strategies are paramount for the development and application of these systems; increased production capabilities, while limiting waste generation and minimizing time requirements, are necessary. The Liberty Blue automated microwave peptide synthesizer offers all of these advantages, plus the flexibility to readily incorporate a multitude of peptide modifications and peptidomimetic systems, including cyclic peptides, peptoids, and glycosylated motifs.

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P03 Structure-based Drug Design (SBDD) and In Silico Pharmacophore Screening Approaches Combined with Biochemical Validation Enabled the Discovery of Di- and Tetrapeptides Inhibitors of Y-49 and ampC Beta Lactamases

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The hydrolysis of β-lactam drugs by β-lactamases is a highly effective resistance mechanism to β-lactam based antibiotics. Among the most promising strategies yet developed for solving this type of resistance problem was the discovery of new non β-lactam scaffolds inhibitors of β-lactamases. Herein we report the SBDD and pharmacophore-based approaches for the discovery of linear and cyclic peptides inhibitors of the Y-49 enzyme and ampC β-lactamases. *In silico* docking experiments were performed with Autodock Vina (Scripps Institute, USA). The beta-lactamases 3M6B.pdb, 1PZP.pdb and 1FSY.pdb were used as target proteins while tetrapeptides with the sequence space [2HN-R-X-H-Y-COOH] were docked as potential active site-directed inhibitors (where X=natural and unnatural L/D-amino acids). We have already reported the discovery of linear tetrapeptides with inhibitory activity in the 50-1.0 uM range for Ki, against Y-49 β-lactamase-mediated hydrolysis of nitrocefin substrate. We further expanded the peptide-pharmacophore space that lead to the discovery of cyclo [RRXY] peptide exhibiting at least six-fold higher affinity for the Y-49 β-lactamase than its linear analogue, RRHY. Moreover, we further discovered novel dipeptide-pharmacophores derived from 1,2,3,4-tetrahydroharmane-3-carboxylic acid exhibiting promising inhibitory activity against Y-49 and ampC β-lactamases. The tetrapeptides and dipeptides lead compounds were tested for their activity on the *in vitro* growth of mc^{2}6230 (*M. tuberculosis* H37Rv *RD1 panCD*) and the lowest concentration of beta lactamase inhibitors that prevented the bacterial growth was in the order of 100-500 uM. Our data support the discovered pharmacophore features as an alternative chemical space aimed to enable the discovery of potent peptide inhibitors of β-lactamases.

P04 The Effects of Novel Myristic Acid Conjugated Protein Kinase C Beta II Activator and Inhibitor Peptides on Phorbol 12-myristate 13-acetate-induced Superoxide Release in Isolated Rat Polymorphonuclear Leukocytes

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Polymorphonuclear leukocyte (PMN) superoxide (SO) production triggered by protein kinase C (PKC) phosphorylation of NADPH oxidase (NOX) contributes to myocardial remodeling following ischemia-reperfusion (I/R) injury. Phorbol 12-myristate 13-acetate (PMA) is a known broad-spectrum PKC agonist that induces PMN SO release. Previous studies have suggested that the PKC beta-II (βII) isoform is a principal mediator of this response. We propose to confirm the role of PKCβII by using selective, cell permeable myristic acid (myr-) conjugated activator (N-myr-SVEIWD; myr-PKCβII+) and inhibitor (N-myr-SLNPEWNET; myr-PKCβII-) peptides that influence PKCβII translocation to cell membrane substrates (e.g. NOX-2). We hypothesize myr-PKCβII+ would augment and myr-PKCβII- would attenuate PMA-induced SO release compared to non-treated and native peptide controls. Rat PMNs (5x10^6) were incubated in the presence/absence of SO dismutase (SOD; 10μg/mL) positive control, native or myr-PKCvII+/- (20 μM). SO release was evaluated by the absorbance change (at 550 nm) due to ferricytochrome c reduction after PMA stimulation (100 nM). Myr-PKCβII- significantly attenuated SO release (0.28±0.03; n=27; p<0.01) when compared to myr-PKCβII+ (0.42±0.03; n=30), non-treated controls (0.41±0.02; n=46), and native PKCβII- (n=20; p<0.05). Native peptides showed similar SO release as non-treated control. SOD (n=8) significantly reduced SO release by 94±7% compared to all groups (p<0.01). Cell viability determined by 0.2% trypan blue exclusion showed no significant reduction among all groups (94±2%). Results suggest myr- conjugation improved myr-PKCβII- delivery compared to native-PKCβII-. Further experiments are required to explain the lack of significance with myr-PKCβII+ to augment PMA-induced SO release, whereas myr-PKCβII- may effectively mitigate PMN SO-mediated I/R injury.

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

P05 The Effects of Novel Myristoylated PKC Epsilon Peptide Activator and Inhibitor on Nitric Oxide Release in Cultured Human Umbilical Vein Endothelial Cells

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Protein kinase C (PKC) isoforms are known to participate in pre-conditioning or post-conditioning against ischemia-reperfusion (I/R) injury. Normally, following diacylglycerol-dependent activation, PKC epsilon (PKCε) binds to isoform-specific receptors for activated C-kinase (RACK) and translocates to phosphorylate its targets, such as endothelial nitric oxide synthase (eNOS) at Ser-1177 to augment nitric oxide (NO) release. Myristic acid conjugated PKCε peptide activator (myr-HDAPIGYD) and inhibitor (N-myr-EAVSLKPT) has been shown to increase and decrease NO release, respectively, in rat aortic tissue by regulating PKCε translocation. However, modulation of PKCε-mediated NO release in human cells remains undetermined. We hypothesize that cell-permeable myristoylated-PKCε activator (Myr-PKCε+) will augment and inhibitor (Myr-PKCε-) will attenuate NO release in cultured human umbilical vein endothelial cells (HUVECs). Real-time HUVEC NO release (pmol) was measured using a calibrated NO electrode following administration of Myr-PKCε+ or Myr-PKCε- ± acetylcholine (Ach; positive control). Data was evaluated with Fishers post-hoc analysis. Basal levels (66±6; n=35) were determined as the picoamp difference between cell-populated (10^6 cells/well) and reagent only wells. Control Ach (10 μM) enhanced NO release (102±7; n=37), Myr-PKCε (10 μM) enhanced NO release with Ach (107±16; n=8) and without Ach (107±13; n=7), and Myr-PKCε- (10 μM) attenuated NO release with Ach (37±9; n=13) and without Ach (7±29; n=9) compared to basal levels (all $p<0.05$). Results suggest that myr-PKCε +/- effects on NO release is translational across mammalian species, presumably through activation and inhibition of PKCε-mediated phosphorylation of eNOS. Future western blot analysis will validate myr-PKCε +/- regulation of PKCε translocation to the cell membrane.

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

P06 Spontaneously Cleavable Glycosylated Linkers for Peptides without Suitable Glycosylation Sites

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Glycan conjugation can greatly improve the solubility and circulation half-life of a peptide. However, some peptides, particularly small peptides such as antigens, do not contain a suitable conjugation site and/or suffer a loss in activity after glycosylation. Here, complex-type biantennary N-linked glycans were attached to peptides through linkers designed to undergo spontaneous cleavage under neutral physiological conditions, with the aim of creating reversibly glycosylated conjugates capable of gradually releasing the active native peptide after administration. The release half-life of Chemerin-9 attached to an asialo N-glycan through such a linker could be varied from 4 h to 45 h in PBS at pH 7 and 37°C depending on the structure of the linker. Decreasing the buffer temperature and pH greatly increased the stability of all investigated linkers, with thioalkylester and ester-type linkers remaining uncleaved at pH 4. Meanwhile, conjugation of a glycosylated linker to a HER2/neu peptide greatly increased its solubility, and a release half-life of 4 h was obtained in PBS (pH 7, 37°C) when a thioaryl-type linker was used. The presented strategy is expected to offer a useful method of not only enhancing the solubility of small peptides but also tuning their release rate to suit their intended therapeutic application.

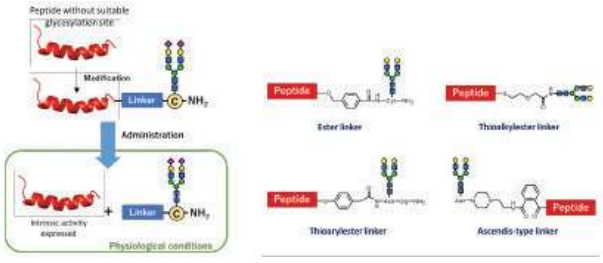

Fig. 1 (L) Spontaneously cleavable glycosylated linker concept. (R) Structures of investigated glycosylated linkers.

References

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P07 Overcoming the Blood-brain-barrier by a Linear 7-mer Peptide, IF7, with Binding Specificity to Annexin A1 in Brain Tumors

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Brain malignancies are difficult to eradicate, as chemotherapeutics injected intravenously cannot reach cancer cells in stroma due to the blood-brain-barrier (BBB). Previously we identified a linear 7-mer peptide that we designate IF7 binds to the N-terminal domain of annexin A1 (Anxa1) (Hatakeyama *et al.*, *Proc. Natl. Acad. Sci. USA*, 108: 19587-92, 2011). Although Anxa1 is normally expressed intracellularly in numerous cell types, Anxa1 is found on the endothelial cell surface in malignant tumors (Oh *et. al.*, *Nature*, 429: 629-35, 2004). When fluorescently labeled IF7 was injected intravenously into brain tumor model mice, IF7 reached tumor vasculature and targeted tumor cells in stroma, overcoming the BBB. In a dual tumor mouse model harboring subcutaneous and brain tumors, IF7-conjugated to the anti-cancer drug SN-38 suppressed growth of both tumors. In a brain metastatic model of syngeneic melanoma, tumors continued shrinking after IF7-SN38 administration. When melanoma cells were injected subcutaneously into recovered mice, CD8+ cytotoxic T cells infiltrated the injection site, suggesting a heightened immune response against tumor cells. These results suggest that IF7-SN38 can overcome BBB and efficiently suppress growth of malignant brain tumors, and also suggest that high efficacy of IF7-SN38 therapy may lead an immunotherapeutic response by the host.

P08 Illuminating the Assembly and Cellular Interactions of a Trimer Derived from Aβ

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Understanding the solution phase and biological behavior of the β-amyloid (Aβ) peptide is key to understanding Alzheimer's Disease (AD). Oligomeric assemblies of Aβ are suspected to be the leading cause of neurotoxicity, but their mechanism of action remains poorly understood. The Nowick lab has established a chemical model for Aβ oligomers by stabilizing fragments of Aβ in a macrocyclic β-hairpin structure and then stabilizing these macrocycles into covalently-linked trimers. Solution phase studies of the covalently-linked trimers have thus far been limited to SEC and SDS-PAGE, but fluorescent labeling has emerged as a new tool to study the solution phase and biological behavior of the trimers. These studies using fluorescence spectroscopy and fluorescence microscopy have provided insights into biological and solution-phase behavior of Aβ oligomers.

P09 Chimeric Interleukin-15 Bovine Ultralong Antibody Fusion for Potential Cancer Therapy

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Interleukin (IL)-15 is a hot immunotherapeutic candidate for cancer treatment because it can stimulate the proliferation and cytotoxicity of cytotoxic T lymphocytes and nature killer (NK) cells, similar to the action of IL-2, which has been used to treat metastatic melanoma and renal cancer. It may be a better candidate drug than IL-2 because it doesn't cause vascular leak syndrome or stimulate regulatory T cells. However, IL-15 is difficult to express as a stable soluble protein and has a short half-life in vitro and in vivo. To solve the problem, we designed an IL-15 ultralong bovine antibody fusion. Bovine ultralong CDR3 antibodies have a unique "stalk and knob" structure in which two antiparallel β-strands support a disulfide bonded knob protruding out of the antibody surface and forms a mini antigen binding domain. By replacing the knob of the bovine antibody BLV1H12 with IL-15, we have created a chimeric BLV1H12_IL-15 IgG which functions the same as IL-15 in in vitro signaling assays but can be easily produced in mammalian cells and with increased stability. Furthermore, as IL-15 needs its high affinity receptor α (Rα) for increased *trans* signaling to the receptor β and γ subunits, two more variant candidates were produced by either co-expressing Rα sushi domain with the chimeric IgG or fusing the Rα sushi domain to the light chain. Both new candidates exhibit higher *in vitro* signaling activity than Blv1h12_IL-15 IgG alone.

P10 Suppression of Hypermetabolic Response with a Peptide Targeting a Brown Fat Vascular Marker

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Dysfunction of adipose tissue (AT), overgrown in obesity, underlies life-threatening pathological conditions. In obesity excessive lipid accumulation in white adipose tissue (WAT) leads to the metabolic syndrome. Brown adipose tissue (BAT) contains adipocytes specialized in adaptive thermogenesis in response to sympathetic nervous system (SNS) activation. Drugs inducing AT "browning" are anticipated for type-2 diabetes treatment. However, hypermetabolic response induced by brown adipocytes is linked to cachexia. Therefore, approaches to reduce excessive activity of BAT are also needed. We have demonstrated that therapy delivery can be directed to tissues of interest trhough markers selectively expressed in the vasculature. We identified peptide Pep3 (CPATAERPC) that homes to the endothelium of BAT. To target BAT vasculature in an experimental therapy setting, we developed a hunter-killer compound D-BAT, composed of Pep3 and a pro-apoptotic domain. We demonstrate that D-BAT selectively kills BAT vascular cells. When administered into mice D-BAT decreased cold tolerance, metabolic rate, and lipid utilization, a phenotype expected of BAT impairment. In a cancer animal model, AT browning and lipolysis induced by tumor growth was suppressed by D-BAT treatment. We identified a receptor of Pep3. We have shown that Pep3 does not home to BAT in mice that lack this receptor in the endothelium. Our results suggest that signaling through Pep3 receptor in BAT endothelium mediates a mechanism through which angiogenesis is coupled with SNS signaling and innervation that underlie AT browning. We conclude that vasculature targeting with D-BAT can be useful as an experimental therapeutic to modulate the function of brown AT and the hypermetabolic response.

P11 An Improved Ornithine Turn-Linker for the Stabilization of a Peptide Derived from Aβ$_{17-36}$

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Soluble oligomers of the β-amyloid protein, Aβ, are associated with the progression of Alzheimer's disease. In order to study these heterogenous oligomers, the Nowick lab has developed β-hairpin macrocycles derived from the Aβ peptide sequence, locked on both sides by two δ-linked L-ornithine turn units. Computational modeling by Monte Carlo stochastic dynamics suggests that introduction of a single methyl group to L-ornithine at the γ-position, with an *R*-configuration, would greatly improve the ability of this turn-linker to stabilize β-hairpins. This work details the synthesis of (*2S,4R*)-N$^{\alpha}$-Boc-N$^{\delta}$-Fmoc-methylornithine containing protecting groups suitable for use in standard Fmoc-based solid-phase peptide synthesis and evaluate for its effectiveness as a turn-linker against L-ornithine and D-proline-glycine in a 12-residue model peptide reported by Gellman. Furthermore, incorporation of (*2S,4R*)-methylornithine into an amyloidogenic macrocycle derived from Aβ$_{17-36}$ previously reported by the Nowick group was able to improve folding of the wild-type peptide from a random coil to a β-sheet by circular dichroism.

P12 Myristoylated Protein Kinase C Beta II Peptide Inhibitor Exhibits Robust Attenuation of Myocardial Ischemia/Reperfusion Injury in Rats

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Ischemia-reperfusion (I/R) injury mediated by excessive reactive oxygen species (ROS) is a well-known phenomenon causing paradoxical myocardial damage after cardio-angioplasty or coronary bypass. Protein kinase C beta-II isoform (PKCβII) inhibition using a cell-permeable myristic acid (myr-) conjugated PKCβII peptide inhibitor (*N*-myr-SLNPEWNET; PKCβII-) given at reperfusion significantly attenuated ROS release in previous animal I/R studies. However, prior studies did not explore the possibility that myr-conjugation, per se, contributes to the attenuation of I/R injury. Therefore, we tested the effectiveness of myr-PKCβII- compared to scrambled myr-PKCβII- (*N*-myr-WNPESLNTE; myr-PKCβII-scram) and plasma controls to reduce infarct size and improve post-reperfused cardiac function.

Isolated, perfused male rat hearts were subjected to global I(30 min)/R(50 min), and myr-PKCβII- (20μM), myr-PKCβII-scram (20μM), or plasma (control) was given at initial reperfusion. We measured left ventricular (LV) cardiac function indices using a pressure transducer, and determined infarct size of frozen post-reperfused hearts using 1% triphenyltetrazolium chloride staining comparing infarcted tissue vs. total tissue weight. Data were evaluated using ANOVA with Bonferroni-Dunn post-hoc analysis. The maximal LV developed pressure rate ($+dP/dt_{max}$; mmHg/s) at 50min postreperfusion significantly improved with myr-PKCβII- (1575±97; n=14) compared to plasma-control (851 ±117; n=13) or myr-PKCβII-scram (543±99; both $p<0.01$; n=6) hearts, and the controls did not differ significantly. Additionally, myr-PKCβII- significantly reduced infarct size (%) to 13±2 compared to either plasma-control (24±4) or myr-PKCβII-scram (25±2; both $p<0.05$). Results suggest that myr-conjugation is not responsible for the cardioprotective effects observed with myr-PKCβII-. Therefore, myr-PKCβII- may be an effective therapeutic to improve clinical outcomes after coronary bypass or cardio-angioplasty.

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P13 Amphiphilic Tricyclic Peptides: Design, Synthesis, and Biological Evaluation of as Molecular Transporters

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The cellular delivery of cell-impermeable and water-insoluble molecules remains a major challenge. Many anticancer, antiviral, and negatively charged molecules (e.g. phosphopeptides and nucleic acids) have limited stability, solubility, and/or cellular uptake. We have previously reported that an optimal balance of positive charge and hydrophobicity in peptides is required for interactions with the cell membrane and drug delivery applications. Cyclic peptides $[WR]_5$ and $[WR]_4$ comprising of alternate arginine and tryptophan residues were found to be nuclear-targeting molecular transporters. To unravel new multivalent cellular interactions, we report the synthesis and biological evaluation of a tricyclic peptide based on naturally occurring amino acids using a simpler biocompatible and biodegradable amidic linkage. Appropriate monomeric cyclic peptides containing carboxylic acid and amine groups were synthesized and were conjugated with each other. The cytotoxicity of the cyclic peptides was evaluated in LLCPK (Normal Kidney Cell Line). Molecular transporter properties of the tricyclic peptides will be discussed.

P14 Searching for Faster and Efficient Solid Phase Peptide Synthesis Methods for Increased Crude Purity in Reduced Time

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Peptide drug development has seen a renewed success, particularly glucagon-like peptide 1 receptor agonists, and with a promising pipeline interest in synthetic peptides has been on the upside. Another field that has been garnering interest is the peptide based materials used for biological applications among others [1]. With higher demand, method development for the synthesis of peptides has become instrumental in accelerating and increasing throughput of peptides. Here, we show the synthesis optimization in parallel including different conditions including multiple coupling reagents, elevated temperatures, and different reaction times for several peptides, including GLP-1 agonist Lixisenatide and others.

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P15 Effects of *N*-terminal Extensions on the Assembly of Macrocyclic β-sheet Peptides Derived from $A\beta_{(16-36)}$

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In Alzheimer's disease (AD), neurodegeneration and synaptotoxicity are tied to the assembly and aggregation of the β-amyloid peptide Aβ. Of the aggregates that form, Aβ oligomers – soluble and metastable assemblies composed of anti-parallel β-sheets, are currently suspected to be the toxic species. Biophysical characterizations of Aβ oligomers by techniques such as X-ray diffraction, NMR spectroscopy, circular dichroism (CD), and sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) have identified two regions of the peptide that contribute to assembly. The packing of hydrophobic residues and intramolecular hydrogen bonding between the central hydrophobic core (CHC), residues 17 – 24, and the hydrophobic C-terminal region, residues 32 – 42 are key interactions that drive aggregation and folding. Though the contributions of the CHC and C-terminal region in oligomer formation have been well established, less is known however, about the role of the *N*-terminal region in assembly. In contrast to the CHC and C-terminal regions, the *N*-terminal region of Aβ is largely hydrophilic, and is subject to a number of diverse post-translational modifications including truncation and pyroglutamation, phosphorylation, racemization, and isomerization. Understanding how the *N*-terminal region effects the assembly and behavior of Aβ oligomers is crucial to further developing our understanding of the molecular basis of Alzheimer's disease. This poster details biophysical characterizations of *N* and C-terminally extended peptides derived from a macrocyclic β-sheet peptide previously characterized by our laboratory.

P16 AutoDock CrankPep: Docking Fully Flexible Large Peptides

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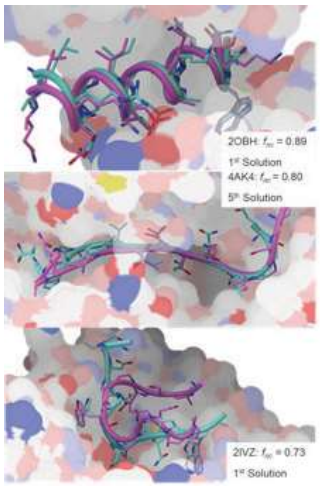

Protein-peptide interactions mediate a wide variety of cellular and biological functions. Methods for predicting these interactions have garnered a lot of interest over the past few years, as witnessed by the rapidly growing number of peptide-based therapeutic molecules currently in clinical trials. The size and flexibility of peptides has shown to be challenging for existing automated docking software programs. Cyclic peptides are particularly interesting as therapeutic molecules, as they are less prone to the liabilities of peptides as therapeutic peptides, and a new therapeutic cyclic peptide is approved nearly every year. However, treating the large macrocycles in these molecules as flexible during docking is a daunting challenge.

Here we present *AutoDock CrankPep* or *ADCP* in short, a novel approach to dock flexible and potentially cyclic peptides, starting from the sequence, into rigid receptors. *ADCP* folds a peptide in the potential field created by the protein to predict the protein-peptide complex. Our results show that *ADCP* outperforms state of the art docking methods for peptides with up to 15 amino acids. In addition, we show that it reliably docks peptides with up to 20 amino acids, which beyond the reach of other current methods. Moreover, it is the first method reported to dock cyclic peptides cyclized through their backbone and/or up to 2 disulfide bonds, thus making it a useful tool for rational peptide-based drug design as well as the study of the interactions between Intrinsically Disordered Proteins (IDPs) and their receptors.

ADCP is available under the LGPL 2.0 opensource license at http://ccsb.scripps.edu/adcp

P17 Distributed Drug Discovery (D3) Synthesis and Testing of Multiple Unnatural Dipeptides Identifies a Subset with Potent Antimicrobial Activity against Pseudomonas aeruginosa *(Pa)*

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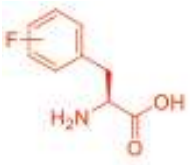
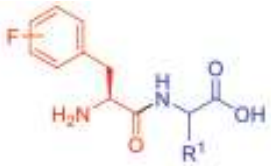
Through Distributed Drug Discovery (D3) the unnatural amino acid (S)-4F-Phenylalanine (**1a:** -4F) was rediscovered as a potent inhibitor of *Pseudomonas aeruginosa (Pa)* growth. Three close analogs (**1b:** -3F; **1c:** -2F; and **1d:** -3,4-diF) were also made and found to be active. Subsequently it was shown that unnatural dipeptides of generic structure **2** and **3** were active, presumably as prodrugs of **1**. The D3 program, which teaches solid-phase organic and peptide synthesis to students at global schools, targeted an 80 compound subset of 2 for distributed synthesis. In this subset, the C-terminal residue is one of 20 natural amino acids and the N-terminal residue is either (S)-4F, -3F, -2F, or 3,4-diF-phenylalanine. A complementary biology laboratory was developed at IUPUI to explore the activity of these compounds against *Pa*. We report the multi-school distributed synthesis and testing of members of this targeted 80 compound subset and the resulting discovery of many unnatural dipeptides **2** active against *Pa*.



1a: 4-F; **b**: 3-F; **c**: 2-F; **d**: 3,4-diF



2: One of four N-Terminal FPhe's, R^1 = C-terminal natural or unnatural amino acid side chain

3: One of four C-Terminal FPhe's, R^2 = N-terminal natural or unnatural amino acid side chain

(Abstract for poster to be presented at the Peptide Therapeutics Foundation in La Jolla California, October 24-25, 2019)

P18 Hyperdisulfide and Cell-Penetrating Cytoprotective Peptides from Medicinal Plants

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A longstanding interest of our laboratory is to study disulfide-rich peptides from medicinal plants as drug leads and as an inspiration for designing orally-active compounds. Plants produce disulfide-rich peptides, also known as cysteine-rich peptides (CRPs), as part of their host-defense mechanism against microbes and insects. Most CRPs contain 15-25% of cysteine per molecule, or about one cysteine per 4 to 7 amino acid residues. Recently, we discovered hyperdisulfide peptides containing >30% cysteine per molecule, or one cysteine per 3 amino acid residues. Here, we report the discovery of β-ginkgotides from *Ginkgo biloba,* as a "first-in-class" hyperdisulfide-constrained peptide family from plants. β-ginkgotides are highly stable against thermal, acid and protease-mediated degradation. They are also cell- penetrating and contain an LC3-interacting region (LIR) motif. Our results showed that β-gB1 promotes autophagosomes formation. We also showed that β-gB1 protects cells from hypoxia- and hypoxia-reoxygenation-induced damages. Taken together, our results suggest that the LIR motif of plant-derived β-gB1 is responsible for its selective autophagy and resulting cytoprotective effects. Moreover, the hyperdisulfide scaffold of β-gB1 holds promise for the engineering of peptidyl therapeutics with enhanced structural and metabolic stability.

Acknowledgement
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P19 Evaluation of Structure-Activity Relationship of Cyclic Peptides [W4R4] and Amphipathic Fatty Acyl-Cyclic [W_4R_4K] as Potential Antibacterial Agents Against Pathogenic Bacteria

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Cyclic peptide [W_4R_4] containing positively-charged arginine (R) and hydrophobic tryptophan (W) residues had antibacterial activity with a minimum inhibitory concentration (MIC) value of 4 μg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA) and 42 μg/mL against *Pseudomonas aeruginosa* (PSA). The purpose of this study was to optimize the antibacterial activity of the cyclic peptide by increasing the number of residues or conjugation with fatty acids. The antimicrobial effects were determined against MRSA, *PSA, Klebsiella pneumoniae (KPC)*, and *Escherichia coli* using meropenem and vancomycin as controls and compared with [W_4R_4]. All the peptides were synthesized using Fmoc-based solid-phase chemistry, characterized by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectroscopy (MALDI-TOF), and purified with high-performance liquid chromatography (HPLC). The cyclic peptide fatty acyl anhydrides (RCO-O-COR) where R = CH_3, $CH_3(CH_2)_{2n-2}$) were conjugated with purified peptides to obtain [$W_4R_4K(C_2)$], [$W_4R_4K(C_4)$], [$W_4R_4K(C_6)$], [$W_4R_4K(C_8)$], [$W_4R_4K(C_{10})$], [$W_4R_4K(C_{12})$], and [$W_4R_4K(C_{14})$]. All the synthesized peptides were evaluated for their MIC values against MRSA, PSA, KPC, and *E. coli*. [$W_4R_4K(C_{10})$] had antibacterial activity against Gram-positive strains like MRSA with MIC value of 4 μg/mL but less activity against Gram-negative bacteria like KPC, PSA, with MIC values of 32 μg/mL and 64 μg/mL, respectively. [$W_4R_4K(C_{10})$] demonstrated 6.4% hemolytic effect on human red blood cells while cyclic peptide [W_4R_4] showed a 10.2% hemolytic effect at a concentration of 128 μg/mL.

P20 N-linked Glycosylation Increases Stability and Efficacy of Bivalirudin In Vitro

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Bivalirudin is an FDA approved thrombin inhibitor used to prevent blood clotting during invasive cardiovascular procedures such as percutaneous coronary intervention. This peptide drug has a short half-life caused by a deamidation reaction of the Asn-9 residue, resulting in impurities during manufacture and rapid storage degradation. This project aimed to determine whether N-linked glycosylation of the Asn-9 residue increases bivalirudin's stability and to compare the inhibitory effects of the glycosylated bivalirudin (glycobivalirudin) to normal bivalirudin. To achieve these goals, four bivalirudin peptides with a variable ninth residue (Asn, GlcNAc-Asn, Asp, or iso-Asp) were synthesized via solid phase peptide synthesis. The Asp and iso-Asp containing chains (deamidation products of bivalirudin) were used to identify the HPLC peaks of degraded bivalirudin. Storage of the bivalirudin and glycobivalirudin peptides in a 25 C water bath determined that glycobivalirudin was more stable over time. A SensoLyte 520 Thrombin Inhibitor Assay determined that glycobivalirudin was a slightly more potent thrombin inhibitor. These results indicate that the glycosylated drug is more stable and effective in vitro. Future projects may explore the efficacy and stability of glycobivalirudin in cytoplasm and animals, with a long-term goal of using this glycosylated drug on patients. Future research may examine the monosaccharide glycosylation method on other peptides and other glycosylation methods, such as conjugation to a dextran scaffold, for bivalirudin.

P21 A Dual apoC-II Mimetic-apoC-III Antagonist Peptide for Lowering Plasma Triglycerides

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Recent epidemiological and genetic studies have established hypertriglyceridemia as causally related to cardiovascular disease, making it an active area for drug development. We describe a new strategy for lowering triglycerides (TG) by means of a novel apolipoprotein (apo) C-II mimetic peptide.

First, using apoC-II as a template, we designed a short peptide, D6PV, lacking the long N-terminal lipoprotein-binding helix. Amino acid substitutions in the central random coil region of the truncated protein were made to enhance its lipid binding capabilities, and it was linked via a proline to lipoprotein lipase (LPL) activation domain in the C-terminal helix of apoC-II. The design of D6PV was motivated by a combination of all-atom molecular dynamics simulations of apoC-II, structural prediction programs, and assorted biophysical techniques. Second, the efficacy of D6VP was assessed ex vivo in human plasma samples and was found to be more potent than apoC-II in activating LPL. D6PV treatment of various mouse models for hypertriglyceridemia led to a rapid and sustained decrease of plasma TG. In human A*POC3*-transgenic mice D6PV lowered total plasma TG and apoC-III levels by over 80% and displaced apoC-III from TG-rich lipoproteins, leading to its increased renal clearance. D6PV was shown to have good subcutaneous bioavailability in mice and non-human primates, and an extended half-life because it binds to high-density lipoproteins, which serve as a long-term reservoir for the peptide.

In summary, D6PV decreases plasma TG by acting as a dual apoC-II mimetic and apoC-III antagonist, thereby demonstrating its potential as a novel therapeutic for hypertriglyceridemia.

P22 Anti-DKK2 D-peptide Inhibitor, One Drug With Multiple Effects on Metastatic Colorectal Cancer Treatment

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Colorectal cancer is the third most common cancer and the second leading cause of cancer death in the United States. Metastatic colorectal cancer (mCRC) is still a non-curable disease (5-year survival rate of 13.5%), so novel treatments are urgently needed[1]. Dickkopf 2 (DKK2) is identified as a secreted modulator of Wnt via binding to LRP5/6. DKK2 can either stimulate or inhibit Wnt signaling depending on cell environment. In colorectal cancer, DKK2 and LRP6 are both upregulated. The interaction of DKK2 and over-expressed LRP6 activates Wnt signaling. A recent study shows that Wnt signaling activation contributes to the resistance toward immune checkpoint inhibitor therapy[2]. Additionally, DKK2 is observed to deactivate NK cell and CD8+ T cell in mCRC[3]. Furthermore, another study reported that DKK2 promotes angiogenesis in mCRC through stimulating lactate secretion[4].

Here, we describe our efforts to develop mirror-image peptide (D-peptide) inhibitors against DKK2 for mCRC. We will screen for such inhibitor using mirror-image phage display, which requires chemical synthesis of the mirror-image version of the functional domain of DKK2 (C-terminus cysteine-rich domain, DKK2C, 88aa). Both the L-DKK2C and D-DKK2C have been chemically synthesized by using a combination of solid-phase peptide synthesis and native chemical ligation. The Wnt reporter activity assay shows that synthetic L-DKK2 has similar activity in inhibiting Wnt signaling as recombinant DKK2C does. We will now use D-DKK2C to screen for D-peptide inhibitor. Subsequently, the D-peptide inhibitor will be tested on several cell cultures to determine its efficacy on inhibiting Wnt signaling under LRP6 overexpression, activating NK cell, and inhibiting angiogenesis. Compared to monoclonal antibodies, D-peptides have several advantages, including lower manufacturing cost, lower immunogenicity, and higher diffusivity into solid tumors. Therefore, D-peptide drug may provide a promising alternative to monoclonal antibodies for treating mCRC.

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P23 Synthesis and Characterization of a Methylene Thioacetal Human Insulin Analog with Enhanced Stability

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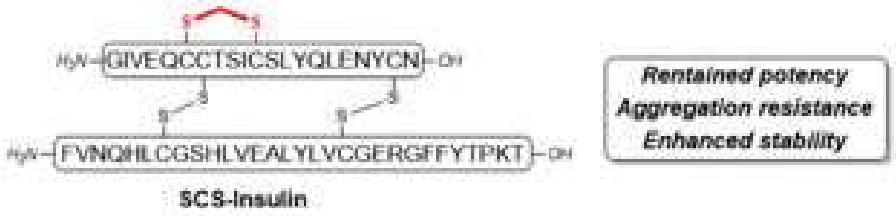
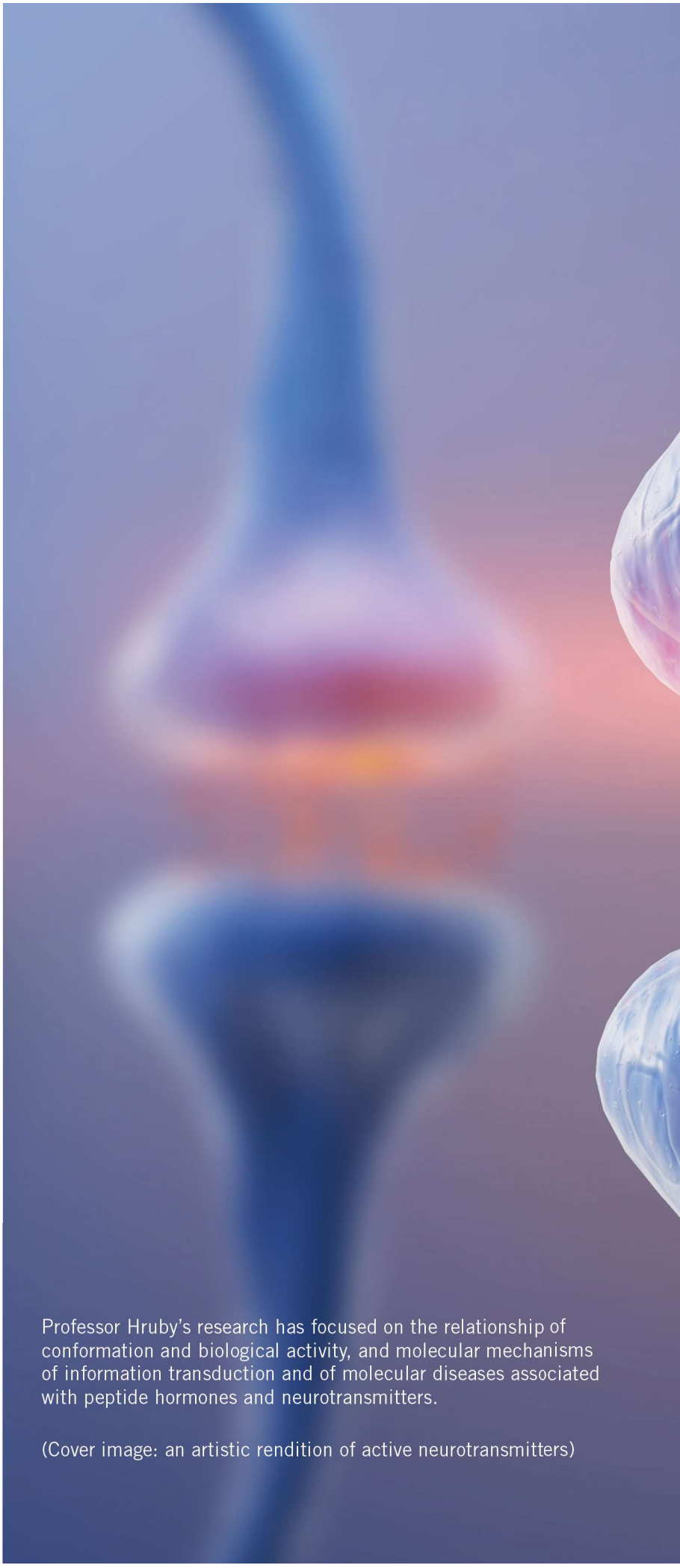
Insulin has been a life-saving drug for millions of people with diabetes since the 1920s. However, several challenges exist which limit therapeutic benefits and reduce patient convenience. One key challenge is the aggregation propensity of insulin, which necessitates its cold-chain delivery and storage. To address this limitation, we chemically synthesized and evaluated a methylene thioacetal human insulin analog (SCS-Ins). To address the hydrophobicity of the insulin A chain, a 2nd generation helping hand (Fmoc-Addhp-OH), linked with a solubilizing hexa-lysine sequence attached to the N-terminal A chain to increase solubility, was used to enable tolerance of both harsh acidic, basic and oxidative reaction conditions. The synthesized SCS-Ins showed enhanced serum stability and aggregation resistance while retaining bioactivity compared with native insulin.





Professor Hruby's research has focused on the relationship of conformation and biological activity, and molecular mechanisms of information transduction and of molecular diseases associated with peptide hormones and neurotransmitters.

(Cover image: an artistic rendition of active neurotransmitters)