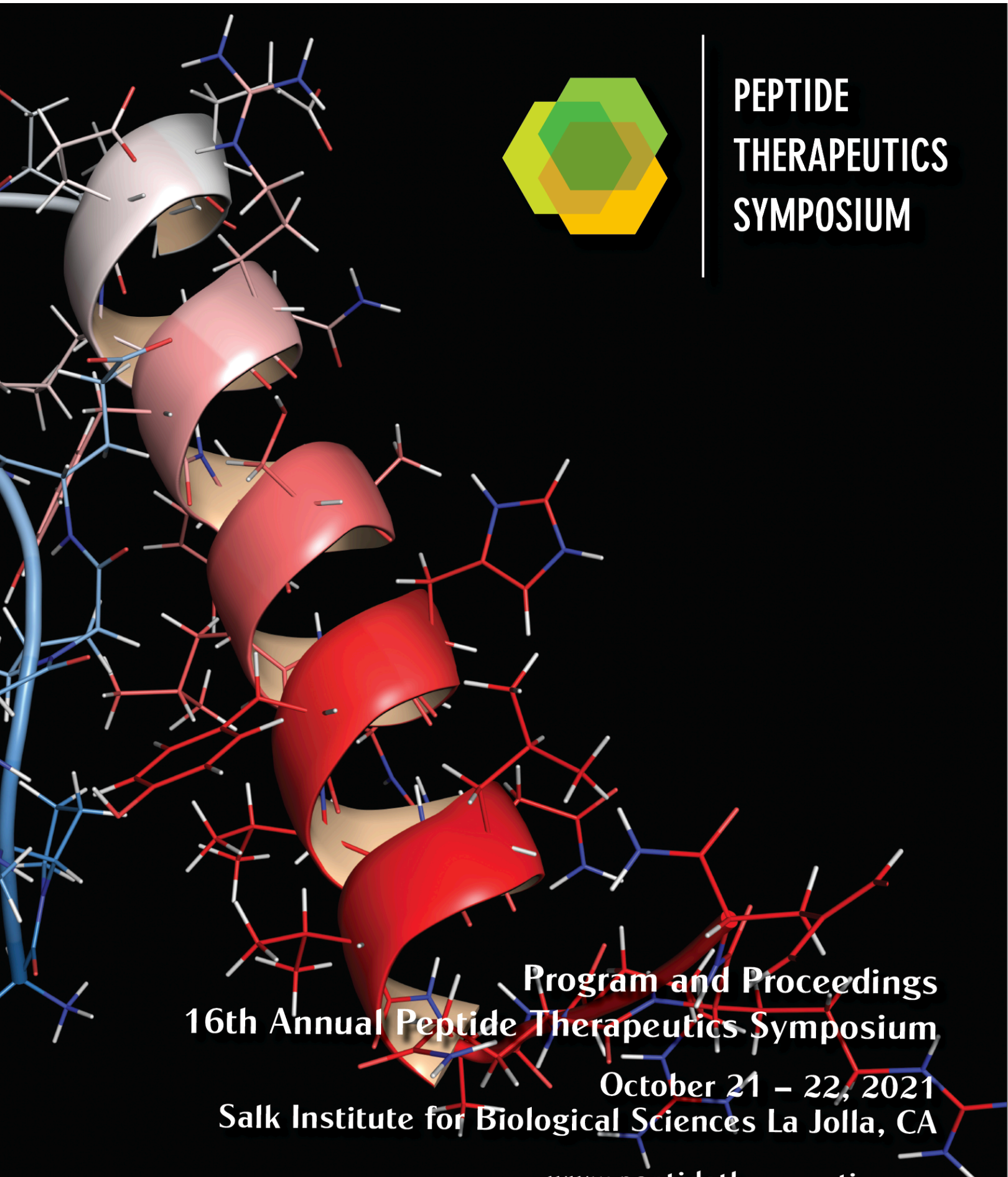


PEPTIDE
THERAPEUTICS
SYMPOSIUM

Program and Proceedings
16th Annual Peptide Therapeutics Symposium

October 21 – 22, 2021
Salk Institute for Biological Sciences La Jolla, CA

www.peptidetherapeutics.org

16th Annual Peptide Therapeutics Symposium

October 21 – 22, 2021

Salk Institute for Biological Studies La Jolla, CA

Virtual and In-Person Meeting

Table of Contents

Table of Contents

16th Annual Peptide Therapeutics Symposium

2021 Travel Grant Awardees

Rachel Heynen, Dordt University
Chelsea Jones, University of California, Irvine
Maj Krumberger, University of California, Irvine
Xingyue Li, University of California, Irvine
Alexi Verwoert, Philadelphia College of Osteopathic Medicine

Symposium Sponsors

AstraZeneca
FERRING PHARMACEUTICALS
novo nordisk®
PolyPeptide GROUP
ZEAL&
REVOLUTIONARY HEALTH SOLUTIONS
PEPTIDE THERAPEUTICS FOUNDATION

16th Annual Peptide Therapeutics Symposium

Dear Colleagues,

We are delighted to be able to welcome you back in person to the new hybrid format for the 16th Annual Peptide Therapeutics Symposium. We will continue to live stream the event to provide colleagues from around the globe with the opportunity to attend virtually. A new interactive platform will allow all attendees to have meaningful dialog with colleagues, poster presenters, and speakers. The taped presentations will be available for viewing for 60 days following the close of the meeting. You may use the Q&A function within the virtual platform to leave questions for the speakers, in the event that they are not answered during the live Q&A.

The hybrid format of the symposium will take place over two days and has been scheduled to align with the time zones of the speakers. On Thursday we have plenary lectures spread throughout the day from Annette Beck-Sickinger, Leipzig University, Daniel Drucker, University of Toronto and Dame Margaret Brimble, University of Auckland. We end the day on Friday with plenary lectures from David Baker, University of Washington and William DeGrado, University of California, San Francisco.

The remainder of the program will include presentations from prominent scientists highlighting the impact of peptides on drug discovery research, as well as current and future peptide therapies.

We have an exciting line-up of speakers and topics that demonstrate the extensive reach of peptide therapeutics in 2021. Not only do we have many diverse therapeutic areas, but also quite diverse "peptide modalities" which speaks to the creativity of our discovery scientists. We will hear lectures touching on: peptides in oncology as radiopharmaceuticals, cancer vaccines and imaging agents, peptides in diabetes therapy as stand-alone agents or as part of connected care devices, as well as peptides for the potential treatment of fibrosis, non-opioid pain management, Parkinson's disease, Rett syndrome, and last but certainly not least, anti-viral peptide approaches including those directed at SARS-CoV-2.

This year we are also excited to recognize and celebrate the 80th birthday and lifetime of scientific achievements of Dr. Waleed Danho. A session in honor of Waleed will include two former colleagues discussing the peptide hormone PYY and the exciting field of RNA therapeutics. Please join us on Friday afternoon for a celebratory reception.

Thank you for joining us, we are grateful for your attendance which is as always key to making this annual scientific event successful.

Sincerely,

Phil Dawson
Chairman of the Board
Peptide Therapeutics Foundation

Adam Mezo
President
Peptide Therapeutics Foundation

Sponsors, Peptide Therapeutics Foundation

Astra Zeneca
Ferring Research Institute Inc.
Novo Nordisk
The PolyPeptide Group
Zealand Pharma

16th 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.

Ferring Research Institute, Inc.
Headquartered in San Diego, California, Ferring Research Institute, Inc., (FRI) is the research and ideas incubator of Ferring Pharmaceuticals. Established in 1996, FRI is located in the heart of the Southern California biopharmaceutical community. The center has attracted a diverse group of highly skilled professionals representing over twenty-four countries of origin. FRI is our key research center for early research and development. Here, our scientists work on small molecule and peptide drug discovery, from new target identification up until early drug development phases. FRI is focused on the following key therapeutic areas: reproductive health and maternal health, uro-oncology, gastroenterology and the microbiome.

Our state-of-the art facility includes small molecule, peptide and protein drug design, medicinal 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.

About Ferring Pharmaceuticals
Ferring is a research-driven, specialty biopharmaceutical group committed to helping people build healthy families and live better lives. Ferring is a leader in reproductive medicine and maternal health, and in specialty areas within gastroenterology and urology. Ferring focuses on developing life-changing innovations that help people live better lives. Grounded in a 70-year commitment to science and research, we are relentless in our pursuit of therapies that help people build families, stay healthy, and fight disease.

Ferring has a strong global profile with offices worldwide and headquartered in Switzerland. We continue to grow through our aim of providing effective treatments for patients. Globally, we reach millions of patients across 110 countries and employ 6,000 employees worldwide. 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.

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.

16th Annual Peptide Therapeutics Symposium

Zealand Pharma

Zealand Pharma A/S (NASDAQ OMX Copenhagen: ZEAL) ("Zealand") is a biotechnology company specialized in the discovery, design and development of peptide-based therapeutics. The company has a portfolio of medicines and product candidates under license collaborations with Sanofi 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)

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 five corporate sponsors; AstraZeneca, Ferring Research Institute, Inc., Novo Nordisk, The PolyPeptide Group, and Zealand Pharma.

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.

Notes

16th Annual Peptide Therapeutics Symposium

October 21 – 22, 2021

Salk Institute for Biological Studies La Jolla, CA

Virtual and In-Person Meeting

Thursday, October 21, 2021

7:00 a.m. – 4:15 p.m.
Registration Check-in
Fritz B. Burns Reception Center, Lower Level

8:00 a.m. – 4:15 p.m.
16th Annual Peptide Therapeutics Symposium
Conrad T. Prebys Auditorium

8:00 a.m. – 8:15 a.m.
Opening Remarks
Phil Dawson, Ph.D.
Chairman of the Board, Peptide Therapeutics Foundation
Professor of Chemistry, Scripps Research
Dean of the Skaggs Graduate School of Chemical and Biological Sciences

8:15 a.m. – 9:00 a.m.
Plenary Lecture
Moderator:
Phil Dawson, Ph.D.

G Protein-Coupled Receptors: From Structure to Cell Specific Drug Targeting
Annette Beck-Sickinger, Ph.D.
Institute of Biochemistry, Faculty of Life Sciences
Leipzig University

9:00 a.m. – 10:00 a.m.
Session I
Moderator:
David Parkes, Ph.D.
President and Founder
DGP Scientific, Inc.

9:00 a.m. – 9:30 a.m.
Toward Novel Therapeutics via Directed Remodeling of the Gut Microbiome
Reza Ghadiri, Ph.D.
Professor
Scripps Research

9:30 a.m. – 10:00 a.m.
Design and Evaluation of Synthetic Lipopeptides as Potent Antivirals
Christopher A. Alabi, Ph.D.
Associate Professor, Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University

16th Annual Peptide Therapeutics Symposium

10:00 a.m. – 11:00 a.m.

Poster Session

Moderator:

Sepideh Asfar
Research Advisor and Peptide Discovery Group Leader
Eli Lilly and Company

Bárbara Matos, *iBiMED Universidade de Aveiro*
Targeting PP1/CAV1 Interaction Using a Bioportide as an Anticancer Strategy

Conor Wynne, *Maynooth University*
Utilising a 1,8-Naphthalimide Probe for the Ratiometric Fluorescent Visualisation of Apoptosis

Anna Kruscha, *Leipzig University*
Addressing of Adipocytes with Peptide-Drug Conjugates

Anne Sophie Czerniak, *Leipzig University*
Establishing a Peptide Based Shuttling System with Chemerin 9 and the CMKLR1 Receptor

Lukas Kogler, *Institute of Biological Chemistry, University of Vienna*
Synthesis of T20K Immunosuppressive Cyclotide

Roland Hellinger, *Medical University of Vienna*
The Cyclic Cysteine Knot Motif: A Structural 'Key' for Immunosuppressive Activity of Cyclotides

Sarah Ruttenberg, *University of California, Irvine*
Uncovering the Secrets of α-Synuclein Oligomers

11:00 a.m. – 11:45 a.m.

Beverage Break & Poster Viewing
Fritz B. Burns Reception Center, Lower Level

11:45 a.m. – 12:30 p.m.

Plenary Lecture

Moderator:

Rie Schultz Hansen, MSc, Ph.D.
Director, Peptide Therapeutics Foundation
Head of Discovery and Innovation
Zealand Pharma A/S Sydmarken

GLP-1 Action-Mechanisms and Future Directions
Daniel Drucker, Ph.D.
Senior Scientist, Lunenfeld Tanenbaum Research Institute,
Mt. Sinai Hospital, University of Toronto

12:30 p.m. – 1:30 p.m.

Lunch Break
Fritz B. Burns Reception Center, Lower Level

16th Annual Peptide Therapeutics Symposium

1:30 p.m. – 2:30 p.m.

Session II
Moderator:
John Dwyer, Ph.D.
CEO, VP Research, 48Hour Discovery Inc.

1:30 p.m. – 2:00 p.m.

Development of Next Generation Incretin Tirzepatide, a Novel Dual GIP and GLP-1 Receptor Agonist Peptide
Jordi Alsina, Ph.D.
Research Fellow, Eli Lilly and Company

2:00 p.m. – 2:30 p.m.

Dasiglucagon, a Next-generation Ready-to-use Glucagon Analog and its Potential use in Bi-Hormonal Artificial Pancreas Pumps
Rie Schultz Hansen, MSc, Ph.D.
Head of Discovery and Innovation
Zealand Pharma A/S Sydmarken

2:30 p.m. – 3:15 p.m.

Plenary Lecture
Moderator:
Robert Hagopian
Director, Peptide Therapeutics Foundation
Director Business Development, PolyPeptide Group

Peptide-Based Therapeutics: Paving the Way from the Lab to the Clinic
Dame Margaret Brimble DNZM, FRS, Ph.D.
Distinguished Professor School of Chemical Sciences and the Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, New Zealand

3:15 p.m. – 4:15 p.m.

Poster Session
Moderator:
Antoine Henninot
Prinicipal Scientist GI Chemistry
Takeda Pharmaceuticals

Ewa Lis, *Kolber Biosciences Inc.*
Understanding and Predicting Peptide Activity Using Artificial Intelligence Approaches

Michel Sanner, *Scripps Research*
Improving Docking Power for Short Peptides using Random Forest

Rachel Heynen, *Dordt University*
Using Picoloyl Ester and 2-Chloro-Trityl Groups for Site-Selective Sulfation of Peptides

Maj Krumberger, *University of California, Irvine*
Design, Synthesis, and Study of a Photoaffinity Label Tailored to Target Trimers Derived from Amyloid-β

Chelsea Jones, *University of California, Irvine*
Teixobactin O-acyl Isopeptide Prodrugs Exhibit Enhanced Antibiotic Activity and Improved Pharmacological Properties

Alexis Verwoert, *Philadelphia College of Osteopathic Medicine*
Myristoylated Protein Kinase C Beta II Peptide Inhibitor Reduces Bilateral Renal Ischemia-Reperfusion Injury in Mice

Xingyue Li, *University of California, Irvine*
Homochiral and Heterochiral Assembly of Peptides Derived from β-Sheet Regions of β-Amyloid

4:15 p.m. – 5:30 p.m. **Opening Reception**
Helen and Morton Adler Memorial Court

Friday, October 22, 2021

7:00 a.m. – 12:30 p.m. **Registration Check-in**
Fritz B. Burns Reception Center, Lower Level

8:00 a.m. – 5:00 p.m. **16th Annual Peptide Therapeutics Symposium**
Conrad T. Prebys Auditorium

8:00 a.m. – 8:15 a.m. **Welcoming Remarks**
Adam Mezo, Ph.D.
President, Peptide Therapeutics Foundation
Senior Director, Discovery Chemistry, Ferring Research Institute, Inc.

8:15 a.m. – 9:45 a.m. **Session III**
Moderator:
Adam Mezo, Ph.D.

8:15 a.m. – 8:45 a.m. **Identification, Characterization and Development of Dual Amylin and Calcitonin Receptor Agonists as Novel Drug Candidates Providing Insulin Sensitization and Weight Loss**
Kim Henriksen, Ph.D.
Director of Endocrinology, Nordic Bioscience A/S
KeyBioscience AG, Denmark

16th Annual Peptide Therapeutics Symposium

8:45 a.m. – 9:15 a.m.

The Use of GLP-1 Receptor Agonists for the Treatment of Parkinson's Disease
Tom Foltynie, Ph.D.
Professor of Neurology
National Hospital for Neurology & Neurosurgery, London, UK

9:15 a.m. – 9:45 a.m.

Personal Peptide Vaccines Directed at Neoantigens for Patients with Advanced Solid Tumors
Patrick A. Ott, MD, Ph.D.
Dana Farber Cancer Institute

9:45 a.m. – 11:00 a.m.

Waleed Danho Celebration

9:45 a.m. – 9:55 a.m.

Welcome and Introduction
Nader Fotouhi, Ph.D.
CSO, TB Alliance

9:55 a.m. – 10:25 a.m.

RNA Therapeutics: New Chemical Modalities Becoming a Therapeutic Reality
Konrad Bleicher, Ph.D.
Expert Scientist, RNA Therapeutics
Roche Innovation Center Basel, Switzerland

10:25 a.m. – 10:55 a.m.

PYY Analogs for the Treatment of Obesity and Type 2 Diabetes
Anish Konkar, Ph.D.
Senior Director, Cardiometabolic & Renal
Diabetes and Complications Therapeutic Area
Eli Lilly and Company

10:55 a.m. – 11:00 a.m.

Presentation of Award

11:00 a.m. – 11:30 a.m.

Poster Session
Moderator:
Rebecca Berlow
Staff Scientist Wright/Dyson Laboratory
Scripps Research

Cristina Clement, *Weill Cornell Medicine*
Development of Qualitative and Quantitative Nano-LC/MS/MS Peptidomics Assays for Mapping the (Neo) Epitopes Derived from Endosomal Processing of Diverse Protein Antigens

Natália Costa, *Institute of Chemistry, UNESP*
Innovative Strategies for the Development of New Anti-leishmania

Ashweta Sahni, *Ohio State University*
Cell Penetrating Peptides and Proteins: Mechanism of Action

Nicolas Varas, *Indiana University*
A Novel Lactam-stapled, Fibrillation-resistant Glucagon Analogue

Sally Wang Ph.D., *PepLib Zonsen Biotech*
Direct Novel Peptide Ligand Identification with HTS Library in Cell-based Functional Assays

11:30 a.m. – 12:30 p.m. **Lunch Break**
Fritz B. Burns Reception Center, Lower Level

12:30 p.m. – 2:00 p.m. **Session IV**
Moderator:
Ewa Lis, Ph.D.
CEO
Koliber Biosciences, Inc.

12:30 p.m. – 1:00 p.m. **Recifin A, a Novel and Selective Allosteric Inhibitor of Tyrosyl-DNA Phosphodiesterase I with a Unique Disulfide-bond Topology**
Christina I. Schroeder, Ph.D.
Stadtman Investigator
Center for Cancer Research, National Cancer Institute, National Institutes of Health

1:00 p.m. – 1:30 p.m. **Defining the Melanocortin GPCR System for Non-Opioid Analgesia and Peripheral Diabetic Neuropathic Pain Therapeutic Development**
Carrie Haskell-Luevano, Ph.D.
Professor & Associate Department Head,
Philip S. Portoghese Endowed Chair in Chemical Neuroscience, University of Minnesota Department of Medicinal Chemistry

1:30 p.m. – 2:00 p.m. **Applications of Covalent Peptide Probes for Imaging of Cancer and Infectious Diseases**
Matthew Bogyo, Ph.D.
Professor of Pathology and Microbiology and Immunology
Stanford University

2:00 p.m. – 3:30 p.m. **Plenary Lectures**
Moderator:
John Zhu
Vice President, Discovery Research
Ferring Research Institute, Inc.

16th Annual Peptide Therapeutics Symposium

2:00 p.m. – 2:45 p.m.

The Coming of Age of De Novo Protein Design
David Baker, Ph.D.
Professor of Biochemistry and Director of the Institute for Protein Design, University of Washington

2:45 p.m. – 3:30 p.m.

Use of Integrin Antagonists to Disrupt Pathological Mechanical Force-Dependent Processes in Fibrosis and Severe Asthma
William DeGrado, Ph.D.
Department of Pharmaceutical Chemistry
University of California, San Francisco

3:30 p.m. – 4:00 p.m.

Beverage Break

4:00 p.m. – 4:45 p.m.

Poster Session
Moderator:
Jason Moss
Seagen Inc.

Mikhail Kolonin, *The University of Texas Health Sciences Center at Houston*
Peptides Targeting Metastatic Tumor Cells as Probes for Cancer Detection and Vehicles for Therapy Delivery

Chelsea Marie T. Parrocha, *University of California, Irvine*
Investigating the Neutralizing Properties of Antibodies Generated Against an Aβ-derived Oligomer: Efforts Toward a Novel Alzheimer's Disease Immunotherapy

Dindyal Mandal, *Chapman University School of Pharmacy*
Peptide-Based Strategy for Nucleic Acid Delivery

Sandeep Lohan, *Chapman University School of Pharmacy*
Short Cationic Membrane Active Antimicrobial Peptides: A Detailed Structural Insight of the Membrane-Bound Peptides Using 2D-NMR and Molecular Dynamic Simulations

Khalid Zoghebi, *Chapman University School of Pharmacy*
Cyclic Peptides Containing Tryptophan and Arginine Residues as Protein Kinase Inhibitors

Muhammad Imran Sajid, *Chapman University School of Pharmacy*
A Physical Mixture of Antibiotic and Synthetic Antimicrobial Cyclic Peptide Proves to be More Effective than Respective Chemical Conjugate Against Multidrug-resistant Bacteria

John J. Dwyer Ph.D., *VP Research, 48Hour Discovery Inc.*
Discovery of Gut-stable, Nanomolar Antagonists from Genetically-Encoded Bicyclic Peptides Displayed on Phage

4:45 p.m. – 5:00 p.m. **Final Remarks**
Adrienne Day, Ph.D.
Director and Treasurer, Peptide Therapeutics Foundation
President, Blue Gum Advisors, LLC

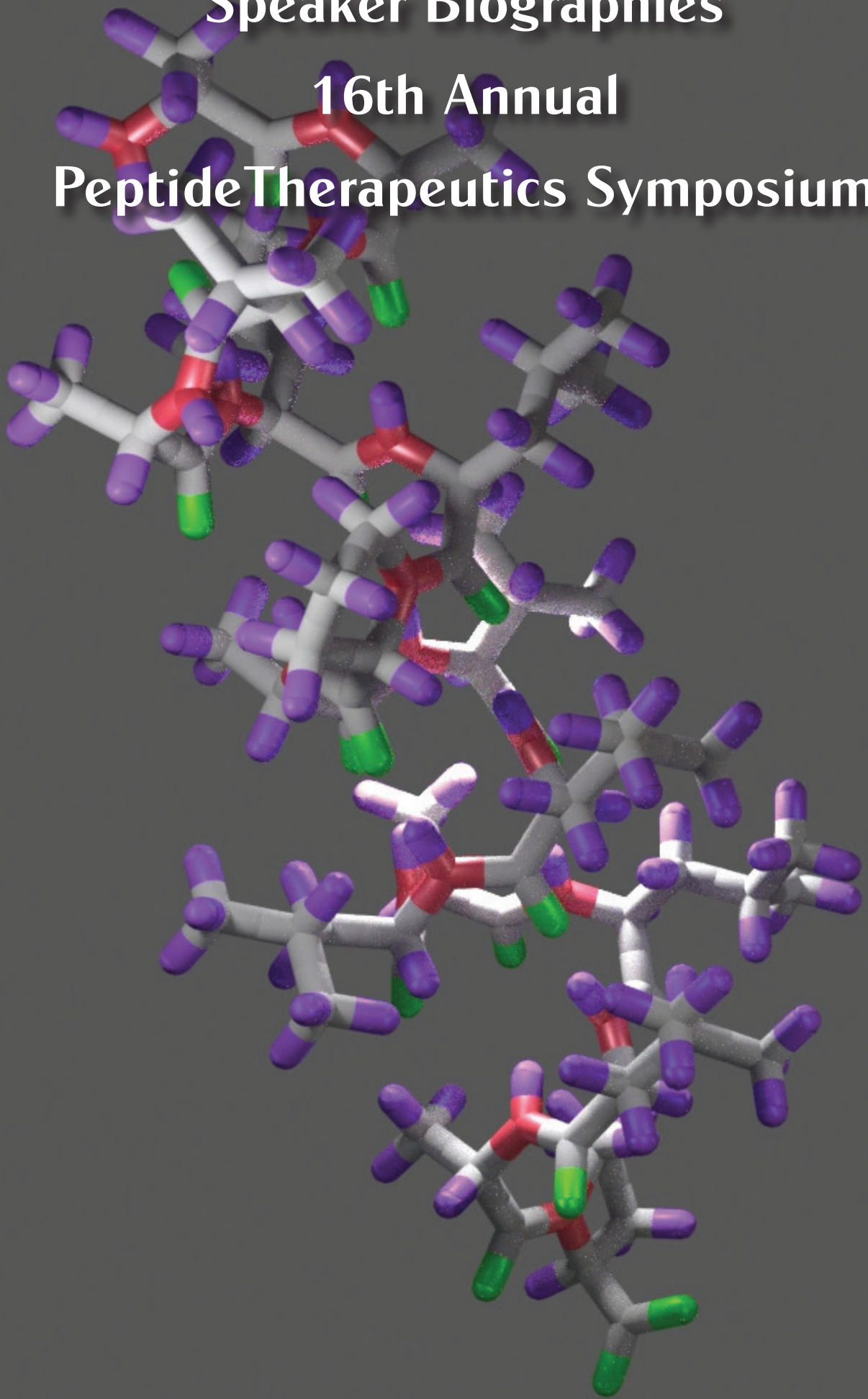
5:00 p.m. – 6:30 p.m. **Celebratory Reception**
Helen and Morton Adler Memorial Court

Notes

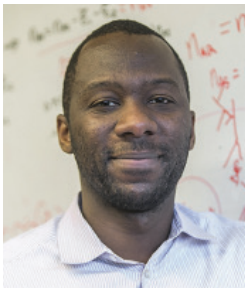
Speaker Biographies

16th Annual

PeptideTherapeutics Symposium



16th Annual Peptide Therapeutics Symposium



Christopher A. Alabi, Ph.D. | Associate Professor, Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University
Design and Evaluation of Synthetic Lipopeptides as Potent Antivirals

Christopher Alabi began his research career as an undergraduate researcher in synthetic organic chemistry under the direction of Dr. David Schuster at NYU. Upon receiving his B.S. in Chemistry from NYU and B.E. in Chemical Engineering from Stevens Institute of Technology, he went on to pursue a graduate degree in Materials Chemistry at California Institute of Technology with Dr. Mark Davis. He then moved to MIT in 2009 and served as NIH Postdoctoral Fellow with Drs. Langer and Anderson. Chris Alabi joined the Cornell faculty in 2013 as an Assistant Professor in the School of Chemical and Biomolecular Engineering. He has won several awards during his short tenure at Cornell including the PhRMA Foundation Research Starter Award, NSF CAREER Award, the 2016 Cornell Engineering Research Excellence Award, the 2017 Tau Beta Pi Professor of the Year Award and the 2018 PMSE Young Investigator Award. In 2019, Chris Alabi was promoted to Associate Professor with Indefinite Tenure. Research in the Alabi lab seeks to understand how the composition and sequence of a macromolecular chain affects its chemical, structural and biological properties with an eye towards engineering sustainable materials and biomolecular therapeutics.

Jordi Alsina, Ph.D. | Research Fellow, Eli Lilly and Company
Developent of Next Generation Incretin Tirzepatide, a Novel Dual GIP and GLP-1 Receptor Agonist Peptide

Dr. Alsina is a Research Fellow and Group Leader for Peptide Discovery at Eli Lilly and Company. His responsibilities include design and synthesis of the next generation of peptide therapeutics, in close collaboration with discovery colleagues in different therapeutic areas within Lilly Research Laboratories. He is one of the coinventors of Tirzepatide and several other peptides in early clinical development at Lilly.

He received his doctorate degree from University of Barcelona, Spain and did postdoctoral studies in the field of peptide chemistry at the University of Minnesota in Minneapolis, Minnesota. He joined Lilly in 2004.

David Baker, Ph.D. | Professor of Biochemistry and Director of the Institute for Protein Design, University of Washington

The Coming of Age of De Novo Protein Design

David Baker is the director of the Institute for Protein Design, a Howard Hughes Medical Institute Investigator, a professor of biochemistry and an adjunct professor of genome sciences, bioengineering, chemical engineering, computer science, and physics at the University of Washington. His research group is a world leader in protein design and protein structure prediction. He received his Ph.D. in biochemistry with Randy Schekman at the University of California, Berkeley, and did postdoctoral work in biophysics with David Agard at UCSF. Dr. Baker is a member of the National Academy of Sciences and the American Academy of Arts and Sciences. Dr. Baker is a recipient of the Breakthrough Prize in Life Sciences, Irving Sigal and Hans Neurath awards from the Protein Society, the Overton Prize from the ISCB, the Feynman Prize from the Foresight Institute, the AAAS Newcomb Cleveland Prize, the Sackler prize in biophysics, and the Centenary Award from the Biochemical society. He has also received awards from the National Science Foundation, the Beckman Foundation, and the Packard Foundation. Dr. Baker has published over 500 research papers, been granted over 100 patents, and co-founded 11 companies. Seventy-five of his mentees have gone on to independent faculty positions.

Annette G. Beck-Sickinger, Ph.D. | Institute of Biochemistry, Faculty of Life Sciences, Leipzig University

G Protein-Coupled Receptors: From Structure to Cell Specific Drug Targeting

Annette G. Beck-Sickinger studied chemistry and biology at the University of Tübingen (Germany). After postdoctorial fellowships in Zürich and Copenhagen, she became assistant professor of Biochemical Pharmacy at ETH Zürich. Since 1999, she is full professor of Biochemistry at Leipzig University.

Annette Beck-Sickinger has been awarded with many prizes including the Leonidas Zervas Award of the European Peptide Society, the gold medal of the Max-Bergmann-Kreis (2009), the Leipzig Science Award (2016), the Albrecht Kossel Award of Biochemistry of the GDCh (2018) and the Du Vigneaud Award of the American Peptide Society (2019). She was honoured with the memberships of the Saxonian Academy of Science in 2009, of the Göttinger Academy of Science (2021), and the German National Academy of Sciences Leopoldina (2012). In 2017, she was awarded with the Saxonian Order of Merit.

Her major reseach fields include structure-activity-relationships of peptide hormones and G protein coupled receptors and protein modification to study function and interaction. A tight connection of chemical methods, bioorganic synthesis and molecular biology tools, including cloning, receptor mutagenesis, protein expression and cell biochemistry is applied. Her interests include further the identification of novel targets, novel therapeutic concepts and innovative approaches to modify proteins as well as concepts for improved enzyme catalysis and biomaterials.

16th Annual Peptide Therapeutics Symposium

Konrad Bleicher, Ph.D. | RNA Therapeutics, Roche Innovation Center Basel, Switzerland

RNA Therapeutics: New Chemical Modalities Becoming a Therapeutic Reality

Konrad Bleicher holds a Ph.D. in Organic Chemistry, which he received from the Tübingen University in Germany (Synthesis and MS characterization of Antisense Oligonucleotides). He started his professional career at Sandoz/Novartis (Switzerland & US) where he gained experience in the area of Combinatorial Chemistry before joining Roche as a scientist in the CNS chemistry department. Since then he has been holding various positions in the Small Molecule Medicinal Chemistry area (Hit ID, Lead ID & Lead Optimization). In 2009 Dr. Bleicher was nominated "Peptide Area Head", overseeing the peptide chemistry activities and building up a peptide portfolio for different indications (mainly CNS-, metabolic- and infectious diseases). Since 2016 he is a member of the RNA Therapeutics group mainly responsible for the RNA chemistry strategy and platform development in Roche Basel. He is the author of over 80 patents/publications.

Matthew Bogyo, Ph.D. | Professor of Pathology and Microbiology and Immunology Stanford University

Applications of Covalent Peptide Probes for Imaging of Cancer and Infectious Diseases

Dr. Bogyo is a Professor of Pathology and Microbiology and Immunology at Stanford University. He received his bachelor's degree in Chemistry from Bates College in 1993 and a doctorate in Chemistry from Massachusetts Institute of Technology in 1997. Dr. Bogyo established an independent scientific career as a Faculty Fellow at the University of California, San Francisco, where he supervised a small laboratory of post-doctoral fellows and students. In 2001, Dr. Bogyo was hired to establish and direct the Chemical Proteomics Department at Celera Genomics focused on applying small molecule probes to the field of drug discovery. Dr. Bogyo then joined the Department of Pathology at Stanford University in July 2003 and was promoted to Associate Professor in 2009 and to full professor in 2013. His laboratory works on the development of new chemical probe technologies that are applied to the study the role of proteases in complex biological pathways associated with human disease. Dr. Bogyo has published over 250 primary research publications and currently serves on the Editorial Board of several prominent research journals. He was the President of the International Proteolysis Society from 2007-2009 and chair of the Gordon Research Conference on Proteolytic Enzymes and Their Inhibitors in 2018 and the Imaging in 2020 meeting in 2016. Dr. Bogyo is also a member of Stanford's Comprehensive Cancer Center, the Molecular Imaging Program at Stanford (MIPS) and is a consultant for several biotechnology and pharmaceutical companies in the Bay Area. He is the recipient of numerous awards including the Searle Scholar Award, The Terman Fellowship and the Burroughs Wellcome Investigators in Pathogenesis award. He is the co-founder of Akrotome Imaging, a small start up company developing imaging contrast agents for detection of surgical margins and Facile Therapeutics which is developing novel anti-virulence agents for the treatment of *C. difficile* infections.

Dame Margaret Brimble DNZM, FRS, Ph.D. | Distinguished Professor, School of Chemical Sciences and the Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, New Zealand

Peptide-Based Therapeutics: Paving the Way from the Lab to the Clinic

Distinguished Professor Dame Brimble studied at the University of Auckland, before being awarded a Commonwealth Scholarship to complete her Ph.D. at the University of Southampton. She is a Fellow of the Royal Society of London, Dame Companion of the New Zealand Order of Merit, has been inducted into the American Chemical Society Medicinal Chemistry Hall of Fame and received the Rutherford, Hector and MacDiarmid medals (Royal Society NZ), the Kiwinet BNZ Supreme award and Baldwins Research Entrepreneur 2019 commercialization awards and the Marsden medal (NZ Association of Scientists). She was awarded the Sosnovsky Award for Cancer Therapy and Natural Products award from the Royal Society of Chemistry. Her research focusses on the synthesis of novel bioactive natural products/peptides and the synthesis of lipopeptides for cancer vaccines and new biomaterials to target dental caries. She discovered the drug candidate trofinetide (NNZ2566) that is in phase 3 clinical trials for Rett Syndrome (Neuren Pharmaceuticals and Acadia Pharmaceuticals) and NNZ2591 (phase 2 clinical trials for Phelan-McDermid syndrome, Angelman syndrome and Pitt Hopkins syndrome). She is co-founder of the cancer immunotherapy company SapVax that has licensed her CLipPA peptide lipidation technology to develop self-adjuvanting peptide-based cancer vaccines. Her laboratory hosts NZ's only laboratory accredited by Medsafe NZ to manufacture peptides under cGMP for human clinical trial. Professor Brimble is currently an Associate Editor for *Organic Letters* (ACS).

Phil Dawson, Ph.D. | Chairman of the Board, Peptide Therapeutics Foundation; Professor of Chemistry, Scripps Research; Dean of the Skaggs Graduate School of Chemistry and Biological Sciences

Opening Remarks

Phil Dawson is a Professor in the Department of Chemistry, Scripps Research in La Jolla, CA and Dean of the Skaggs Graduate School of Chemical and Biological Sciences. He received an A.B. (1992) in Chemistry from Washington University, and Ph.D. (1996) from Scripps Research under the guidance of Steve Kent. After pursuing postdoctoral work at Caltech, he returned to Scripps as an Assistant Professor. He has served as President of the American Peptide Society, the Board of Directors for FASEB and co-chaired the 22nd American Peptide Symposium and the 2016 GRC. He has published over 180 papers, and has been honored with an Alfred P. Sloan Foundation fellowship, the Vincent du Vigneaud Award, the Max Bergmann Kreis Gold Medal, the Zervas Award and the RSC MedImmune Protein and Peptide Science Award and the Akabori Memorial Award. Professor Dawson is a pioneer of chemoselective ligation methods for macromolecule synthesis and modification and has applied these tools broadly to better understand biological systems.

16th Annual Peptide Therapeutics Symposium

Adrienne Day, Ph.D. | Director and Treasurer, Peptide Therapeutics Foundation; President, Blue Gum Advisors
Closing Remarks

Dr. Day is the President of Blue Gum Advisors LLC, a consulting firm focused on providing business development support to the life science industry. She is a seasoned business development professional with more than 30 years of experience in the biotechnology and biopharmaceutical industries. Dr. Day has hands-on operational and executive management experience in the non-profit, for-profit and startup environments.

Most recently she was Senior Director of Business Development for Ferring Pharmaceuticals. Prior to that 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 and the La Jolla Cancer Research Foundation.

William DeGrado, Ph.D. | Department of Pharmaceutical Chemistry, University of California, San Francisco
Use of Integrin Antagonists to Disrupt Pathological Mechanical Force-Dependent Processes in Fibrosis and Severe Asthma

William (Bill) DeGrado's work focuses on the design of small molecule drugs, peptides, and proteins to address biological and mechanistic questions. Since 2011, Bill has been a professor in the Department of Pharmaceutical Chemistry at the University of California San Francisco. Prior to UCSF, he was a member of DuPont Central Research and DuPont Merck Pharmaceutical Company from 1981 to 1996, and then the Raiziss Professor in the Department of Biochemistry and Biophysics at the University of Pennsylvania (1996–2011). He graduated from Kalamazoo College in 1978, received his Ph.D. in organic chemistry from the University of Chicago (1981), and joined DuPont Central Research without an intervening postdoctoral position.

Daniel Drucker, Ph.D. | Senior Scientist, Lunenfeld Tanenbaum Research Institute, Mt. Sinai Hospital, University of Toronto
GLP-1 Action-Mechanisms and Future Directions

Dr. Drucker was among the first to describe the processing of proglucagon into smaller peptides, and he characterized actions of GLP-1 on insulin biosynthesis and secretion in the mid 1980s. The actions of GLP-1 were validated in human studies and supported development of new drug classes for the treatment of type 2 diabetes and obesity. In 1996, he described the first actions of GLP-2, and identified a degradation-resistant GLP-2 analogue, teduglutide, enabling the clinical development and approval of teduglutide for the treatment of intestinal failure. His studies spanning 3 decades have utilized molecular and cellular biology and mouse genetics to define multiple new mechanisms for gut hormone action. This body of work has provided new insights into additional therapeutic opportunities, while enhancing our understanding of pathways informing the safety of these agents.

Tom Foltynie, Ph.D. | Professor of Neurology, National Hospital for Neurology & Neurosurgery, London, UK
The Use of GLP-1 Receptor Agonists for the Treatment of Parkinson's Disease

Professor Tom Foltynie is Professor of Neurology in the Department of Clinical and Movement Neurosciences, UCL Institute of Neurology and Consultant Neurologist at the National Hospital for Neurology and Neurosurgery, Queen Square, London. He is responsible for Movement disorder patients, particularly Parkinson's disease (PD) patients undergoing advanced treatments such as Deep Brain Stimulation (DBS), Apomorphine and Duodopa. He is chief investigator for a series of trials of Exenatide- a potential neurorestorative treatment for PD, as well as the lead clinician at UCL for trials of alpha synuclein antibosy treatment for PD and Oxford Biomedica/ Axovant's gene therapy product for PD, and the Transeuro PD cell transplantation programme.

Professor Foltynie has published clinical trials of DBS as a treatment for the cognitive problems associated with advanced PD/DLB, as well as successful results of a trial of Deep Brain Stimulation for the treatment of patients with severe Tourette syndrome. He is interested in the mechanisms of action of DBS as elucidated using functional MRI, and developing ways of providing therapeutic DBS with better benefit to side effect ratios.

He trained in medicine at UCL, qualifying in 1995 then working in Addenbrooke's Hospital, in Cambridge. From 1999 to 2003, he undertook his Ph.D. in Cambridge looking at the heterogeneity of Parkinson's disease, describing differences in cognitive abilities between patients under the influence of various genes including COMT and BDNF, and Tau. He finished his neurology training between Addenbrooke's Hospital, Cambridge and the National Hospital for Neurology and Neurosurgery in London, before taking up his consultant clinical academic position in London in 2008. He was promoted to Professor in 2016.

16th Annual Peptide Therapeutics Symposium

Nader Fotouhi, Ph.D. | CSO, TB Alliance
Welcome and Introduction: Waleed Danho Celebration

As Chief Scientific Officer for the TB Alliance, Dr. Nader Fotouhi guides and oversees the organization's research and preclinical development activities.

Dr. Fotouhi has 30 years of experience in the pharmaceutical industry, with significant research and early development expertise in a variety of therapeutic areas. Prior to joining the TB Alliance, Dr. Fotouhi held various leadership positions at Hoffmann-La Roche, including the head of the Discovery Chemistry group at the Nutley New Jersey site, the global head of Discovery Technologies, and served as the Nutley New Jersey Pharma Research and Early Development Site Leader.

Dr. Fotouhi holds a Ph.D. and Postdoctoral fellowship in Organic Chemistry from the Massachusetts Institute of Technology. Dr. Fotouhi has authored or co-authored more than 50 articles and presentations and holds 22 patents.

M. Reza Ghadiri, Ph.D. | Professor, Department of Chemistry and The Skaggs Institute for Chemical Biology, Scripps Research

Covalent Non-hydrolyzable Peptides with Human Caspase Prime-side Specificities

Dr. Ghadiri is Professor, Department of Chemistry and Member of the Skaggs Institute for Chemical Biology, at The Scripps Research. He received his B.A. in Chemistry from University of Wisconsin- Milwaukee in 1982 and Ph.D. in 1987 in Synthetic Organic Chemistry with Professor B. M. Trost at the University of Wisconsin-Madison. Dr. Ghadiri joined the faculty of The Scripps Research Institute in 1989 after completing a postdoctoral appointment at The Rockefeller University in the laboratory of Professor E. T. Kaiser.

Dr. Ghadiri has a broad and multidisciplinary research interest that include: de novo design of synthetic peptides and catalysts; rational design of DNA-programmed intrasterically regulated enzymes and therapeutics; prebiotic chemistry, design of self-replicating molecular systems, complex self-organized networks, and adaptive informational biopolymers; design and discovery of bioactive and antimicrobial agents; single-molecule nanopore DNA sequencing; DNA-based molecular computation; and chemical biology of the gut microbiome.

He is recipient of the Arnold and Mabel Beckman Young Investigators Award (1991); Searle Scholars Award (1991); Alfred P. Sloan Research Fellow (1993); Eli Lilly Grantee (1994); American Chemical Society Award in Pure Chemistry (1995); Feynman Prize in Nanotechnology (1998); Arthur C. Cope Scholar Award, American Chemical Society (1999); Elected Fellow, The American Association for the Advancement of Science (2001); Vincent du Vigneaud Award, The American Peptide Society (2010); and Ronald Breslow Award for Achievement in Biomimetic Chemistry, American Chemical Society (2021).

Rie Schultz Hansen, Ph.D. | Director, Peptide Therapeutics Foundation, Head of Discovery and Innovation, Zealand Pharma A/S Sydmarken
Dasiglucagon, a Next-generation Ready-to-use Glucagon Analog and its Potential Use in Bi-Hormonal Artificial Pancreas Pumps

Rie holds a Ph.D. in medicinal research from the University of Copenhagen. She is the author of more than 50 publications, published conference reports and patent applications.

Rie has 18 years of work experience from biotech companies. Working at Zealand Pharma for the last 9 years, the focus of her research and industrial work has been in translational pharmacology for peptide and small molecule drug candidates. Prior to joining Zealand, Rie was a research scientist at NeuroSearch.

Carrie Haskell-Luevano, Ph.D. | Professor & Associate Department Head, Philip S. Portoghese Endowed Chair in Chemical Neuroscience, University of Minnesota Department of Medicinal Chemistry
Defining the Melanocortin GPCR System for Non-Opioid Analgesia and Peripheral Diabetic Neuropathic Pain Therapeutic Development

Carrie Haskell-Luevano, Ph.D., earned her BS degree in Chemistry from the California State University of Fresno (1990) and her Ph.D. degree in Chemistry (1995) at the University of Arizona. Subsequently, she performed NIH NRSA supported postdoctoral studies at the University of Michigan and Vollum Institute in Portland Oregon (OHSU). In 1998, she established an independent research program at the University of Florida Department of Medicinal Chemistry in the College of Pharmacy. In 2011 Dr. Haskell-Luevano was recruited to join the University of Minnesota Department of Medicinal Chemistry as a Professor and the inaugural Philip S. Portoghese Endowed Chair in Chemical Neuroscience as well as a University of Minnesota Institute for Translational Neuroscience Science Scholar. Professional contributions and service to the Peptide scientific community include: Associate Editor for the *Journal of Medicinal Chemistry* (2012-2020), ACS Medicinal Chemistry (MEDI) councilor (2015–2020), American Peptide Society (APS) Councilor (2005–2017, 2021–2027) as well as a charter member of Drug Discovery for the Nervous System (division DDNS 2015-2019) the Synthetic and Biological Chemistry-B (SBC-B 2005–2009) NIH Study Sections. Dr. Haskell-Luevano was elected as Vice Chair (2006) and Chair (2008) Gordon Research Conference “Peptides, Chemistry & Biology Of”.

Dr. Haskell-Luevano’s research focuses upon GPCRs, the neuroendocrine regulation of pain (opioid receptors), food intake, and energy homeostasis (melanocortin receptors). Research approaches utilize a variety of multidisciplinary techniques including peptide, small molecule, and combinatorial chemistry synthesis and cell based assays, chemical biology, neuromolecular pharmacology, working with knock out mice, and neuroscience. Dr. Haskell-Luevano’s research has been continuously and generously supported by NIH (NIDDK & NIDA) as well as the American Heart Association, American Diabetes Association, and the United States-Israel Binational Science Foundation. The Haskell-Luevano laboratory has published >150 peer-reviewed manuscripts, reviews, book chapters, and conference proceedings. Six patents have been issued based upon the discovery of novel ligands towards anti-obesity therapeutic applications.

16th Annual Peptide Therapeutics Symposium

Kim Henriksen, Ph.D. | Director of Endocrinology, Nordic Bioscience A/S & KeyBioscience AG, Denmark
Identification, Characterization and Development of Dual Amylin and Calcitonin Receptor Agonists as Novel Drug Candidates Providing Insulin Sensitization and Weight Loss

Dr. Henriksen joined Nordic Bioscience as a member of the pharmacology division in 2002. In 2010 he joined the endocrinology effort at Nordic Bioscience, and assumed the position as Director of Endocrinology in 2018.

His research is focused on identification and development of novel peptide therapies for metabolic conditions, such as obesity, NASH and type 2 diabetes. The primary target was the identification of peptides belonging to the calcitonin family of peptides with superior activity on the amylin and calcitonin receptors, also called Dual Amylin and Calcitonin Receptor Agonists (DACRAs). The work has resulted in patenting and development of a series of DACRAs, including KBP-042 and KBP-089, and more recently the long acting KBP-066A.

Dr. Henriksen has published more than 160 peer-reviewed papers, many of which are about the development of the DACRAs. He presently has an H-Factor: 52, an i10 index of 129 and a total of 9837 citations.

Anish Konkar, Ph.D. | Senior Director, Cardiometabolic & Renal Diabetes and Complications Therapeutic Area, Eli Lilly and Company
PYY Analogs for the Treatment of Obesity and Type 2 Diabetes

Anish Konkar is currently working in the Cardiometabolic and Renal department within the Diabetes and Complications Therapeutic Area at Eli Lilly and Company. He received his PhD in pharmacology from the Ohio State University, and performed postdoctoral research at Wayne State University and Parke-Davis (now Pfizer) with Dr. James Granneman investigating the pharmacology of beta-adrenergic receptors. Subsequently, Anish has worked at several pharmaceutical companies in the area of metabolic diseases research, including Bayer, Roche, MedImmune and Sanofi. Anish had the opportunity to work with several excellent cross-functional teams that advanced preclinical candidates into the clinic, including PYY analogs, dual and triple agonists of GLP-1, GIP and/or glucagon receptors.

Adam Mezo, Ph.D. | President, Peptide Therapeutics Foundation, Senior Director, Discovery Chemistry, Ferring Research Institute, Inc.
Opening Remarks

Dr. Adam Mezo is currently Senior Director, Discovery Chemistry at the Ferring Research Institute, Inc. in San Diego, part of the global research organization at Ferring Pharmaceuticals. Since 2017, he has led teams of chemists and drug hunters aimed at the discovery and development of next-generation peptide and small molecule therapeutics across a range of therapeutic areas including reproductive and women's health, gastroenterology and immunology.

Prior to this role, Dr. Mezo was Sr. Research Advisor and Group Leader at Eli Lilly for 5 years leading teams of peptide chemists that delivered several clinical candidate molecules for the treatment of diabetes. He started his career in at Syntonix Pharmaceuticals / Biogen Idec, working at the interface of peptide, protein and small molecule chemistry, generating semi-synthetic peptide-Fc fusion proteins, peptide antagonists and small molecules. Dr. Mezo held a variety of roles, including Director, Molecular Discovery, where he led a diverse team of chemists, biochemists and molecular biologists in search of novel therapeutics in the fields of hemophilia and immunology.

Dr. Mezo has over 50 published manuscripts and conference presentations, along with 20 issued US patents. He received his undergraduate degree in chemistry from Queen's University (Canada), PhD from the University of British Columbia in organic chemistry, and performed postdoctoral work at the Massachusetts Institute of Technology in the field of bioorganic chemistry.

Patrick A. Ott, MD, Ph.D. | Dana Farber Cancer Institute
Personal Peptide Vaccines Directed at Neoantigens for Patients with Advanced Solid Tumors

Dr. Patrick Ott is the Clinical Director of both the Melanoma Disease Center and the Center for Immuno-Oncology at DFCI, serves as attending physician in the Department of Medicine at Brigham and Women's Hospital, and has an appointment as Associate Professor at Harvard Medical School in Boston, MA. Dr. Ott received his MD and Ph.D. from Ludwig Maximilians University of Munich, Germany. He completed post-doctoral training in Immunology and residency training in Medicine at Case Western Reserve University. After a fellowship in Hematology-Oncology and 4 years on the faculty at New York University, he moved to Dana Farber Cancer Institute (DFCI) in 2012.

He is a clinical investigator and an integral member of the clinical trials program at Dana Farber/ Harvard Cancer Center, where he designs and conducts phase 1 immunotherapy trials for patients with melanoma and a wide range of other tumors. His primary research interests are in melanoma and immunotherapy, specifically the development of innovative tumor vaccine approaches. Dr. Ott has been the Principal Investigator of a first in man clinical trial testing a personalized neoantigen vaccine (NeoVax) in patients with melanoma. The results of the study, reported in in *Nature* in 2017 and in *Nature Medicine* in 2021, established the feasibility and safety of this novel cancer vaccine approach for the first time in a coordinated clinical trial setting. Strong and consistent immunogenicity was demonstrated in patients with high risk melanoma, providing the basis for further testing of this innovative new treatment concept in other cancers. A study using an almost identical personal neoantigen vaccine in combination with nivolumab in patients with metastatic melanoma, non-small cell lung cancer, and urothelial cancer also demonstrated that robust vaccine specific responses were generated. Furthermore, complete pathologic responses and epitope spreading were associated with clinical benefit, suggesting vaccine induced anti-tumor activity (Ott et, Cell, 2020). Dr. Ott has been the Principal Investigator and co-investigator on over 30 treatment trials, including those that have been instrumental in the clinical development of the newly FDA approved drugs pembrolizumab and nivolumab for the treatment of advanced melanoma, small cell lung cancer, and many other cancers. This work has resulted in numerous high impact publications including the New England Journal of Medicine, the Lancet Oncology, and the Journal of Clinical Oncology.

16th Annual Peptide Therapeutics Symposium

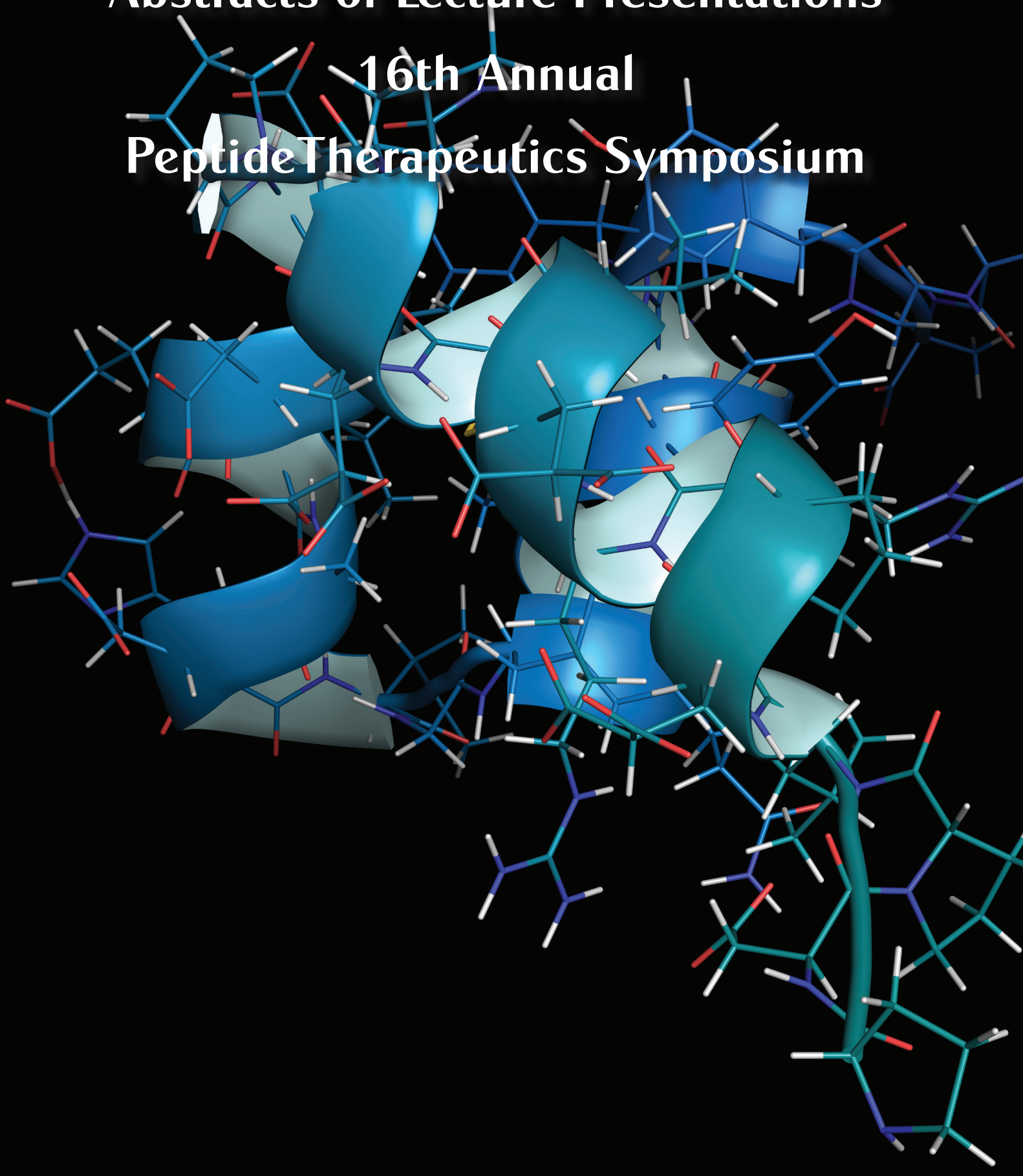
Christina I. Schroeder, Ph.D. | Stadtman Investigator, Center for Cancer Research, National Cancer Institute, National Institutes of Health
Recifin A, a Novel and Selective Allosteric Inhibitor of Tyrosyl-DNA Phosphodiesterase I with a Unique Disulfide-bond Topology

Dr. Schroeder holds a M.Sc. in Chemistry from University of Kalmar, Sweden, a Ph.D. in Pharmacology from the University of Queensland, Australia, and a Graduate Certificate in Research Management from Southern Cross University, Australia. She has carried out postdoctoral training at Scripps Research Institute, the University of Queensland, and the University of New South Wales, Australia, and has led an independent research group at the University of Queensland's Institute for Molecular Bioscience since 2014, focusing her research on biodiscovery and peptide engineering of venom-derived bioactive peptides for the development of novel peptide-based drug leads for pain and cancer. She was the recipient of the inaugural Lord Mayor Convention Trailblazer grant in 2018, and in 2019 she was awarded the prestigious Treager award from the Australian Peptide Association. In 2020 she joined the Chemical Biology Laboratory at the National Cancer Institute, NIH, USA as a Stadtman Investigator and she holds an adjunct Associate Professor position at the University of Queensland, Australia.

Abstracts of Lecture Presentations

16th Annual

Peptide Therapeutics Symposium



Design and Evaluation of Synthetic Lipopeptides as Potent Antivirals

Christopher A. Alabi. Ph.D.
Associate Professor
Robert Frederick Smith School of Chemical and Biomolecular Engineering
Cornell University

Viral infection of target cells occurs via the coordinated action of binding and fusion proteins. Upon receptor engagement, the fusion protein undergoes a structural transition that leads to its insertion and subsequent fusion with the target cell membrane. Peptides derived from the heptad repeat region of the fusion protein can interfere with the structural transition of the fusion protein, thus inhibiting infection at the entry stage. In a collaborative effort with the Porotto and Moscona research groups, our team has developed multivalent lipopeptide inhibitory ligands that self-assemble into serum stable nanoparticles with potent antiviral activity. Self-assembly of the amphipathic lipopeptides enhances their biodistribution and half-life and contributes to enhanced *in vivo* efficacy. In the first part of this talk, I will describe the development of an antiviral peptide that targets the measles virus (MeV) fusion protein. This antiviral peptide construct comprises a fusion inhibitor tripeptide (FIP) conjugated to a lipidated MeV fusion C-terminal heptad repeat (HRC) domain. Chemical conjugation of both components resulted in markedly increased antiviral potency. Surprisingly, in vitro mechanistic experiments revealed the FIP-HRC lipid conjugate exerted its antiviral activity predominantly by stabilizing the fusion protein in its prefusion state, while HRC-derived peptides alone largely act on the fusion protein after activation. Coupled with in vivo experiments showing effective prevention of MeV infection in cotton rats, FIP-HRC lipid conjugates show promise as potential MeV antivirals. In the second half of this talk, I will discuss the design of a fusion inhibitory dimeric lipopeptide with potent activity against the SARS-CoV-2 virus. Proposed anchoring of the dimeric lipopeptide in the host cell membrane, interactions with the viral proteins, and retention in the lungs contributed to preventing direct-contact transmission in ferrets.

Development of Next Generation Incretin Tirzepatide, a Novel Dual GIP and GLP-1 Receptor Agonist Peptide

Jordi Alsina, Ph.D.
Research Fellow
Eli Lilly and Company

Tirzepatide (LY3298176) is a dual GIP and GLP-1 receptor agonist peptide currently under development for the treatment of type 2 diabetes mellitus (T2DM), obesity, nonalcoholic steatohepatitis (NASH), and heart failure with preserved ejection fraction (HFpEF). Phase 3 clinical trials in T2DM indicate that tirzepatide improves clinical outcomes beyond those achieved by selective GLP-1 receptor peptide agonists and could represent a novel incretin treatment paradigm. During this talk, preclinical data that led to the discovery of this multi-functional peptide will be presented including description of key aspects of the chemical structure, its unique in vitro pharmacological profile, *in vivo* characterization in diabetic/obese rodent models, and ADME properties.

The Coming of Age of De Novo Protein Design

David Baker, Ph.D.
Professor of Biochemistry
Director of the Protein Design
University of Washington

Proteins mediate the critical processes of life and beautifully solve the challenges faced during the evolution of modern organisms. Our goal is to design a new generation of proteins that address current-day problems not faced during evolution. In contrast to traditional protein engineering efforts, which have focused on modifying naturally occurring proteins, we design new proteins from scratch based on Anfinsen's principle that proteins fold to their global free energy minimum. We compute amino acid sequences predicted to fold into proteins with new structures and functions, produce synthetic genes encoding these sequences, and characterize them experimentally. In this talk, I will describe general methods for designing proteins which bind with high affinity to arbitrary protein targets, the use of these methods to design 55 residue proteins that bind to the SARS-COV-2 Spike with picomolar affinity and block infection, and the systematic design of membrane permeable macrocycles with high structural accuracy.

G Protein-Coupled Receptors: From Structure to Cell Specific Drug Targeting

Annette G. Beck-Sickinger, Ph.D.
Institute of Biochemistry, Faculty of Life Sciences
Leipzig University

Peptides hormones play an important role in the regulation of manifold activi-ties in the body. Many of them transmit their activity through G protein-coupled receptors (GPCR), which are among the most promising drug targets nowadays. However, in addition to their direct activity, indirect mechanisms have been shown to play a role. This includes their use as drug shuttles, e. g. in tumour targeting. Accordingly, in addition to ligand binding, internalization has to be addressed and to be studied, including arrestin recruitment. The neuropeptide Y/pancreatic polypeptide family contains 36 amino acid peptides that bind in human to four different so-called Y-receptors. By a combination of X-ray analysis, NMR, molecular modelling and crosslinking combined with mass spectrometry, we have recently identified the distinct binding modes of NPY peptides to their Y-receptors[1-4]. Furthermore, we found that clustering of receptors plays an important role in ligand independent signalling[5].

All neuropeptide Y receptors have been shown to play a relevant role in the regulation of food intake. Furthermore, they participate in adipogenesis and some of them overexpressed in tumours. By knowing the receptor bound structure, specific ligands as well as peptide-drug conjugates have been de-signed to selectively address Y receptors in different tissues including allo-steric modulators[6].

In breast cancer we demonstrated that human Y_1 receptors are addressable by peptide conjugates using ^{18}F PET-tracers[7]. We now designed Y_1 receptor selective peptides linked to different toxophors[8]. We identified novel linkers that lead to a rapid and efficient release of the toxin inside of the cell and sub-sequently to cell death.

In the field of tumour therapy, peptide-drug conjugates are already well ac-cepted. However, the concept of receptor-mediated internalisation and subsequent tissue specific intracellular application is not limited to the selective addressing of tumours. We recently demonstrated that peptide-drug conjugates are suitable to selectively shuttle drugs into adipocytes by NPY-meditated peptide-drug conjugates. Tesaglitazar that addresses the transcription factor PPARg led to metabolic changes in cells and mice after NPY mediated internalisation[9].

1 Kaiser A et al. *Angew, Chem Int Ed Engl.* **2015**, 54:7446-7449.
2 Yang Z, Han S, Keller M, Kaiser A, Bender B *et al.*, *Nature* **2018**, 556:520-524.
3 Tang T, Hartig C, *et al., Nat Commun.* **2021**,12:737.
4 Krug U et al. Angew, *Chem Int Ed Engl.* **2020**, 59:23854-23861.
5 Sánchez MF, *et al.*, *Science.* **2021**; 371(6536): eabb7657.
6 Schüß C, Vu O, Schubert M, *et al.*, *J Med Chem.* **2021**, 64:2801-2814.
7 Hofmann S *et al.*, *Mol Pharm.* **2015**, 12:1121-30.
8 Böhme D, Krieghoff J, Beck-Sickinger AG., *J Med Chem.* 2016;59:3409-17.
9 Wittrisch S *et al.*, *Mol Metab.* **2020**, 31:163-180.

RNA Therapeutics: New Chemical Modalities Becoming a Therapeutic Reality

Konrad Bleicher, Ph.D.
RNA Therapeutics
Roche Innovation Center Basel, Switzerland

Conceptually, RNA based molecules have been introduced as a potential therapeutic modality already back in 1978 by Zamecnik and Stephenson, demonstrating *in vitro* that the protein biosynthesis machinery can be modulated through Watson-Crick based interactions between a target RNA (Rous sarcoma virus) and a complementary oligonucleotide strand, in this particular case a 13-mer DNA oligonucleotide. Twenty years after this pioneering study, the first therapeutic Antisense Oligonucleotide (ASO) was approved by the FDA for the treatment of cytomegalovirus-induced retinitis in immunocompromised AIDS patients (Vitravene). Despite this successful launch, the field has been in turmoil and it took fifteen more years for yet another important breakthrough with the launch of Kynamro for the treatment of homozygous familial hypercholesterolemia. While this product was commercially not very successful, it demonstrated that indeed the systemic administration of such a modality is feasible and clinically functional. Since then, a couple of drugs have been marketed, which also go beyond RNase-H mediated RNA knockdown, and a rather impressive clinical portfolio has been built for different targets and indications.

Since the first published study by Zamecnik and Stephenson, it has been recognized that oligonucleotides need to be chemically modified to induce functional activity and over the years a broad range of chemical modifications have been investigated with the main purpose to positively modulate the pharmacokinetic profile of RNA drug candidates. Unarguably, the most fundamental invention in this respect was the introduction of the phosphorothioate internucleoside linkage, with most of the current oligonucleotide drugs and clinical assets carrying such a modification to various extents. While thereby introducing a chiral centre, recent developments in oligonucleotide chemistry now make the synthesis of fully stereodefined phosphorothioate ASOs possible, by generating these phosphorothioate linkages in an enantioselective fashion, allowing us to investigate the drug properties of single molecules rather than otherwise huge mixtures of diastereoisomers.

This presentation will give an overview of the current status of RNA therapeutics and particularly cover the medicinal chemistry aspects of single stranded antisense oligonucleotides. We will also discuss the impact of stereochemistry to the drug profile of antisense oligonucleotides in general, present discovery tactics for the identification of such stereodefined ASOs and particularly focus on the combination of achiral phosphorodithioates with stereodefined phosphorothioate internucleoside linkages. Both, *in vitro* potency and *in vivo* efficacy data will be presented and a particular focus will be given on drug metabolism. The data package will demonstrate the superiority of such chimeric thioate/dithioate-designs over their stereomixed as well as their fully stereodefined phosphorothioate ASO counterparts and exemplify what impact medicinal chemistry strategies may have for the further development of RNA therapeutics.

Applications of Covalent Peptide Probes for Imaging of Cancer and Infectious Diseases

Matthew Bogyo, Ph.D.
Professor of Pathology and Microbiology and Immunology
Stanford University

Hydrolases are enzymes (i.e. proteases, esterases, lipases) that often play pathogenic roles in many common human diseases such as cancer, asthma, arthritis, atherosclerosis and infection by pathogens. Therefore, tools that allow dynamic monitoring of their activity can be used as diagnostic agents, as imaging contrast agents and for the identification of novel enzymes as drug leads. In this presentation, I will describe our efforts to design and build peptide-based covalent probes that can be used to identify, inhibit and image various hydrolase targets in models of cancer and infectious disease. This will include recent advances in protease activated fluorescent probes for real-time visualization of tumors during surgery as well our efforts to identify several new classes of serine hydrolases in pathogenic and commensal bacteria. We believe many of these enzymes will represent valuable imaging and therapy targets that can be used to visualize and disrupt various aspects of colonization and community formation inside a host.

Peptide-Based Therapeutics: Paving the Way from the Lab to the Clinic

Dame Margaret Brimble DNZM, FRS
Distinguished Professor, School of Chemical Sciences and the Maurice Wilkins Centre for Molecular Biodiscovery
University of Auckland, New Zealand

This lecture will showcase our research on the synthesis of peptides, lipopeptides and glycopeptides as a platform for the discovery and development of peptide therapeutics as agents to treat neurogenetic disorders, infectious disease, cancer and diabetes. Professor Brimble discovered the peptide drug candidate trofinetide (NNZ2566) that has been granted orphan drug status and fast track designation by the US FDA and is currently being evaluated in a final phase III clinical trial undertaken by Neuren Pharmaceuticals (see: http://www.neurenpharma.com/IRM/content/default.aspx) to treat Rett Syndrome. Professor Brimble recently co-founded the spin-out company SapVax with US$6 million investment from BioMotiv in Cleveland, Ohio to develop a suite of "first-in-class cancer vaccines" based on a novel self-adjuvanting peptide chemistry platform for immuno-oncology applications (see: https://sapvaxllc.com). She also established a Medsafe NZ-approved laboratory that has manufactured clinical grade peptide antigens for use as vaccines in human clinical trials to treat melanoma

Use of Integrin Antagonists to Disrupt Pathological Mechanical Force-Dependent Processes in Fibrosis and Severe Asthma

William DeGrado, Ph.D.
Department of Pharmaceutical Chemistry
University of California, San Francisco

Hyunil Jo, Aparna Sundaram, Dean Sheppard, William DeGrado

Integrins are a class of dimeric membrane proteins, which connect extracellular protein ligands to cytoplasmic alpha-smooth muscle actin (αSMA). The force generated by αSMA contraction is transmitted through integrins to the bound extracellular ligands, playing an important in a number of biological and pathophysiological processes. For example, transforming growth factor beta is expressed as a latent precursor that is simultaneously bound to the extracellular matrix and one of a number of integrin subtypes, including $\alpha v\beta 1$ and $\alpha v\beta 6$. In stiff fibrotic tissue this results in activation of TGF-β in an actin-dependent manner. This pathological feed-forward loop can be inhibited using small molecule inhibitors of the appropriate integrins. We are also examining the role of integrin-mediated force generation in the exaggerated airway narrowing and contraction observed in severe asthma. We are designing integrin antagonists to disrupt the actin-extracellular bridge between airway smooth muscle and the extracellular matrix. In animal models, these antagonists are able to decrease the transmission of force and they mitigate hyper-responsiveness, while leaving normal contractile responses intact.

GLP-1 Action-Mechanisms and Future Directions

Daniel Drucker, Ph.D.
Senior Scientist
Lunenfeld Tanenbaum Research Institute, Mt. Sinai Hospital
University of Toronto

Enthusiasm for gut-derived therapies stems in part from a precise scientific understanding of the physiological and pharmacological roles of gut hormones in control of energy homeostasis. Interrogation of gut hormone action, ranging from studies of hormone synthesis and secretion, clearance and degradation, receptor signaling and PK-PD relationships, receptor desensitization, communication with the CNS, and understanding the utility of preclinical models has yielded a number of gut-derived therapies for treatment of diabetes and obesity. The success and tolerability of the DPP-4 inhibitors, the pleiotropic actions and striking efficacy of the GLP-1R agonists, and the enigmatic yet unparalleled benefits of bariatric surgery, have validated the enteroendocrine system as a viable target for the treatment of disorders of energy homeostasis. Here I review key principles, opportunities, challenges and pitfalls for ongoing efforts directed at development of novel, improved and safe gut-derived therapies, with a focus on where and how GLP-1 acts to control metabolism.

The Use of GLP-1 Receptor Agonists for the Treatment of Parkinson's Disease

Tom Foltynie, Ph.D.
Professor of Neurology
National Hospital for Neurology & Neurosurgery
London UK

The major unmet need in Parkinson's disease is the identification of interventions that may slow down, stop or even reverse the neurodegenerative process of Parkinson's disease. To date no therapies have been definitively proven to have such effects. There are laboratory data to confirm neuroprotective properties of a large number of agents but thus far, none have demonstrated definitive efficacy when evaluated in formal clinical trials.

The GLP-1 receptor agonists are licensed for the treatment of patients with Type 2 diabetes mellitus and in addition to effects on glucose level dependent insulin release, also have effects on preserving pancreatic beta cell mass. They have neuroprotective properties in-vitro and across a broad range of in-vivo models of Parkinson's disease. The epidemiological data also suggest that they ameliorate the elevated risk of Parkinson's disease seen among patients with type 2 diabetes. Two small clinical trials have reported an advantage in PD severity among people treated with exenatide, the original GLP-1 receptor agonist. These data have triggered major interest in whether this class of drug may indeed have neuroprotective properties that may be relevant to the broader population of PD patients.

Covalent Non-hydrolyzable Peptides with Human Caspase Prime-side Specificities

M. Reza Ghadiri, Ph.D.
Department of Chemistry and The Skaggs Institute for Chemical Biology
Scripps Research
10550 North Torrey Pines Road
La Jolla, California 92037

In humans and mammals, Western-type diet can induce imbalances in gut microbiota that strongly correlate with the development of several chronic diseases including atherosclerosis. I will describe that self-assembling cyclic D,L-α-peptides selected by using an *in vitro en masse* screening protocol can function as bacterial growth modulators to remodel a Western diet-induced imbalance in the gut microbiome and thus prevent atherosclerosis development in *LDLr*$^{-/-}$ mice. Daily oral administration of selected peptides to mice remodeled the gut microbiome and caused diverse effects in the host, including marked reductions in plasma cholesterol levels and atherosclerotic plaques. There was extensive reprogramming of the microbiome transcriptome and host gene expression levels, suppressed production of several pro-inflammatory cytokines, improved gut barrier integrity, increased populations of intestinal Helios positive regulatory T cells (Helios+ Treg) and rebalanced levels of disease-relevant metabolites, such as short-chain fatty acids (SCFAs) and bile acids. The ability to chemically manipulate the gut microbiome in a targeted fashion within a living organism provides not only an additional tool for deciphering the chemical biology of the gut microbiome, but also an avenue for advancing personalized therapeutics.

Dasiglucagon, a Next-generation Ready-to-use Glucagon Analog and its Potential use in Bi-Hormonal Artificial Pancreas Pumps

Rie Schultz Hansen, MSc, Ph.D.
Director, Peptide Therapeutics Foundation
Head of Discovery and Innovation,
Zealand Pharma A/S Sydmarken, Denmark

Hypoglycemia is a condition in which blood glucose drops to unsafe levels. It is frequently seen in people with diabetes treated with insulin which include all people with type 1 and approximately 20% of people with type 2 diabetes in the United States.
Type 1 diabetes is caused by insulin deficiency and is also associated with inappropriate glucagon secretion. Both hormones are essential to ensure stable and healthy blood glucose levels.

Dasiglucagon is an analog of human glucagon that is stable in aqueous formulation, approved by the US FDA for treatment of severe hypoglycemia in people with diabetes age 6 years and up. It is also being investigated for use in bi-hormonal artificial pancreas (BHAP) pumps containing both insulin and dasiglucagon for automated management of Type 1 diabetes, and in a low-dose pen for treatment of exercise-induced hypoglycemia.

A Phase 2 study is ongoing for dasiglucagon in a low-dose pen and a Phase 3 trial for use of dasiglucagon in a BHAP is expected to start late in 2021.

Defining the Melanocortin GPCR System for Non-Opioid Analgesia and Peripheral Diabetic Neuropathic Pain Therapeutic Development

Carrie Haskell-Luevano, Ph.D.
Professor & Associate Department Head
Philip S. Portoghese Endowed Chair in Chemical Neuroscience
University of Minnesota Department of Medicinal Chemistry

Carrie Haskell-Luevano, Philip S. Portoghese, Mary Lunzer, Danielle Adank, Mark Ericson

The first observations linking the melanocortins an1d nociception (pain) was in the 1970's (Gispen *et al.* and Bertolini *et al.*). The endogenous agouti-related peptide (AGRP) antagonist, endogenous pro-opiomelanocortin (POMC) derived agonists, and the melanocortin-4 receptor (MC4R) mRNA are expressed in the dorsal root ganglia (DRG) and spinal cord. A 2018 publication (Alhadeff *et al.*) studying hunger and pain as competing survival neuronal circuitry signals identified AGRP expressing neurons projecting to the hindbrain parabrachial nucleus (PBN) as important for acute thermal pain. Given the current state of the "opioid crises," and global search for non-opioid alternatives [for the treatment of pain without addictive and adverse side effects associated with morphine and drugs associated with substance use disorder (SUD)], data will be present clarifying and elaborating the role of the central melanocortin receptors (MC3R and MC4R) and ligands as potential therapeutic targets for analgesia.

Identification, Characterization and Development of Dual Amylin and Calcitonin Receptor Agonists as Novel Drug Candidates Providing Insulin Sensitization and Weight Loss

Kim Henriksen, Ph.D.
Director of Endocrinology
Nordic Bioscience A/S & KeyBioscience AG
Herlev Hovedgade 207
DK-2730 Herlev
Denmark

Obesity and type 2 diabetes (T2D) are two of the largest health challenges, and there is a need for drugs that effectively provide weight loss, insulin sensitivity, and preferably in combination with already existing drugs.

Amylin is a natural hormone, which is co-secreted with insulin from the β-cells, and which possesses an important ability to regulate food intake, and hence it has raised significant interest as a therapeutic option for obesity and T2D.

Through exploration of phylogenetically related peptides, we identified a series of peptides possessing amylin receptor agonistic potential, and with the most potent molecules being dual amylin and calcitonin receptor agonists (DACRAs). Using these peptide backbones as starting points, we devised a series of analogues called KBPs with the aim of identifying a DACRA peptide with increased potency and duration of action on the two target receptors, while showing no off-target activities. We identified a group of highly potent and specific lead compounds, including KBP-042 and KBP-089, which we selected for further testing.

Applying obese and diabetic rat models, we demonstrated the ability of the KBPs to reduce weight through the classical amylin receptor mediated mechanisms involving reduced food intake and increased energy expenditure. More surprisingly, the KBPs potently reduced fasting blood glucose and improved insulin sensitivity, effects not classically associated with amylin receptor agonists.

To deconstruct the involvement of the two receptors in the weight and glucose regulation by a KBP, we compared it to the selective ligands, amylin and calcitonin, in vivo. Here, we documented that calcitonin receptor agonism led to improved glucose control through improved insulin sensitivity, explaining the additional glucoregulatory potential of the DACRAs when compared to selective amylin receptor agonists.

Furthermore, we performed a series of combination studies, where the KBP was combined with the leading incretins. In these studies, we found a unique ability of the KBPs to combine with the incretin mode of action, which clearly illustrates their potential as combination partners.

In a first-in-man trial, KBP-042 showed the classical amylin receptor agonism induced nausea, while also showing indications of target receptor engagement indication pharmacodynamic effects. However, as peptide therapies for obesity and T2D were transitioning towards once weekly molecules, it was decided to pursue a long-acting molecule to replace the once daily KBP-042.

To address the short plasma half-life, a long acting KBP was developed through addition of a fatty acid side chain to the peptide backbone. Importantly, this led to a molecule with a PK profile compatible with once weekly dosing in humans. Importantly, the long acting KBP showed in vivo efficacy exceeding that of the short acting, both with respect to weight loss and glucose control, while maintaining its unique ability to combine with incretins.

In summary, the long-acting DACRA possesses potent weight and gluco-regulatory effects, and therefore is a highly relevant molecule for clinical development as a once weekly injection therapy for obesity and/or type 2 diabetes.
oral route.

PYY Analogs for the Treatment of Obesity and Type 2 Diabetes

Anish Konkar, Ph.D.
Senior Director, Cardiometabolic & Renal
Diabetes and Complications Therapeutic Area
Eli Lilly and Company

Obesity is as a major risk factor for numerous chronic diseases, including type 2 diabetes (T2D), chronic kidney disease, NASH, cardiovascular diseases, several cancers and an array of musculoskeletal disorders. The prevalence of overweight and obesity is increasing worldwide and the number of individuals who are classified as obese by the WHO has risen to >650 million people. Similarly, prevalence of diabetes has reached epidemic proportions, and the International Diabetes Federation estimated that 425 million adults between the ages of 20 and 79 years live with diabetes. Unlike bariatric surgery, which has a profound impact on obesity and T2D, currently available pharmacotherapies do not adequately treat these diseases. At present, only one drug class, glucagon-like peptide-1 receptor agonists (GLP-1RAs), has shown the potential to effectively treat both T2D and obesity. Based on the learning's from bariatric surgery-mediated body weight loss and resolution of T2D, a number of pharmaceutical companies have focused their efforts on identifying and developing gut peptides as treatments for chronic diseases associated with obesity and T2D. PYY(3-36) is an enteroendocrine hormone released by L-cells along with GLP-1 and has been shown to reduce excess caloric intake in preclinical and clinical studies. Similar to GLP-1, the peptide is cleared rapidly from circulation, which severely limits its therapeutic utility. Novel long acting PYY analogs offer the potential to produce significant body weight loss and glucose control when administered alone or in combination with GLP-1-based analogs.

Personal Peptide Vaccines Directed at Neoantigens for Patients with Advanced Solid Tumors

Patrick A. Ott, MD, Ph.D.
Dana Farber Cancer Institute

Cancer vaccines have been envisioned as an effective tool to generate, amplify, and diversify T cell responses against tumors. Neoantigens encoded by tumor mutations are key targets of effective anti-tumor immune responses. The majority of neoantigens are specific to each individual patient's tumor, and therefore targeting these antigens necessitates a personal approach i.e. vaccines need to be custom-made for each patient. In patients with high risk melanoma we have demonstrated that a personal neoantigen vaccine (NeoVax), consisting of up to 20 long peptides and poly-ICLC, induced strong polyfunctional neoantigen-specific T cells that recognized patient tumor in vitro. Generating the personalized vaccine was found to feasible and safe. Using ELISPOT, vaccine specific tetramers, and single cell RNAseq and TCRseq, we observed long-term persistence of neoantigen-specific T cell responses following vaccination, with ex vivo detection of neoantigen-specific T cells exhibiting a memory phenotype. We also observed diversification of neoantigen-specific T cell clones over time, with emergence of multiple T cell receptor clonotypes exhibiting distinct functional avidities. Furthermore, we detected evidence of tumor infiltration by neoantigen-specific T cell clones after vaccination and epitope spreading, suggesting on-target vaccine-induced tumor cell killing. Personal neoantigen peptide vaccines thus induce T cell responses that persist over years and broaden the spectrum of tumor-specific cytotoxicity in patients with melanoma. In a clinical trial testing NeoVax in patients with glioblastoma multiforme we also found NeoVax to be safe and immunogenic and demonstrated trafficking of vaccine specific T cells into intracranial tumor. In phase 1b study in patients with metastatic melanoma, non-small cell lung cancer, and urothelial cancer who received personal peptide vaccines in combination with anti-PD-1 therapy we found encouraging prolonged median PFS compared to historical controls, evidence of vaccine-specific T cells in post-vaccine metastatic tumors, as well as evidence for epitope spreading which was associated with durable clinical benefit.

Recifin A, a Novel and Selective Allosteric Inhibitor of Tyrosyl-DNA Phosphodiesterase I with a Unique Disulfide-bond Topology

Christina I. Schroeder, Ph.D.
Stadtman Investigator
Center for Cancer Research, National Cancer Institute, National Institutes of Health
Frederick, MD 21704

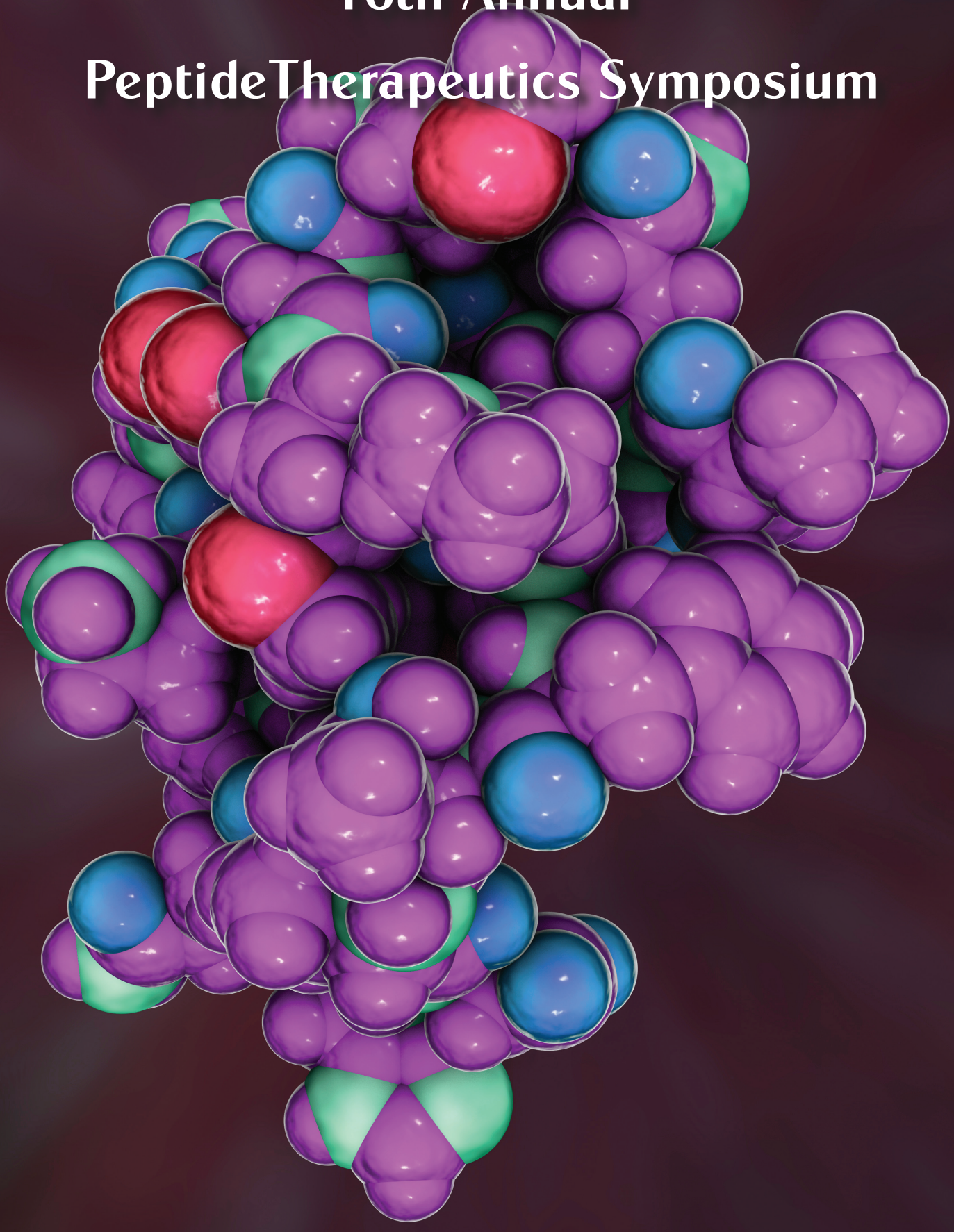
Tyrosyl-DNA phosphodiesterase 1 (TDP1) is a molecular target for the sensitization of cancer cells to the FDA-approved topoisomerase inhibitors topotecan and irinotecan. However, current TDP1 inhibitors have low binding affinity or are substrate mimics with low specificity. Through high-throughput screening of natural products and extracts library in the search for novel TDP1 inhibitors, we identified a new class of complex knotted peptides with a unique disulfide-bond topology from the marine sponge Axinella sp.(1) The active component was a 42-residue peptide named recifin A. Unlike previously described TDP1 inhibitors which bind to the C-terminal catalytic domain of TDP1, recifin A acts as an allosteric inhibitor and binds to the N-terminal regulatory domain. The three-dimensional NMR structure revealed a novel fold comprising a four-strand antiparallel β-sheet and two helical turns stabilized by a complex disulfide-bond network that creates an embedded ring around one of the strands. The structure is locked in place by a centrally located tyrosine residue, resulting in the Tyr-lock family name. Recifin A represents both the first of a unique structural class of knotted disulfide-rich peptides and defines a previously unseen mechanism of TDP1 inhibition that could lead to the development of a new class of TDP1 inhibitors with improved specificity that could be exploited for potential anticancer applications. .

[1] Krumpe, L.R.H. *et al.*, *JACS*, **2020**, *142*, 21178-21188

Abstracts of Poster Presentations

16th Annual

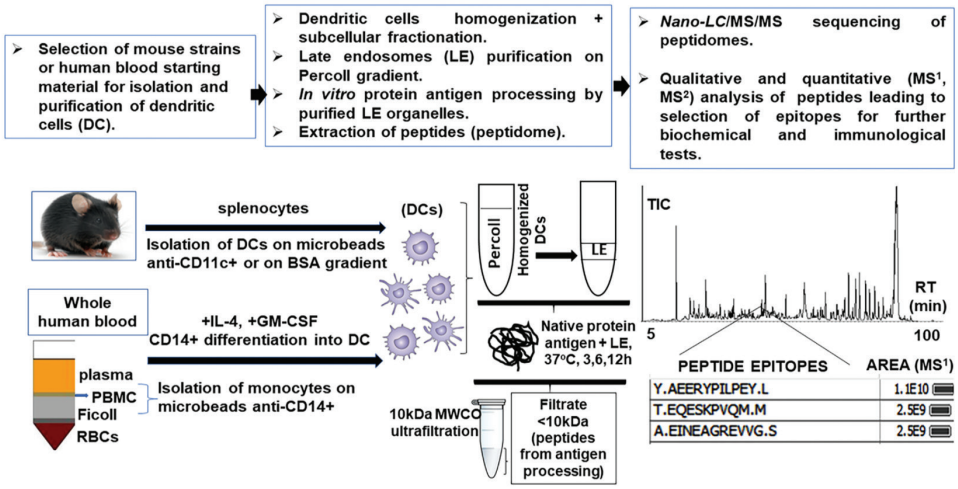
PeptideTherapeutics Symposium



Development of Qualitative and Quantitative Nano-LC/MS/MS Peptidomics Assays for Mapping the (Neo) Epitopes Derived from Endosomal Processing of Diverse Protein Antigens

Cristina C. Clement Ph.D.
[1]Radiation Oncology Department, Weill Cornell Medicine, New York, 10021, USA
*Corresponding author: ccc4002@med.cornell.edu

A comprehensive qualitative and quantitative analysis of a wide spectrum of antigen processing by endosomal compartments would provide important insights into the mechanisms of protein digestion by enzymes residing in these subcellular organelles, under different physiological states, including pathological conditions, such as the T2DM metabolic syndrome, mediated by redox stress under high concentration of oxidized sugars, and lipids. In addition, such analysis would enable the discovery of novel epitopes and neoepitopes of immunological importance, allowing downstream screening of a wide landscape of novel peptides, potential binders to MHC-II molecules. Herein, we describe a nano-LC/MS/MS based platform that combines gradient purified endosomes from mouse and human dendritic cells (DC)s, incubated with antigens, such as insulin, transthyretin, model protein antigens like ovalbumin (OVA), and hen egg lysozyme (HEL), followed by targeted, hot spot assessment of MS/MS-sequenced peptides. The analysis allowed the discovery of peptides epitopes potential binders to MHC-II that were further validated using immunological and biochemical assays. Moreover, further *in vivo* experiments proved that the endosomal compartment of (DC)s from obese Ob/Ob mice, displays dramatic changes in the overall pattern of antigen processing as compared with control C57Bl6 mice. The findings of this research emphasize that, at least in the case of specific antigens, the oxidative stress and metabolic syndrome (like T2DM) could compromise the proteostasis of endosomal compartments and the generation of MHC-class-II immunodominant epitopes.



Innovative Strategies for the Development of New Anti-leishmanial

N.C.S. Costa[1]; Batista, V. S.[1]; Nascimento Junior, N. M. J[1], E.M. Cilli[1], Marcia A.S. Graminha[2]
[1] Institute of Chemistry, UNESP - Univ. Estadual Paulista, Araraquara, São Paulo, Brazil.
[2] Department of Clinical Analysis, School of Pharmaceutical Sciences,[2] São Paulo State University (UNESP), Araraquara, São Paulo, Brazil

Leishmaniasis corresponds to a group of diseases caused by protozoa of the genus Leishmania. These, among other parasitic diseases, represent a major public health problem, especially considering the growing number of patients at risk, including immunocompromised and those living in poverty, however, there are few therapeutic options available, ineffective and toxic. Within this scenario, part of our objectives is aimed at the functionalization and characterization of the leishmanicidal activity of antimicrobial peptides from the temporal and histatin classes to the antileishmanial amphotericin B. Additionally, from the molecular modeling by homology of the Leishmania MCU (mitochondrial calcium unipoter) protein, studies of molecular docking will be used for planning and synthesis of novel peptides capable of interfering with the influx of $Ca2^{+}$ to the mitochondria of the parasite. So far, the peptides TSHa, Histatin 5 and TSHd and their equivalents functionalized to amphotericin B (Amph B-Histatin, Amph B *-TSHd, Amph B*-TSHa, Amph B+ (Collateral reaction)- TSHa have been synthesized with a high degree of purity. and also, the dimer of TSHa, called $(TSHa)_2K$. Among the main findings in biological assays, it was found that the derived peptides are more potent against intracellular amastigotes ($IC50 < 1$ µmol L^{-1}) and more selective for parasites when compared to host cells (SI>= 600). Additionally, the model of the structure of the MCU protein of Leishmania mexicana was developed, which allowed the realization of a molecular docking study against the inhibitors of this protein using the GOLD program and, from this, linear peptides and cyclic peptides were planned for future analysis of its potential to interfere with Ca^{2+} flux into the mitochondria of *L. mexicana*.

Establishing a Peptide Based Shuttling System with Chemerin 9 and the CMKLR1 Receptor

Anne Sophie Czerniak, Tobias Fischer and Annette G. Beck-Sickinger
Institute of Biochemistry, Faculty of Life Sciences, Leipzig University

Chemerin is a small chemotactic protein and a modulator of the innate immune system that mainly operates by activating the chemokine-like receptor 1 (CMKLR1), a receptor expressed by immune cells like natural killer cells, dendritic cells or macrophages. The formation of a chemerin gradient plays an important role in the recruitment of these mobile cells to inflammatory tissue. Recently, the overexpression of the CMKLR1 receptor on different tumors was described, offering a potential entry point for targeted delivery of chemotherapeutics. In this study, we evaluate small synthetic peptides derived from the nine C-terminal amino acids of chemerin (chemerin 9) that are biologically active and stable in blood plasma. First studies using bioluminescence resonance energy transfer (BRET) show efficient recruitment of arrestin to the CMKLR1 receptor after stimulation with chemerin 9 and its cyclic derivatives. In addition, we are able to confirm the peptide uptake into a model cell lines using fluorescencently labeled variants of the peptides. Furthermore, we were able to design a model shuttling system with the small molecule methotrexate, a chemotherapeutic agent, attached to cyclic chemerin 9. We demonstrate the efficiency and specificity of the peptide shuttling system in different cell types.

Altogether, we present a promising approach to specifically shuttle drugs into cancer cells using chemerin and the CMKLR1 receptor system, which can be easily translated to targeted drug delivery for other diseases.

Discovery of Gut-stable, Nanomolar Antagonists from Genetically-Encoded Bicyclic Peptides Displayed on Phage

John J. Dwyer,[1,2] Amanda Marra,[2] Jessica Cao,[1] Wenrui Huang,[1] Zoe O'Gara,[1] Ratmir Derda[1,2]
[1]48Hour Discovery, Inc. Edmonton, Canada; [2]48HD BioPharma, Inc. San Diego CA

Oral delivery of biologic and peptide/macrocycle therapeutics has traditionally been very challenging due to the lack of oral bioavailability and rapid degradation in the gut. Development of proteolytically stable peptides for gut-restricted targets presents an opportunity to identify new classes of drugs for chronic illnesses such as colitis and Crohn's disease, as well for emerging indications related to the microbiome. To this end, 48Hour Discovery (48HD) employs novel 2-fold symmetric linchpins (TSLs) to chemically modify peptide libraries fused to the pIII coat protein on M13 phage and produce value-added, genetically-encoded, billion-scale bicyclic library with exquisite gut stability. A proprietary 48HD selection platform coupled to next generation sequencing (NGS) and 48HD.cloud-based informatics screened over a billion genetically encoded bicycles and identified potent nanomolar antagonist against a clinically validated target. The NGS analysis not only identified the binders / antagonists but also provided positional SAR information for subsequent optimization. A series of TSL-bicyclic ligands synthesized from this analysis were shown to have IC50 values in the nanomolar range in a competition ELISA assay. Interestingly, these de novo discovered bicycles contains peptide motifs that share sequence similarity with the natural binding partner for this target. Importantly, the bicyclic hits have half-lives of 3-8 hours in an in vitro simulated intestinal fluid (SIF) assay. These results demonstrate that potent and proteolytically stable peptides can be identified for clinically validated targets in the gut.

The Cyclic Cysteine Knot Motif: A Structural 'Key' for Immunosuppressive Activity of Cyclotides

Roland Hellinger[#], Edin Muratspahic[#], Seema Devi[$], Johannes Koehbach,[§] Mina Vasileva[#], Peta J. Harvey[§], David J. Craik[§], Carsten Gründemann[&] and Christian W. Gruber[*,#]
[#]Center for Pharmacology and Physiology, Medical University of Vienna, Schwarzspanierstr. 17, 1090 Vienna, Austria; [§]Institute for Molecular Biosciences, Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, The University of Queensland, Brisbane, 4072 Queensland, Australia; [$]Institute for Infection Prevention and Hospital Epidemiology, Center for Complementary Medicine, Faculty of Medicine, University of Freiburg, Freiburg, Germany, Breisacher Str. 115B, 79111 Freiburg, Germany; [&]Translational Complementary Medicine, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstr. 80, 4056 Basel, Switzerland;*Corresponding author: christian.w.gruber@meduniwien.ac.at

'T20K' a synthetic analogue of the plant cyclotide kalata B1 is well known for its anti-proliferative activity towards immune cells. This peptide is currently under clinical investigation as drug candidate for multiple sclerosis[1,2]. However, its mode of action is not fully explored. Thus mechanistic studies are needed that shed light on the biological function of the drug. In this study, we explored the structure-activity relationship of T20K, with respect to the importance of the prototypic cyclic cysteine knot structural motif for their immunosuppressive activity[3]. Partial or full reduction of the cystine knot or incorrect folding of T20K resulted in a loss of function in proliferation experiments. Similarly, an acyclic analogue of T20K was inactive. The lack of immunosuppressive activity of all non-native T20K peptides appeared associated with the ability of cyclotides to interact with and penetrate cell membranes, since cellular uptake studies demonstrated fast fractional transfer into the cytosol of human immune cells only of the native peptide. The changes in cellular activity were compared to structural differences between cyclic and linear peptides by NMR. Although the cyclic cystine knot truncated analogue had native-like confirmation, the backbone of the acyclic T20K was less rigid and there were considerable structural changes in intercysteine loops 1 and 6 as compared to the native cyclic T20K. These findings support the notion that the cyclic cystine knot motif is a unique bioactive scaffold which governs interactions with, and transport across cellular membranes. Due to the conservation of the cystine knot motif across evolution, these observations could provide guidance for the design of novel cyclic cysteine-stabilized molecules.

References

[1] Thell, K., Hellinger, R., Sahin, E., Michenthaler, P., Gold-Binder, M., Haider, T., Kuttke, M., Liutkeviciute, Z., Goransson, U., Grundemann, C., Schabbauer, G. and Gruber, C.W. **2016**, *Proc Natl Acad Sci USA 113* (15), 3960-5.
[2] Thell, K., Hellinger, R., Schabbauer, G. and Gruber, C.W. **2014**, *Drug Discov Today 19* (5), 645-53.
[3] Hellinger, R., Muratspahic, E., Devi, S., Koehbach, J., Vasileva, M., Harvey, PJ., Craik, DJ., Gründemann, C., and Gruber, CW. *ACS Chem Biol*, *in press*.

Using Picoloyl Ester and 2-Chloro-Trityl Groups for Site-Selective Sulfation of Peptides

Rachel Heynen, Jake Thorsteinson, Graham Howerzyl, and Hailiang Joshua Zhu
Departments of Chemistry and Biology, Dordt University, Sioux Center, IA 51250

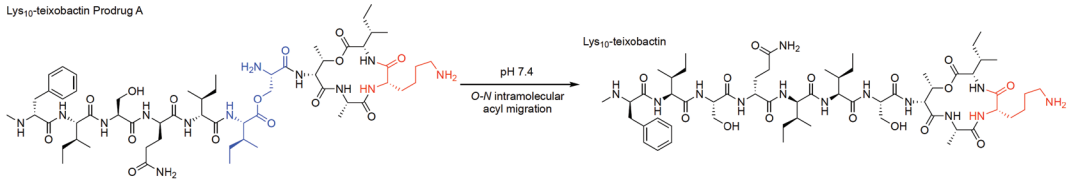
Protein sulfation on Tyrosine (Tyr) residues is a widely existing post-translational modification (PTS) on human proteins such as C-C chemokine receptors (CCRs) and P-Selectin glycoprotein ligand-1 (PSGL-1). Sulfate groups are essential for their ligand binding processes which trigger downstream signal transduction pathways. Multiple sulfation sites are common on CCRs and PSGL-1. For example, the N-terminal of CCR5 contains four potentially sulfated tyrosines, Tyr3, Tyr10, Tyr14 and Tyr15. Variations of Tyr sulfation, even on the same protein, are dependent on specific biological stages and processes. Therefore, development of efficient processes for the synthesis of libraries of sulfated peptides is needed in order to study the roles of sulfation in the binding processes. Site-selective solid-phase sulfation has been demonstrated as an efficient process for the synthesis of a sulfated peptide library. However, the options for Tyr side chain protecting groups are limited for site-selective solid-phase sulfation as compatibility issues arise when solid-phase deprotection and sulfation are applied multiple times. In our work, we developed two new protection strategies for Tyr side chain protection. These include the Picoloyl ester (Pico) group and the acid-sensitive group, 2-Cl-Trityl. Fmoc protected Tyrs with these two respective protecting groups have been successfully synthesized, and in test reactions, the Pico group on Fmoc-Tyr has been readily removed using Cu(OAc)2 as a catalyst and methanol-containing solvents. Application of the two Fmoc-Tyrs for site-selective solid-phase sulfation of CCR5 N-terminal peptide is in progress.

Teixobactin O-acyl Isopeptide Prodrugs Exhibit Enhanced Antibiotic Activity and Improved Pharmacological Properties

Chelsea R. Jones[1], James S. Nowick[1,2]
[1] Department of Chemistry, University of California, Irvine, Irvine, CA 92697, USA
[2] Department of Pharmaceutical Sciences, University of California, Irvine, Irvine, CA 92697, USA

In 2015, Lewis and coworkers discovered teixobactin, a non-ribosomal undecapeptide consisting of a linear tail and a macrolactone ring.[1,2] Teixobactin inhibits gram-positive bacteria—including MRSA, VRE, and MDR-TB—by binding the prenyl-pyrophosphate groups of lipid II, a precursor to peptidoglycan cell wall synthesis.[1] Teixobactin and active analogues of teixobactin form gels in aqueous conditions.[3,4] This tendency to gelate in aqueous conditions is a limitation to teixobactin and administration of the compound. Particularly, at high concentrations required for IND-enabling toxicity studies, teixobactin aggregates and forms gels.

In the present work, we have synthesized and investigated novel antibiotics comprising of O-acyl isopeptide prodrugs of teixobactin analogues. These teixobactin prodrugs vary in the position at which the Oacyl isopeptide linkage is present, either between Ile_6 and Ser_7, Ile_2 and Ser_3, or between both Ile_6 and Ser_7 and Ile_2 and Ser_3. The teixobactin O-acyl isopeptide derivatives undergo conversion to the corresponding teixobactin analogue when exposed to neutral or basic conditions. The Lys10-teixobactin O-acyl isopeptide derivatives have improved antibiotic activity, with minimum inhibitory concentrations of 0.5–1 μg/mL across a panel of Grampositive bacteria, as compared to 2–4 μg/mL for Lys_{10}-teixobactin. Gelation assays of the Lys_{10}-teixobactin Oacyl isopeptide prodrugs demonstrate that these derivatives do not gelate immediately upon exposure to buffer. The greater solubility of the prodrugs imparts better pharmacological properties than the parent antibiotics, which are difficult to administer intravenously, making them superior drug candidates.



References:
[1] Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schäberle, T. F.; Hughes, D. E.; Epstein, S.; *et al.* A New Antibiotic Kills Pathogens without Detectable Resistance. *Nature* **2015**, 517, 455–459.
[2] Yang, H.; Pishenko, A. V.; Li, X.; Nowick, J. S. Design, Synthesis, and Study of Lactam and Ring-Expanded Analogues of Teixobactin. *J. Org. Chem.* **2020**, 85, 1331–1339.
[3] Chen, K. H.; Le, S. P.; Han, X.; Frias, J. M.; Nowick, J. S. Alanine Scan Reveals Modifiable Residues in Teixobactin. *Chem. Commun.* **2017**, 53, 11357–11359.
[4] Yang, H.; Wierzbicki, M.; Du Bois, D. R.; Nowick, J. S. X-Ray Crystallographic Structure of a Teixobactin Derivative Reveals Amyloid-like Assembly. *J. Am. Chem. Soc.* **2018**, 140, 14028–14032.

Synthesis of T20K Immunosuppressive Cyclotide

Lukas Kogler,[1] Roland Hellinger,[2] Kirtikumar B Jadhava *
[1]Institute of Biological Chemistry, University of Vienna, Währinger str. 38, 1090 Vienna, Austria
2Center for Physiology and Pharmacology, Medical University of Vienna, Währinger str. 13a, 1090 Austria
lukas.kogler1@gmail.com

T20K is an immunosuppressive cyclotide derived from the naturally occurring plant peptide katala B1. It has been shown to suppress T-lymphocytes in an IL-2 dependent pathway.[1] T20K is currently in phase I clinical trials for the treatment of multiple sclerosis (MS), a neurodegenerative disease driven by autoreactive T-cells.[1] Besides interesting bioactivity, cyclotide T20K also features unique chemical features. It is a cyclic peptide composed of 29 amino acid residues, and 3 disulfide bonds, referred to as the cyclic cysteine knot motif (Figure 1) [2]. These unique structural features confer a high chemical, enzymatic and thermal stability. This makes them good potential candidates for drug development e.g., molecular grafting and receptor ligand design. Here we describe the comparison of two synthetic strategies to produce T20K in sufficient quantities.

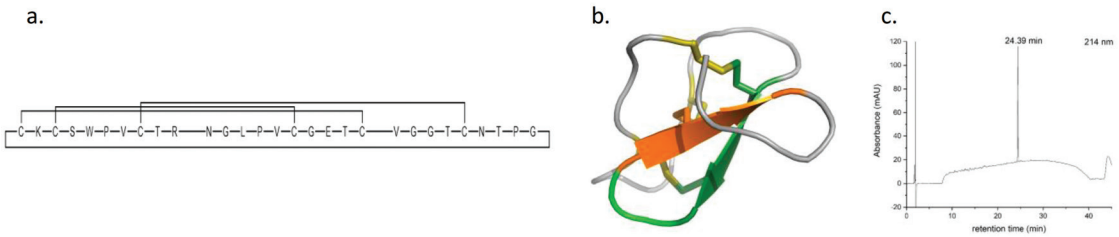


Figure 1: T20K a) sequence and b) crystal structure (PDB: 1NB1) and c) HPLC chromatogram of pure T20K
We chose to retro-synthetically disconnect cyclic T20K between Gly11-Gly12 for the first strategy, whereas second strategy involved Gly18-Cys19 retrosynthetic disconnection. For first strategy, side chain protected linear peptide was cyclized between Gly11¡-Gly12, whereas the second strategy took the advantage of native chemical ligation (NCL) to effect cyclization. Linear peptides were synthesized by Fmoc SPPS on an automated synthesizer. Upon cyclization, the peptides were folded under redox conditions to form thermodynamically stable T20K. HPLC analysis with the natural product confirmed the correct disulfide connectivity. This synthetic access to large quantities of T20K would help us elucidate its molecular mode of action in multiple sclerosis.

References

[1] Thell, K.; Hellinger, R.; Sahin, E.; Michenthaler, P.; Gold-Binder, M.; Haider, T.; Kuttke, M.; Liutkeviciuté, Z.; Göransson, U.; Gründemann, C.; Schabbauer, G.; Gruber, C. W. Oral Activity of a Nature-Derived Cyclic Peptide for the Treatment of Multiple Sclerosis. *Proc. Natl. Acad. Sci.* **2016**, 113, 3960–3965.
[2] Cheneval, O.; Schroeder, C. I.; Durek, T.; Walsh, P.; Huang, Y.-H.; Liras, S.; Price, D. A.; Craik, D. J. Fmoc-Based Synthesis of Disulfide-Rich Cyclic Peptides. *J. Org. Chem.* **2014**, 79, 5538–5544.

Peptides Targeting Metastatic Tumor Cells as Probes for Cancer Detection and Vehicles for Therapy Delivery

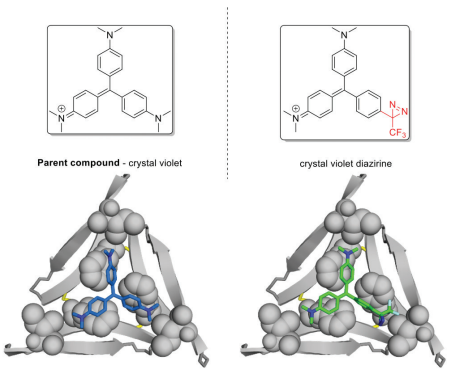
Shraddha Subramanian, Alexes Daquinag, Solmaz AghaAmiri,
Sukhen C. Ghosh, Ali Azhdarinia, and Mikhail G. Kolonin*
The Brown Foundation Institute of Molecular Medicine for the Prevention of Disease, The University of Texas Health Sciences Center at Houston, Houston, Texas 77030; *Presenting author
The Brown Foundation Institute of Molecular Medicine; University of Texas Health Science Center at Houston
1825 Pressler St. 430E, Houston, TX 77030
Office: 713-500-3146; FAX 713-500-2208

Metastatic cancers remain largely uncurable due to chemotherapy often overcome by chemoresistance and being toxic systemically. Biomarkers of metastatic cells are lacking and probes that could be used for their detection and therapy targeting would be highly valuable. Here, we hypothesized that metastatic cancer cells express cell surface receptors that can be harnessed for identification of molecules homing to metastases. We used phage display to isolate peptides binding to metastatic cells. By screening a combinatorial library in a mouse model of spontaneous breast tumor metastasis, we identified cyclic peptides with tropism for cancer cells disseminated to the lung and validated them as metastases-specific probes. We show that peptides CLRHSSKIC and CRAGVGRGC bind to murine and human cells derived from breast carcinoma and melanoma in culture and are selective for metastatic cells *in vivo*. Peptide CRAGVGRGC was radiolabeled with ^{67}Ga and validated as a selective probe enabling non-invasive detection of lung metastases in mice. Moreover, ^{67}Ga-labeled CRAGVGRGC, administered systemically, enabled non-invasive imaging of lung metastases in mice by positron emission tomography. We also used CRAGVGRGC to design a peptide inducing apoptosis upon cell internalization and demonstrated that the resulting hunter-killer peptide kills cancer cells and has a potential to suppress metastatic burden *in vivo*. Localization analysis *in vivo* and *ex vivo* indicate that CLRHSSKIC, as well as CRAGVGRGC, is selective for cancer cells undergoing epithelial-to-mesenchymal transition. We conclude that peptide CRAGVGRGC, selective for metastatic cells, may be useful as a lead for the development of imaging modalities and therapies targeting metastases.

Design, Synthesis, and Study of a Photoaffinity Label Tailored to Target Trimers Derived from Amyloid-β

Maj Krumberger[1], Yang Bi[1], Patrick J. Salveson[1], James S. Nowick[1,2]
[1]University of California Irvine, Department of Chemistry
[2]University of California Irvine, Department of Pharmaceutical Sciences

Amyloid-β (Aβ) soluble oligomers play a central role in the pathogenesis of Alzheimer's disease. While many small molecules that bind to these toxic oligomers exist, the molecular interactions that lead to the binding are not fully understood. Our laboratory has previously reported that C3-symmetric dyes, such as crystal violet (CrV), bind to a C3-symmetric trimer derived from $A\beta_{17-36}$. To investigate the molecular motifs that lead to this binding we designed a crystal violet isostere that contains a reactive photoaffinity labeling diazirine to capture the interactions. Docking studies revealed that both CrV and crystal violet diazirine (CrVD) bind similarly to the trimer derived from $A\beta 1_{7-36}$, with Phe^{20} and Ile^{31} being the two residues that lie most closely to the reactive diazirine motif. We established a synthesis of CrVD from α,α,α-trifluoroacetophenone by an oxime formation, tosylation of the oxime and displacement of the leaving group with ammonia to generate a diaziridine. The diaziridine was then oxidized to a diazirine via an iodine oxidation, formylated through a Rieche formylation, condensed with N,N-dimethyaniline, and oxidized to the final product. Absorbance studies have revealed that CrVD binds to the trimer, similarly to the parent compound CrV. Mass spectrometric analysis has confirmed that covalent binding between CrVD and the trimer occurs upon UV irradiation. Currently, mass spectrometric studies are ongoing to determine which residues in the peptide are being labeled by the photoaffinity label CrVD.



Selective Adressing of Adipocytes with Peptide-Drug Conjugates

Anna Kruscha, Annette G. Beck-Sickinger
Institute of Biochemistry, Faculty of Life Sciences, Leipzig University

Obesity is a global epidemic that even more strengthens due to the Covid-19 pandemic and results in increased incidence of associated long-term effects such as type 2 diabetes. A promising approach for an effective therapy is the application of peptide-drug conjugates, as the peptide enables the highly selective addressing of the adipose tissue. Agonistic binding peptides induce internalization by binding to a cell-specific surface receptor with high affinity. Cleavable linker systems allow the endosomal release of the peptide-bound drug. Intracellularly, the free drug can act on either metabolic or transcriptional level to modulate cell behavior and activity. Neuropeptide Y (NPY) is an appropriate peptide for selective adressing of adipocytes, since the associated neuropeptide Y1 receptor (NPY1R) is highly expressed on the cell surface of adipocytes. We already used the NPY/NPY1R system to shuttle the PPARγ agonist tesaglitazar in adipocytes successfully and demonstrated its intracellular release[1].

We aim to specifically transport selective estrogen receptor modulators (SERMs) into adipocytes. For this, we synthesized several NPY1R-selective SERM-[F^7,P^{34}]-NPY conjugates by solid-phase peptide synthesis. The purity and identity of these conjugates were confirmed by reversed-phase high-performance liquid chromatography and mass spectrometry. Receptor activation, selectivity and internalization was proved and the attachment of the cleavable linker and SERMs did not alter the behavior of the carrier peptide [F^7,P^{34}]-NPY. The newly synthesized conjugates display high affinity and selectivity for NPY1R and induce internalization. In conclusion, the new conjugates represent an attractive system for the selective transport of SERMs into adipocytes.

[1] Wittrisch, S.; Klöting, N.; Mörl, K.; Chakaroun, R.; Blüher, M.; Beck-Sickinger, A. G. (2020), NPY1R-targeted peptide-mediated delivery of a dual PPARα/γ agonist to adipocytes enhances adipogenesis and prevents diabetes progression, *Mol Metab* 31; 163–180.

Homochiral and Heterochiral Assembly of Peptides Derived from β-Sheet Regions of β-Amyloid

Xingyue Li, Nick Truex, James S. Nowick
Department of Chemistry, University of California, Irvine, California 92697-2025, United States

Abstract: There is growing interest to better understand the preference between a homochiral versus hetereochiral assembly from a racemic mixture of L- and D- peptides. Recent studies suggest that the β-amyloid (Aβ) peptide forms more stable, and kinetically favored rippled β-sheet fibrils from the mixing of the L- and D- peptides. Pauling and Corey first postulated the rippled β-sheet structure in 1953 to account for the hydrogen-bonding interactions that would occur between mixed enantiomers, as opposed to the pleated -sheet from pure L- or D- enantiomers. This preference for the rippled -sheet fibrils has been used to propose the idea of "Aβ chiral inactivation", in which the D-Aβ peptide can be used to suppress the formation of toxic Aβ oligomers, and instead accelerate the formation of fibrils. Using NMR spectroscopy, I aim to gain insights into the chemical interactions involved in assembly of racemic peptides containing a sequence from A. The Nowick laboratory has previously reported peptide 1a, a macrocyclic peptide containing the Aβ sequence LVFFAED, and assembles as a homotetramer by NMR under aqueous conditions. In this work, equimolar concentrations of peptide 1a and its enantiomer (ent-1a) were mixed to determine if the peptide would still prefer to assemble as a homotetramer. By NMR, I observe the formation of both the homochiral and a hetereochiral species, with the major species being the homochiral assembly in approximately 2:1 ratio. In addition, HSQC experiments with equimolar ^{15}N labeled peptide **1a** and unlabeled *ent*-**1a** reveal the formation of a new species in addition to the monomer and homotetramer that was previously reported. While this initial finding does not fully contradict with recent results in the field, it does pose an interesting question for what interactions drive one type of assembly over the other; future results from NMR experiments will attempt to shed light on such important contributing factors.

Understanding and Predicting Peptide Activity Using Artificial Intelligence Approaches

Ewa Lis Ph.D., Leonard Dieguez, Lily Lindmeier, Billyana Tsvetanova Ph.D.
Koliber Biosciences Inc.

Artificial Intelligence (AI) is becoming widely adopted for small molecule drug discovery, yet the methods for leveraging AI for peptide drug discovery are lagging far behind. These challenges are primarily driven by limited availability of large peptide datasets coupled with the difficulty of encoding peptides to a machine learning algorithm, especially when the datasets are small. In this poster presentation we will discuss the advances that were made in developing feature encodings for peptides to enable development of high performing models for immunology applications. We will demonstrate how AI can be utilized to prioritize peptide variants for testing through in silico prediction of performance. Moreover, methods to explain the models and visualize feature importance will be presented. Lastly, we will provide results from wet-lab validation for immunogenicity prediction, an area of immense importance for fast and reliable vaccine development.

Short Cationic Membrane Active Antimicrobial Peptides: A Detailed Structural Insight of the Membrane-Bound Peptides Using 2D-NMR and Molecular Dynamic Simulations

Sandeep Lohan, Dindyal Mandal, Rakesh Tiwari, Keykavous Parang
Center for Targeted Drug Delivery, Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, CA
AJK Biopharmaceutical, Irvine, CA

We designed and synthesized a series of small amphiphilic macrocyclic peptides. Antibacterial screening results revealed broad-spectrum activity of lead peptides **8C** and **9C** with predominant activity against most Gram-positive bacteria, including the drug-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE) with MIC values in the range of 1.5 to 3.1 μg/mL. Moderate activity was observed against Gram-negative bacteria with MIC values of 12.5 to 25 μg/mL. Toxicity study results (against hRBCs and eukaryotic cells) revealed the predominant lethal action of lead peptides against bacterial cells versus mammalian cells. Kill kinetic study revealed the rapid killing action of **8C** and **9C** against resistant strains of *S. aureus* and *E. coli*. A calcein dye leakage experiment showed the membranolytic effect of **8C** and **9C**, which was further confirmed by flow cytometry analysis, fluorescent microscopy, and scanning electron microscopy. The membrane interaction behavior was studied by assessing three-dimensional structures in aqueous solution and in phospholipid bilayers using nuclear magnetic resonance spectroscopy (NMR) and molecular dynamics (MD) simulations. Moreover, both lead cyclic peptides displayed high stability in human plasma. These results highlight the potential of newly designed cyclic amphiphilic peptides as the next generation of peptide-based antibiotics.

Peptide-Based Strategy for Nucleic Acid Delivery

Dindyal Mandal, Sandeep Lohan, Eman H. M. Mohammed, Rakesh Tiwari, Hamidreza Montazeri Aliabadi, Keykavous Parang
Center for Targeted Drug Delivery, Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, CA; AJK Biopharmaceutical, Irvine, CA

siRNA technique has gained tremendous attention for therapeutic applications due to its target-specific gene silencing through RNA interference (RNAi). However, the delivery of siRNA remains challenging because of several limitations. To address this issue, a number of disulfide-constrained cyclic amphipathic peptides and fatty acid conjugated linear amphiphilic peptides were designed and synthesized. Among the studied peptides, PAB001, PAB005, and PAB006 demonstrated significant transfection efficiency (~70%) of Alexa488-labelled siRNA in MDA-MB-231 (Triple negative breast cancer cells) as evidenced from FACS analysis. The above peptides effectively formed complexes with siRNA, exhibiting favorable hydrodynamic size (100-300 nm) and zeta potential (20-40 mV). Moreover, confocal microscopy indicated significant internalization of fluorescence-labeled siRNA/peptide complex when compared with control cells labeled with siRNA only. Additionally, the protein silencing efficiency of the newly developed peptide/siRNA complex was evaluated in the same cell line targeting signal transducer and activator of transcription 3 (STAT3) as a model protein. Western blot results suggested substantial silencing (80-90%) of STAT3 protein with minimal cytotoxicity, which is comparable to commercial transfection agent Lipofectamine 2000. In summary, this study indicates that newly developed peptides are promising and efficient nonviral vectors for siRNA delivery

Targeting PP1/CAV1 Interaction Using a Bioportide as an Anticancer Strategy

Bárbara Matos[1], John Howl[2], Carmen Jerónimo[3,4], Margarida Fardilha[1]
[1]Laboratory of Signal Transduction, Department of Medical Sciences, Institute of Biomedicine – iBiMED, University of Aveiro, 3810-193 Aveiro, Portugal; [2]Molecular Pharmacology Group, Research Institute in Healthcare Science, University of Wolverhampton, Wolverhampton WV1 1LY, UK; [3]Cancer Biology and Epigenetics Group, IPO Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO Porto), Research Center-LAB 3, F Bdg., 1st floor, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal; [4]Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar, University of Porto (ICBAS-UP), Rua Jorge Viterbo Ferreira 228, 4050-513, Porto, Portugal

The development of effective therapeutic approaches for prostate cancer is currently one of the major challenges of the scientific community in oncology. Protein phosphatase 1 (PP1) emerged as a promising drug target in this context. Thus, the main goal of this work is to establish an efficient strategy to disrupt a key PP1 complex with important roles in prostate cancer progression. To accomplish this goal, a peptide sequence derived from the region that include the PP1-binding motif of caveolin-1 (CAV1) was synthetised and coupled to a cell penetrating peptide (CPP). The ability of this combined peptide (named bioportide) to affect prostate cancer cells progression was evaluated *in vitro*. In this context, prostate cancer cells (PC-3 cell line) were incubated with different concentrations of the bioportide and a mutated homologue (control) and cells viability (PrestoBlue cell viability assay) and the expression of various protein biomarkers (Western blotting) were measured.

We found that, despite the incubation with the bioportide for 24h did not significantly affect the prostate cancer cells viability, after 48h incubation, the bioportide significantly reduced the prostate cancer cells viability. At a concentration of 10 μM, the bioportide decreased the phosphorylation of AKT, (Ser473), suggesting an increased activity of PP1 and consequent disruption of PP1/CAV1 interaction. These results highlight the potential of the synthesized bioportide to affect the target interaction and negatively impact the prostate cancer cells proliferation. Further analyses are now required to confirm the disruption of the target interaction and to better elucidate the mechanisms of cell death.

Investigating the Neutralizing Properties of Antibodies Generated Against an Aβ-derived Oligomer: Efforts Toward a Novel Alzheimer's Disease Immunotherapy

Chelsea Marie T. Parrocha[1*], Adam G. Kretuzter[2*], Prof. James S. Nowick[1,2]
Department of Pharmaceutical Sciences[1], University of California Irvine; Department of Chemistry[2], University of California, Irvine

β-amyloid (Aβ) oligomers are responsible for the progression and pathogenesis of Alzheimer's disease (AD); they are neurotoxic, induce cognitive impairment, and inhibit long-term potentiation which makes them an attractive target to develop therapies against AD. Aβ oligomers are elusive with no structural definition due to their diversity in size, heterogeneity, and propensity to self-assemble into fibrils. To study the properties of Aβ oligomers, we synthesize peptides derived from fragments of full-length Aβ that share properties similar to Aβ oligomers in the literature. The Aβ-derived oligomer (**Trimer 1**) is well characterized by X-ray crystallography, SDS-PAGE, SEC, cytotoxicity assays, and incorporates native residues that stabilizes its unique confirmation.

Immunotherapies are a promising therapy against AD. Thus far, no AD immunotherapies slow the progression of AD and rely on non-toxic monomeric fragments of full-length AV. We hypothesize that by utilizing **Trimer 1**, it will stimulate the production of antibodies that target endogenous Aβ and may ameliorate cognitive impairment and pathology in transgenic AD mice. This poster summarizes efforts towards developing a potential immunotherapy against AD in C57/BL6J mice. Rabbits immunized with **Trimer 1** to generate polyclonal antibodies sustained a strong immune response (reported antibody titer >1:5000). Through immunofluorescent staining, the rabbit polyclonal antibodies recognize Aβ pathology in AD transgenic mice brain and AD human tissue. Thus far, C57/BL6J immunized with **Trimer 1** demonstrates a sustained and increased immune response. Future directions will include to determine if this potential immunotherapy can ameliorate cognitive impairment and pathology in transgenic AD mice.

Uncovering the Secrets of α-Synuclein Oligomers

Sarah Ruttenberg
Ph.D. Candidate, Department of Chemistry, University of California-Irvine; Advisor: Professor James S. Nowick

Parkinson's Disease (PD) is a neurodegenerative disorder which has been linked to formation of soluble β-sheet rich oligomers of the amyloid protein α-synuclein. The smallest of these oligomers are dimers, which have been shown to seed the aggregation of α-synuclein into cytotoxic oligomers and fibrils, and trimers, which have been observed and correlated with loss of dopaminergic neurons in vivo. Unfortunately, elucidating the structures of these oligomers as well as their mechanism of toxicity has been difficult due to their heterogeneity and rapid interconversion between species. To combat these limitations, we designed and covalently cross-linked macrocyclic β-hairpin peptide mimics of α-synuclein36-55 to create stable oligomers. This region of alpha-synuclein plays a significant role in the formation and toxicity of the cytotoxic oligomers implicated in PD, and the stabilization of the oligomeric assemblies allows for easier characterization while still demonstrating many of the same behaviors as the native species. In solution, the synthetic oligomers assemble into higher-order species like oligomers of full-length α-synuclein do. *In vitro* (using SH-SY5Y neuroblastoma cells), the presence of these oligomers leads to increased membrane permeability and decreased production of ATP, indicating cell death. Currently, high-resolution structures of several of these oligomers (some of the first high-resolution structure of any oligomers of α-synuclein) are being determined using X-ray crystallography. Continuing to elucidate and study the structures of the covalently cross-linked oligomers described here should provide significant insight to the structures of oligomers formed by full-length α-synuclein.

Cell Penetrating Peptides and Proteins: Mechanism of Action

Ashweta Sahni, Dr. Ziqing Qian, Prof. Dehua Pei
Ohio State University

The selective permeability of the cell membrane allows small uncharged molecules (such as oxygen) to easily permeate through by passive diffusion, while preventing large entities from entering the cell. Despite this, there are a handful of natural and unnatural entities that can topologically cross the lipid bilayer, such as cell penetrating peptides (CPPs), certain proteins (e.g., bacterial toxins) and pathogens (e.g., viruses). Since these entities are too large to passively diffuse across the cell membrane, they employ alternative mechanisms to access the cytosol of the cell. Most CPPs and some bacterial toxins enter cells by endocytosis, however, the mechanism of their subsequent endosomal escape is poorly understood. Our work addresses this knowledge gap, by providing direct evidence for this process in live cells. Previously, we designed a series of confocal microscopic experiments to visualize the endosomal escape of CPPs using a pH-sensitive fluorescent dye. We established that CPPs escape the endosome by inducing vesicle budding and collapse (VBC). Experiments with enlarged endosomes allowed us to identify the intermediates along the VBC pathway. Our findings resolved a long-lasting mystery in the field of CPPs and provide a toolbox of experimental techniques for probing endosomal escape. Our recent results show that bacterial toxins (such as diphtheria toxin) also undergo VBC, indicating that this may be a universal mechanism for endosomal escape. Ultimately, my research will provide a deeper understanding of the mechanisms that govern cellular uptake of biomolecules. These findings can be applied to design efficient cell penetrating molecules for the purpose of drug design and delivery.

A Physical Mixture of Antibiotic and Synthetic Antimicrobial Cyclic Peptide Proves to be More Effective than Respective Chemical Conjugate Against Multidrug-resistant Bacteria

Muhammad Imran Sajid[1, 2], Sandeep Lohan[2], Shun Kato, Keykavous Parang[1], Rakesh Kumar Tiwari[1]*
[1]Center for Targeted Drug Delivery, Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Harry and Diane Rinker Health Science Campus, Irvine, CA 92618; [2]Faculty of Pharmacy, University of Central Punjab, Lahore 54000, Pakistan; *Tel: +1-714-516-5483; Fax: +1-714-516-5481; Email: tiwari@chapman.edu

Bacterial resistance is a growing global concern necessitating the discovery and development of antibiotics effective against the drug-resistant bacterial strain. Synthetic antimicrobial peptides gained the attention of scientists due to their low cytotoxicity, cost-effectiveness, and increased potency against drug-resistant bacterial strains. Previously, we reported a potent cyclic synthetic antimicrobial peptide with a MIC of 2.97 µg/mL against MRSA. Herein, we investigated its conjugate with another potent antibiotic, Levofloxacin, with the intent to make the drug-resistant strains sensitive. Surprisingly, levofloxacin activity was remarkably reduced against *S. aureus* (ATCC 29213), *S. aureus* (ATCC BAA-1556), *E. coli* (ATCC 25922), *E. coli* (ATCC BAA-2452), *P. aeruginosa* (ATCC 27883) *P. aeruginosa* (ATCC BAA-1744), *K. pneumoniae* (ATCC 13883) and *K. pneumoniae* (ATCC BAA-1705). Besides, the physical mixture of the Levofloxacin with the cyclic [R_4W_4] proved to be significantly effective against all strains. Furthermore, the checkerboard assay revealed the significant synergistic effect of the Peptide against all studied strains except for the wild type *S. aureus*, in which the partial synergy was observed. Hemolysis assay points to the superiority of the physical mixture of the Levofloxacin with cyclic [R_4W_4]. We can confidently speculate that exploring the physical combinations of several potent antibiotics with synthetic antimicrobial peptides may solve this intriguing puzzle of bacterial resistance.

Improving Docking Power for Short Peptides Using Random Forest

Michel F. Sanner[1]*, Leonard Dieguez[2], Stefano Forli[1], Ewa Lis[2]
[1]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 93037; [2]Koliber Biosciences Inc., 12265 World Trade Drive, Suite G,
San Diego, CA 92128

Corresponding author: Michel F. Sanner, Department of Integrative Structural and Computational Biology, MB5, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. Tel.: +1 858 829 8587; Fax: +1 858 784 2341; E-mail: sanner@scripps.edu

In recent years, therapeutic peptides (TPs) have gained a lot interest as demonstrated by the 60 peptides approved as drugs in major markets and 150+ peptides currently in clinical trials. However, while small molecule docking is routinely used in rational drug design efforts, docking peptides has proven challenging partly because docking scoring functions, developed and calibrated for small molecules, perform poorly for these molecules. Here, we present Random Forest classifiers trained to discriminate correctly docked peptides. We show that, for a testing set of 47 protein-peptide complexes, structurally dissimilar from the training set and previously used to benchmark AutoDock Vina's ability to dock short peptides, these Random Forest classifiers improve docking power from ~25% for AutoDock scoring functions to an average of ~70%. These results pave the way for peptide-docking success rates comparable to those of small molecule docking. To develop these classifiers, we compiled the ProptPep37_2021 dataset, a curated, high-quality set of 322 crystallographic protein-peptides complexes annotated with structural similarity information. The dataset also provides a collection of high-quality putative poses with a range of deviations from the crystallographic pose, providing correct and incorrect poses (i.e., decoys) of the peptide for each entry. The ProptPep37_2021 dataset as well as the classifiers presented here are freely available.

16th Annual Peptide Therapeutics Symposium

A Novel Lactam-stapled, Fibrillation-resistant Glucagon Analogue

Nicolas Varas[1], Rachel Grabowski[2], Shayan Poordian[2], Nelson Phillips[2], Mark Jarosinski[2], Faramarz Ismail-Beigi[2], Michael Weiss[1]
[1]Indiana University;[2]Case Western Reserve University

Insulin treatment is lifesaving for type 1 diabetic patients, but unfortunately, it comes with an ever-present threat of hypoglycemic episodes caused by over-insulinization. Most of these episodes are manageable by sugar ingestion, but their continuous occurrence increases the probability of ischemic events, neurological damage, coma, and even death[1, 2]. Consequently, the risk of hypoglycemia remains the most significant obstacle that prevents insulin-dependent patients from attaining adequate metabolic control. Glucagon is insulin's natural counter-regulatory hormone and therefore is used in emergency settings to treat severe hypoglycemia. Previous clinical studies have shown a significant decrease in hypoglycemic events when using a dual-hormone, insulin/glucagon, artificial pancreas[3-5], and recent seminal studies have shown prevention of hypoglycemia when glucagon is co-administrated with insulin[6,7]. Despite its potential as a drug, native glucagon is chemically and physically unstable with an intrinsic propensity to rapidly form β-amyloid fibrils, nullifying its activity and making it cytotoxic[8-11]. To address this problem, our group is developing fibrillation-resistant glucagon analogues through lactam side-chain cyclization (at positions i,i+4) with the purpose of lock-in an α-helix turn and prevent β-sheet formation. This strategy gave us a fully active and fibrillation-resistant analog that retained its in vivo activity for up to 2-weeks when continuously agitated at 45°C. Ongoing studies aim to test its ability to protect from hypoglycemia when co-administered with a single-chain insulin analogue. These results validate side-chain cyclization as an approach to develop fibrillation-resistant glucagon analogues and give hope in the search for treatments that allow better glycemic control in Diabetes Mellitus.

Disclosure: Patent Pending

[1] Freeland, B. Hypoglycemia in Diabetes Mellitus. *Home Healthc Now 35*, 414-419 (**2017**).
[2] Cryer, P.E. Hypoglycemia in type 1 diabetes mellitus. *Endocrinol Metab Clin North Am 39*, 641-654 (**2010**).
[3] Peters, T.M. & Haidar, A. Dual-hormone artificial pancreas: benefits and limitations compared with single-hormone systems. *Diabet Med 35*, 450-459 (**2018**).
[4] Taleb, N. *et al*. Glucagon in artificial pancreas systems: Potential benefits and safety profile of future chronic use. *Diabetes Obes Metab 19*, 13-23 (**2017**).
[5] Wilson, L.M. & Castle, J.R. Stable Liquid Glucagon: Beyond Emergency Hypoglycemia Rescue. *J Diabetes Sci Technol 12*, 847-853 (**2018**).
[6] Bruce W. Bode, J.B., Anne Shah, David Parkes, Soumitra Ghosh and Alan D. Cherrington 7-LB: Insulin and Glucagon Coadministration in Type 1 Diabetes Prevents Hypoglycemia without Worsening Hyperglycemia. *Diabetes 69* (**2020**).
[7] Pedersen, C., Bouman, S.D., Porsgaard, T., Rosenkilde, M.M. & Roed, N.K. Dual treatment with a fixed ratio of glucagon and insulin increases the therapeutic window of insulin in diabetic rats. *Physiol Rep 6*, e13657 (**2018**).
[8] Beaven, G.H., Gratzer, W.B. & Davies, H.G. Formation and structure of gels and fibrils from glucagon. *Eur J Biochem 11*, 37-42 (**1969**).
[9] Onoue, S. *et al*. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. *Pharm Res 21*, 1274-1283 (**2004**).
[10] Gelenter, M.D. *et al*. The peptide hormone glucagon forms amyloid fibrils with two coexisting beta-strand conformations. *Nat Struct Mol Biol 26*, 592-598 (**2019**).
[11] Taleb, N. *et al*. Stability of Commercially Available Glucagon Formulation for Dual-Hormone Artificial Pancreas Clinical Use. *Diabetes Technol Ther 19*, 589-594 (**2017)**.

Myristoylated Protein Kinase C Beta II Peptide Inhibitor Reduces Bilateral Renal Ischemia-Reperfusion Injury in Mice

Alexis Verwoert[1], Tameka Dean[1], Sunit Singh[1], Qian Chen[1], Robert Barsotti[1], Yanlin Jiang[2], James George[2], Anupam Agarwal[2], and Lindon Young[1]
[1]Philadelphia College of Osteopathic Medicine, Philadelphia, PA; [2]University of Alabama at Birmingham School of Medicine, Birmingham, AL

Acute kidney injury (AKI) due to ischemia-reperfusion (I/R) insult involves oxidative stress and inflammation leading to rapid renal decline. Without effective therapies available, I/R-induced AKI remains a significant cause of post-operative mortality. Cell-permeable myristoylated protein kinase C beta II peptide inhibitor (N-myr-SLNPEWNET; myr-PKCβII-) is known to attenuate myocardial I/R injury in ex vivo rat hearts. We hypothesized that myr-PKCβII- would attenuate severe renal I/R injury characterized by a reduction in serum creatinine (Cr) compared to scrambled control peptide (N-myr-WNPESLNTE; myr-PKCβII-scram). Renal pedicles of anesthetized male C57BL/6J mice (25–30g) were clamped bilaterally for 20min. Five minutes before unclamping, 2.0 mg/kg myr-PKCβII- (n=9) or myr-PKCβII-scram (n=8) was given i.v. Cr (mg/dL) was measured at baseline (0.13±0.013 [myr-PKCβII-] vs. 0.11±0.005 [myr-PKCβII-scram]), 24h, 72h, and 96h post-injury. Data were evaluated by a Student's t-test. Myr-PKCβII- significantly reduced Cr vs. myr-PKCβII-scram at 24h (1.36±0.11 vs. 1.59±0.06; *p=.040) and 72h (0.73±0.15 vs. 1.28±0.25;*p=.042), but not at 96h (0.51±0.09; n=8 vs. 0.72±0.15; n=6, p=.12). Three animals died prior to 96h. Therefore, we tested 19min bilateral renal ischemia in non-treated control mice (n=8) and observed no fatalities at 96h. A significant increase in Cr from baseline (0.076±0.003) was observed at 24h (0.28±0.07; *p=.017), 72h (0.163±0.03; *p=.016) and 96h (0.146±0.02; *p=.039), which suggests AKI is prevalent up to 96h. Collectively, the data suggest that myr-PKCβII- attenuated renal I/R injury. Future experiments will determine whether myr-PKCβII- will mitigate AKI in a 19min bilateral renal ischemia model based on kidney function tests and AKI biomarkers.

Direct Novel Peptide Ligand Identification with HTS Library in Cell-based Functional Assays

Dr. John Wang
CSO, PepLib Zonsen Biotech

Presented by Sally Wang, Ph.D.
VP of Business Development, PepLib Zosen Biotech

A novel high diversity cyclic peptide library has been constructed based on the Peptide Information Compression Technology (PICT) and designed to have high accuracy and functional assay capabilities. The library consists of ~75,000 80-mer cyclic peptide constructs that contain all possible 3,200,000 (205) pentapeptide sequences, and in total half a billion unique peptide (up to 80 amino acids) sequences. Innovatively, the library can be used with functional whole cell assays to enable direct identification of novel functionally active peptide ligands.

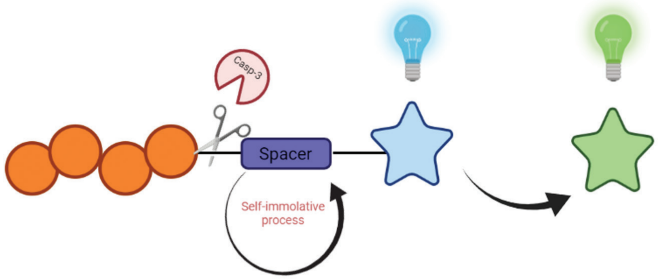
The PepLib ZSenithFive™ library was screened with a rat osteoblast culture model to identify novel 80-mer peptide agonists. We observed more than two folds activity over control Bone Morphogenetic Protein-2 (BMP-2) in initial hits. The two cycles of decompression to reduce the size of the 80-mer peptide hits resulted into octapeptide leads with 9-fold activity. The osteoblast stimulation by peptide leads from PICT may offer new approaches to bone disorders such as osteoporosis and nonunion.

Utilising a 1,8-Naphthalimide Probe for the Ratiometric Fluorescent Visualisation of Apoptosis

Conner Wynne
Maynooth University
Synthesis & Solid-State Pharmaceutical Centre (SSPC)

The ability to visualize specific intracellular biological activities is essential to our understanding of cell behaviour(s). One such behaviour that has attained great interest in recent times, is the programmed cell death in multicellular organisms, apoptosis. Caspase-3 is a key regulator of apoptosis; thus, its activity is an effective way for monitoring apoptosis.[1] Many fluorescent probes have synthesized for the detection of Caspase-3. However, the real-time quantification of Caspase-3 *in cellulo* remains a challenge. Ratiometric probes may provide an elegant solution to this problem, owing to their advantages in quantitative detection. In this work, we report a peptide probe (Ac-DEVD-PABA-Naph) with a fluorescence response specifically for Caspase-3, highlighting the ability of peptides to act as bioconjugates for probes & biomarkers.

We have developed a versatile methodology for the synthesis of novel fluorescently-labelled peptides. The essence of this strategy involves a cell-permeable 1,8-naphthalimide (Naph)[2] fluorescent probe that is covalently bound to the peptide (Ac-DEVD) backbone via a p-aminobenzyl alcohol (PABA) spacer. The spectroscopic response toward Caspase-3 was investigated, with the probe displaying a time-dependent ratiometric change in fluorescence. Response kinetics toward different concentrations of Caspase-3, probe-activity in the presence of Caspase-3 inhibitors, and the selectivity of the probe for Caspase-3 over other endogenous species were examined. Together, these experiments suggest that Ac-DEVD-PABA-Naph may be able to estimate the apoptotic stages and evaluate apoptosis-related disease progression.



Cyclic Peptides Containing Tryptophan and Arginine Residues as Protein Kinase Inhibitors

Khalid Zoghebi, Michel F. Sanner, Samara Hanna, Saghar Mozaffari, Simin Rahighi, Rakesh K. Tiwari, and Keykavous Parang
Center for Targeted Drug Delivery, Department of Biomedical and Pharmaceutical Sciences; Chapman University School of Pharmacy, Irvine, California 92618; Department of Pharmaceutical Chemistry, College of Pharmacy, Jazan University, Jazan 45142, Saudi Arabia.

Abnormal protein kinases expression has been shown to be associated with unregulated cell transduction signals in cancer cells. We have previously reported cyclic peptides containing alternative arginine (R) and tryptophan (W) residues [WR]5 as a noncompetitive c-Src tyrosine kinase inhibitor. Herein, larger cyclic peptides $[WR]_x$ (x= 6–9) and hybrid cyclic-linear peptides, $[R6K]W_6$ and $[R5K]W_7$, containing R and W residues were synthesized and evaluated for their protein kinase inhibitory activity. $[WR]_9$ was found to be the most potent tyrosine kinases inhibitor among all the peptides. $[WR]_9$ showed high inhibitory activity (IC50 = 0.21 μM) against c-Src kinase. Furthermore, $[WR]_9$ inhibited other protein kinases such as Abl kinase and PKCa kinase activity IC50 values of 0.35 μM and 2.86 μM, respectively. $[WR]_9$ exhibited IC50 values of <0.25 μM against Akt1, Alk, and Btk. It was found that $[R6K]W_6$ and $[R5K]W_7$ were generally less potent than $[WR]_9$, $[WR]_8$, $[WR]_7$, and $[WR]_6$ against Abl1, Alk, Braf, Cdk1/cyclin A1, and PKCa. Based on these results, the presence of R and W residues in the ring, ring size, and the number of amino acids in the structure of the cyclic peptide were found to be critical in protein kinase inhibitory potency. Molecular modeling studies revealed 3 putative binding pockets for $[WR]_{(5–9)}$ through automated blind docking. The most populated pocket is located between the SH2, SH3, and N-lobe domains on the opposite side of the ATP binding site of c-Src. The second pocket is formed by the same domains and located on the ATP binding site side of the protein. Finally, a third pocket was identified between the SH2 and SH3 domains. Molecular dynamics simulations of the protein–peptide complexes indicate that in the presence of $[WR]_9$, the plasticity of the protein is affected. Based on the modeling data, the second pocket is most likely the site where these peptides bind and offer a plausible rationale for the increased affinity of $[WR]_9$.

Notes

Notes

AstraZeneca

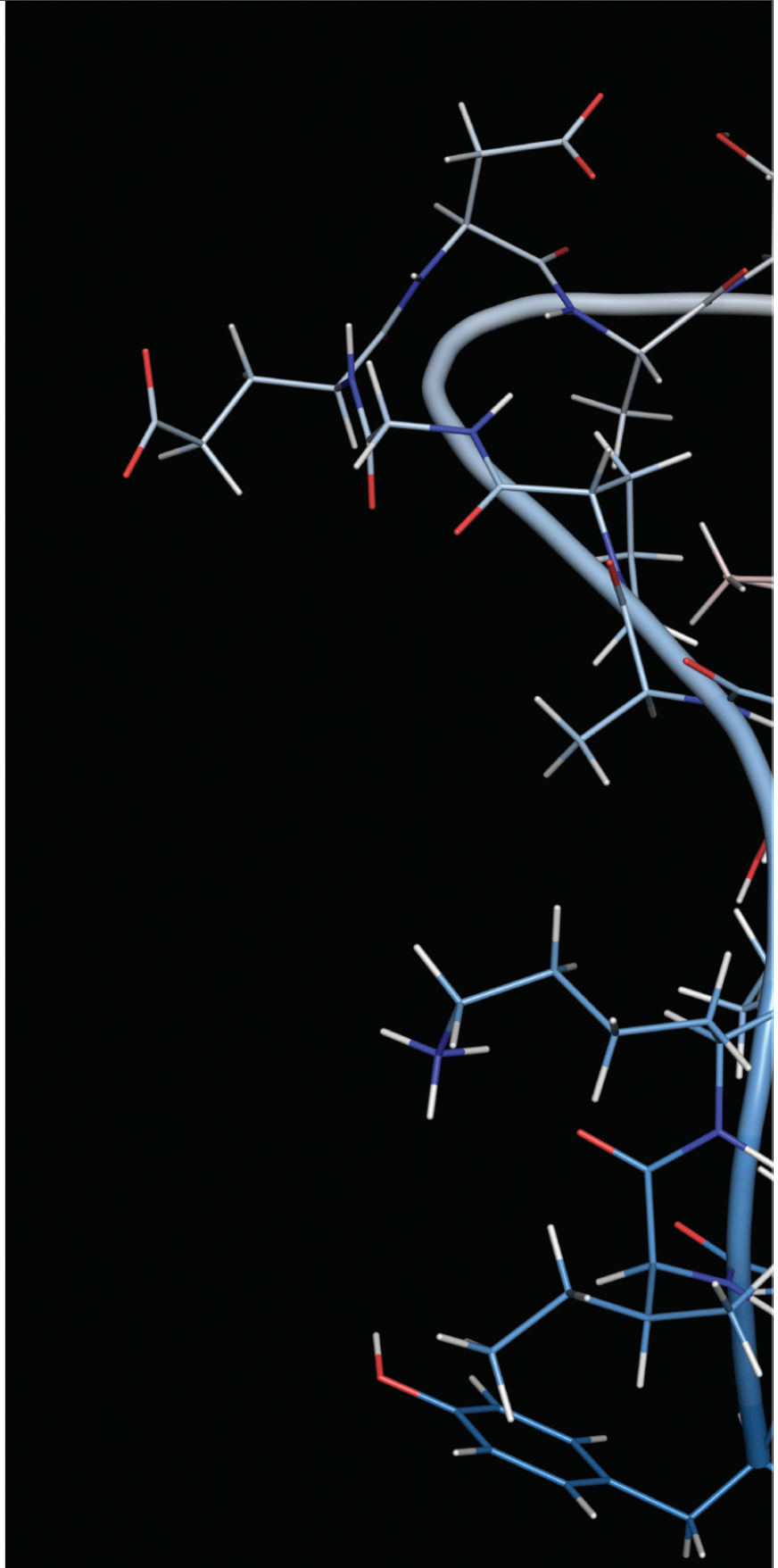
FERRING
PHARMACEUTICALS

PolyPeptide
GROUP

novo nordisk®

ZEAL&
REVOLUTIONARY HEALTH SOLUTIONS

PEPTIDE
THERAPEUTICS
FOUNDATION



Cover image: Peptide YY.
Waleed Danho responsible for synthesis of fully active truncated analogs.