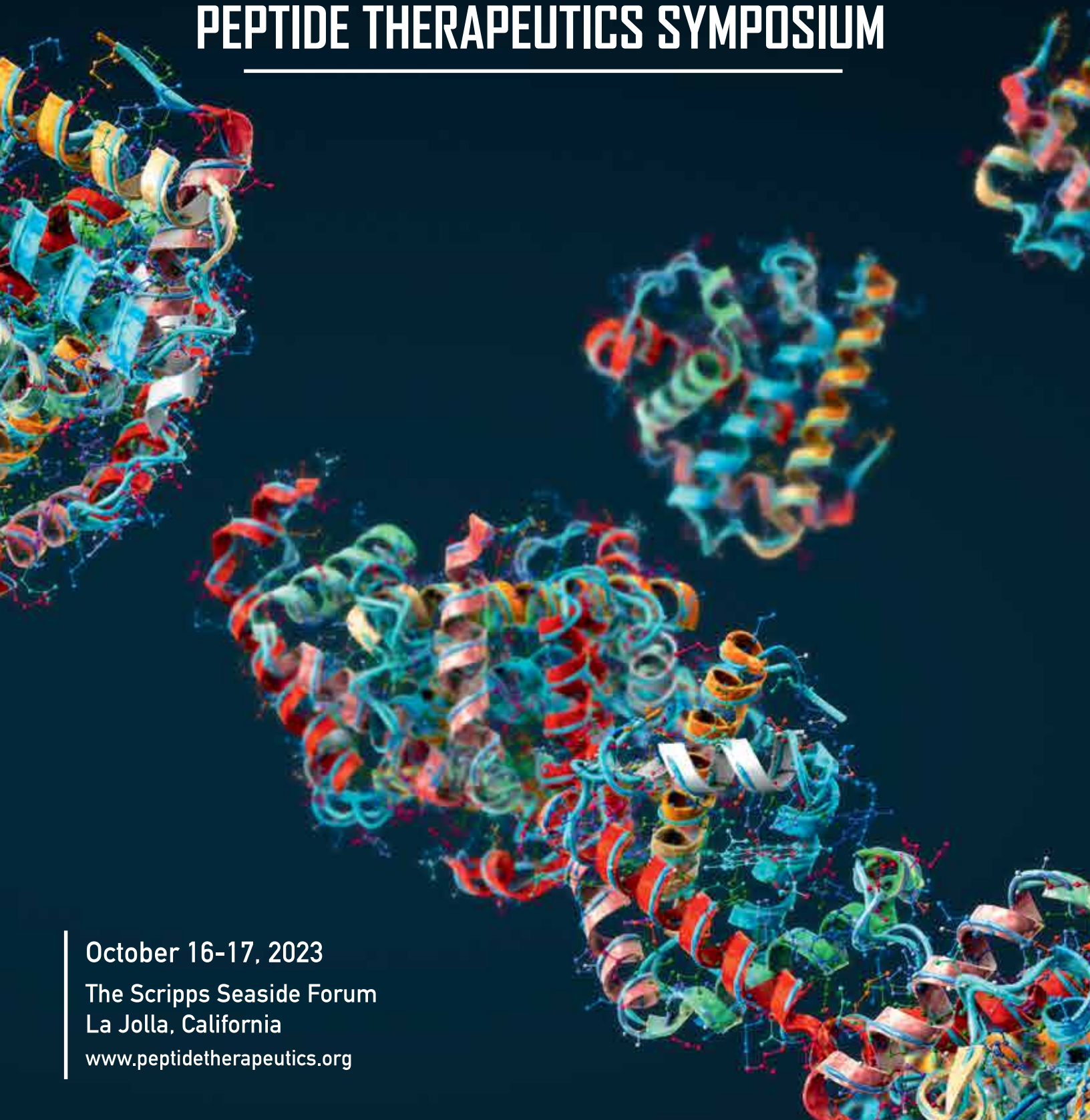
PEPTIDE
THERAPEUTICS
SYMPOSIUM

Program and Proceedings for the 18th Annual

PEPTIDE THERAPEUTICS SYMPOSIUM



October 16-17, 2023

The Scripps Seaside Forum
La Jolla, California

www.peptidetherapeutics.org

18th Annual Peptide Therapeutics Symposium

October 16-17, 2023

The Scripps Seaside Forum, La Jolla, CA

Virtual and In-Person Meeting

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2023 Travel Grant Awardees

Shambhu Chandra, University of British Columbia, Vancouver
Devani Johnson, Philadelphia College of Osteopathic Medicine
Chelsea Jones, University of California, Irvine
Jonathan Moreno, Chapman University
James Ramsarran, Philadelphia College of Osteopathic Medicine
Sagarika Taneja, The Ohio State University
Zachary Urbach, University of California, Irvine

Symposium Sponsors

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PEPTIDE THERAPEUTICS FOUNDATION

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18th Annual Peptide Therapeutics Symposium

Dear Colleagues,

The Peptide Therapeutics Foundation and its sponsors welcome you to the 18th Annual Peptide Therapeutics Symposium taking place for the first time at the Scripps Seaside Forum. We are excited to have all of our speakers presenting in person this year but will continue to support a hybrid format to engage our colleagues from around the world. We encourage wherever possible the live participation of our virtual attendees in the post-lecture Q&A.

The symposium opens on Monday morning with two plenary lectures. Ruth Gimeno, from Eli Lilly, will review key events leading to the development of peptide therapeutics for obesity and type II diabetes. In the second plenary lecture, Tom Lutz from the University of Zurich will discuss amylin biology and its continuing therapeutic potential. The next session will feature state-of-the-art talks from Weibo Cai, Kenn Hermann, and Jinsha Liu covering the potential uses of radionuclides, radiolabeled peptides and the development of a novel platform for the discover of cell-penetrating peptides.

After lunch topics include FGF-21 biology, a proteolipid-base nucleic acid delivery system and a PNA-based in vivo gene editing technology; these will be presented by Akero Therapeutics, Entos Pharmaceuticals and Neubase Therapeutics. The last session of the day will include lectures from Jiandie Lin, Eunheeni Choi and Archita Agrawal that discuss novel peptide drugs that have the potential for the development of new class of peptide therapeutics. We encourage you to join us for the Opening Reception and poster viewing that will immediately follow.

Tuesday morning will kick off with Plenary Lectures from David Maggs and Annelise Baron, highlighting the interplay of insulin and glucagon and cathelicidin-mimicking peptides, respectively. In the last session of the day we will hear from speakers at Apellis, Advidity Bioscience and ProteinQure who will describe novel therapeutics relating to C3, advances in delivery of oligonucleotides, and a new AI-based approach for peptide drug discovery. A networking lunch will follow the close of the Symposium and we invite you to join your colleagues and enjoy the view.

The taped presentations will be available online for viewing for 60 days following the close of the meeting. After the conference, you may use the Q&A function within the virtual platform to ask questions of the speakers, should they not have time to answer them during the live Q&A.

As in previous years our primary goal is to present new advances and discoveries in the field of peptide-based therapeutics, both from a research and development perspective. We encourage you to take full advantage of the networking opportunities and make sure to swing by the symposium sponsor tables.

Sadly, this year we will miss the probing questions and pointed comments of Waleed Danho who passed at the end August. Waleed was a fierce supporter of the peptide community and gave encouragement and support to many of us. He was much loved and will be greatly missed. We encourage you to take the mic and challenge the speakers with thought provoking questions to honor the memory of Waleed.

Phil Dawson
Chairman of the Board
Peptide Therapeutics Foundation

Adam Mezo
President
Peptide Therapeutics Foundation

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Ferring Pharmaceuticals

Ferring Pharmaceuticals is a research-driven, specialty biopharmaceutical group committed to helping people around the world build families and live better lives. Founded in 1950, privately-owned Ferring employs over 7,000 people worldwide. Headquartered in Saint-Prex, Switzerland, Ferring is a leader in reproductive medicine and maternal health, and in specialty areas within microbiome/gastroenterology and uro-oncology/urology. The company has emerged as a world leader with one of the largest peptide therapeutics portfolios in the industry. As part of its commitment to developing innovative products to treat diseases with high unmet medical need, Ferring invests heavily in its research infrastructure both in terms of people and technology. The company has operating subsidiaries in more than 50 countries and markets its products in over 100 countries.

Neurocrine Biosciences Inc.

Neurocrine Biosciences is a neuroscience-focused, biopharmaceutical company with a simple purpose: to relieve suffering for people with great needs, but few options. We are dedicated to discovering and developing life-changing treatments for patients with under-addressed neurological, neuroendocrine, and neuropsychiatric disorders. The company's diverse portfolio includes FDA-approved treatments for tardive dyskinesia, Parkinson's disease, endometriosis* and uterine fibroids*, as well as over a dozen mid- to late-stage clinical programs in multiple therapeutic areas. For three decades, we have applied our unique insight into neuroscience and the interconnections between brain and body systems to treat complex conditions. We relentlessly pursue medicines to ease the burden of debilitating diseases and disorders, because you deserve brave science. (*in collaboration with AbbVie)

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.

PolyPeptide

PolyPeptide is a publicly listed organization, traded on the Swiss stock exchange, which focuses on manufacturing proprietary and generic GMP-grade peptides and oligonucleotides for the pharmaceutical and biotechnological market. With more than 60 years of experience, PolyPeptide is committed to the highest quality of manufacturing for commercial peptide drug substances, GMP peptides in clinical trials, or small-scale pre-clinical custom syntheses.

As an organization, PolyPeptide has grown by selective acquisition of existing expertise, culminating in its position today as a leader in peptide manufacturing. With three manufacturing facilities in Europe, two facilities in the US , and one in Asia, PolyPeptide is a multinational company with about 1200 employees worldwide with a diversity which brings breadth and depth of knowledge, as well as unique experience to the organization.

PolyPeptide's long-established core strength is in GMP manufacturing, and a broad range of services which supports peptide & peptide-like projects, including conjugation to non-peptide moieties, from bench scale through to commercialization. With continually increasing capacity for GMP manufacturing, PolyPeptide is uniquely placed to serve the needs of its customers at all stages of pharmaceutical peptide development with a proven track record in security of supply to its clients.

Takeda Pharmaceuticals

Takeda is a global, values-based, R&D-driven biopharmaceutical leader headquartered in Japan, committed to discover and deliver life-transforming treatments, guided by our commitment to patients, our people and the planet. Takeda focuses its R&D efforts on four therapeutic areas: Oncology, Rare Genetics and Hematology, Neuroscience, and Gastroenterology (GI). We also make targeted R&D investments in Plasma-Derived Therapies and Vaccines. We are focusing on developing highly innovative medicines that contribute to making a difference in people's lives by advancing the frontier of new treatment options and leveraging our enhanced collaborative R&D engine and capabilities to create a robust, modality-diverse pipeline. Our employees are committed to improving quality of life for patients and to working with our partners in health care in approximately 80 countries and regions.

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)

WuXi TIDES

WuXi TIDES, a unique Contract Research and Development Manufacturing Organization (CRDMO) platform, is an integral part of WuXi STA, a subsidiary of WuXi AppTec. WuXi TIDES offers our worldwide partners efficient, flexible, and high-quality solutions for the drug development of oligonucleotides, peptides and related synthetic conjugates ("TIDES" drugs). We greatly simplify the TIDES drug development by providing all discovery, CMC development and the entire manufacturing supply chain under one roof.

With over 1,000 scientists from 9 R&D and manufacturing sites, we offer discovery compound screening and synthesis, process development and manufacturing of novel monomers, linkers and ligands, oligonucleotides, peptides and complex synthetic conjugates at any scale. Beyond chemistry, we offer formulation development, manufacturing, labeling and distribution services in a variety of injectable dosage forms

and filling formats including the Lipid Nanoparticle (LNP) drug delivery platform. Our comprehensive analytical method development, validation and testing platform will support your needs in TIDES drug development from discovery through clinical to commercial. Moreover, our Regulatory Affairs CMC team is experienced in preparing CMC dossiers to support global filings for TIDES new drug applications.

PEPTIDE THERAPEUTICS FOUNDATION

Peptide Therapeutics Foundation

Peptide Therapeutics Foundation is a not-for-profit 501C (3), established in 2008 to promote research and development of peptide therapeutics. The Foundation is currently supported by six corporate sponsors; Ferring Pharmaceuticals, Neurocrine Biosciences Inc., Novo Nordisk, PolyPeptide, Takeda Pharmaceuticals 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.

18th Annual Peptide Therapeutics Symposium

October 16-17, 2023

The Scripps Seaside Forum, La Jolla, CA

Virtual and In-Person Meeting

| Monday, October 16, 2023

8:00 a.m. - 4:00 p.m.
Registration Check-In
The Scripps Seaside Forum Lobby

8:00 a.m. – 8:30 a.m.
Breakfast
The Scripps Seaside Forum Lobby

8:30 a.m. – 4:45 p.m.
18th Annual Peptide Therapeutics Symposium
Samuel H. Scripps Auditorium

8:30 a.m. – 8:35 a.m.
Opening Remarks
Adam Mezo, Ph.D.
President, Peptide Therapeutics Foundation
Executive Scientific Director, Discovery Biology Research
Neurocrine Biosciences, Inc.

8:35 a.m. – 10:05 a.m.
Plenary Lectures
Moderator: Sepideh Afshar
Senior Director and Peptide Discovery Group Leader
Eli Lilly and Company

8:35 a.m. – 9:20 a.m
Peptide Therapeutics for the Treatment of Obesity and Type 2 Diabetes
Ruth E. Gimeno, Ph.D.
Group Vice President, Diabetes, Obesity and Cardiometabolic Research
Eli Lilly and Company

9:20 a.m. – 10:05 a.m.
Amylin: State of the Union
Thomas Lutz, Ph.D.
Professor in Veterinary Physiology, Institute of Veterinary Physiology, Vetsuisse Faculty
University of Zurich

10:05 a.m. – 10:35 a.m.
Beverage Break & Poster Viewing
The Scripps Seaside Forum Lobby

10:35 a.m. – 12:05 p.m.
Session I
Moderator: Bryan Fuchs, Ph.D.
Vice President, Gastroenterology
Ferring Pharmaceuticals

| Monday, October 16, 2023 (Continued)

10:35 a.m. – 11:05 a.m.

Harnessing the Power of Molecular Imaging for Precision Medicine: Radiolabeled Peptides, Antibodies, and Everything in Between
Weibo Cai, Ph.D.
Vilas Distinguished Achievement Professor of Radiology, Medical Physics, Materials Science & Engineering, Pharmaceutical Sciences
University of Wisconsin - Madison

11:05 a.m. - 11:35 a.m.

Radionuclide Theranostics: Current State and Future Perspectives
Ken Herrmann, MD, MBA
Department Chair, Nuclear Medicine
Universitätsklinikum Essen, Germany

11:35 a.m. – 12:05 p.m.

Establishing a Novel Platform for Discovery of CPPs that Localize in Cytoplasm
Jinsha Liu, Ph.D.
Advisor
Eli Lilly and Company

12:05 p.m. – 1:05 p.m.

Lunch & Poster Viewing
The Scripps Seaside Forum Lawn

1:05 p.m. – 2:35 p.m.

Session II
Moderator: Antoine Henninot
Director
Takeda Pharmaceuticals

1:05 p.m. – 1:35 p.m.

Development of Efruxifermin, an FGF21 Analog, for Treatment of Nonalcoholic Steatohepatitis (NASH)
Jonathan M. Young, Ph.D., J.D.
Co-Founder and Chief Operating Officer
Akero Therapeutics, Inc.

1:35 p.m. – 2:05 p.m.

Safe and Effective Delivery of Nucleic Acids Using Proteolipid Vehicles Formulated with Fusion-Associated Small Transmembrane Proteins
John D. Lewis, Ph.D.
Founder & CEO
Entos Pharmaceuticals

2:05 p.m. – 2:35 p.m.

Stealth Editing™ - A Novel Non-Immunogenic In Vivo Gene Editing Technology
Dietrich A. Stephan, Ph.D.
Founder & CEO
NeuBase Therapeutics

18th Annual Peptide Therapeutics Symposium

| Monday, October 16, 2023 (Continued)

2:35 p.m. – 2:55 p.m.
Beverage Break & Poster Viewing
The Scripps Seaside Forum Lobby

2:55 p.m. – 4:25 p.m.
Session III
Moderator: Ron He, PhD, MBA
Principal Scientist
Peptide Chemistry | Discovery Biologics
Neurocrine Biosciences, Inc.

2:55 p.m. – 3:25 p.m.
Neuregulin 4 (NRG4) as a New Hormonal Therapy for NASH-related Liver Cancer
Jiandie Lin, Ph.D.
Bradley M. Patten Collegiate Professor of Life Sciences, Professor of Cell & Developmental Biology, Research Professor in the Life Sciences Institute
University of Michigan

3:25 p.m. - 3:55 p.m.
Activation of the Insulin Receptor by Insulin and Non-insulin Peptide: Application in Human Disease
Eunhee Choi, Ph.D.
Assistant Professor, Department of Pathology and Cell Biology
Columbia University

3:55 p.m. - 4:25 p.m.
The Structure-function of the Endocrine FGFs: Nature Repeats Itself
Archita Agrawal, Ph.D.
Postdoctoral Researcher
Salk Institute for Biological Studies

4:25 p.m. - 4:45 p.m.
Tribute to Waleed Danho

4:45 p.m. - 6:15 p.m.
Opening Reception
The Scripps Seaside Forum Lawn

| Tuesday, October 17, 2023

8:00 a.m. - 10:00 a.m.
Registration Check-In
The Scripps Seaside Forum Lobby

8:00 a.m. - 8:30 a.m.
Breakfast & Poster Viewing
The Scripps Seaside Forum Lobby

8:30 a.m. - 12:30 p.m.
18th Annual Peptide Therapeutics Symposium
Samuel H. Scripps Auditorium

8:30 a.m. - 8:45 a.m.
Welcoming Remarks
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

18th Annual Peptide Therapeutics Symposium

| Tuesday, October 17, 2023 (Continued)

8:45 a.m. - 10:15 a.m.

Plenary Lectures
Moderator: David Parkes
President
DGP Scientific Inc.

8:45 a.m. - 9:30 a.m.

The Interplay of Insulin and Glucagon: From Physiology to Pharmacology
David Maggs, MD, FRCP
Advance Therapeutics
UT Health San Antonio TX

9:30 a.m. - 10:15 a.m.

One Ellipsoid to Rule Them All — Cathelicidin-mimicking Peptoids that are Antiviral, Antibacterial & Antifungal and Well-tolerated in the Airways
Annelise E. Barron, Ph.D.
Associate Professor of Bioengineering
Stanford University, Schools of Medicine and Engineering

10:15 a.m. - 10:45 a.m.

Beverage Break & Poster Viewing
The Scripps Seaside Forum Lobby

10:45 a.m. - 12:15 p.m.

Session IV
Moderator: Robert Hagopian
Director, Peptide Therapeutics Foundation
Director, Business Development, Group Pipeline
PolyPeptide

10:45 a.m. - 11:15 a.m.

Computational Design of Novel Peptides for SORT1 and Their Use in Oncology
Lucas Siow
Co-Founder & CEO
ProteinQure Inc.

11:15 a.m. - 11:45 a.m.

Pegcetacoplan: the CMC Journey through Development to FDA Approval
Christopher P. Holmes, Ph.D.
VP of Chemical and Pharmaceutical Development
Apellis

11:45 a.m. - 12:15 p.m.

Advances in Oligonucleotide Delivery to Muscle Using Antibody Oligonucleotide Conjugates (AOCs): Transferrin Receptor 1 (TfR1) Mediated Uptake of AOCs for the Treatment of Muscular Dystrophies
Arthur A. Levin, Ph.D.
Avidity Board Member and Distinguished Scientist & Strategic Leader
Avidity Biosciences, Inc.

| Tuesday, October 17, 2023 (Continued)

12:15 p.m. - 12:30 p.m.

Closing Remarks
Nick Cox, Ph.D.
Director, Peptide Therapeutics Foundation
Senior Director of Discovery Chemistry
Novo Nordisk

12:30 p.m. - 1:30 p.m.

Networking Lunch
The Scripps Seaside Forum Lawn

Waleed Danho Tribute

We are deeply saddened by the loss of our dear friend and colleague Dr. Waleed Ohan Danho who passed away on Aug 28, 2023 in Del Mar, California. He was 82 years of age. He will be greatly missed by all of us in the peptide community and we extend our heartfelt condolences to his family and friends.

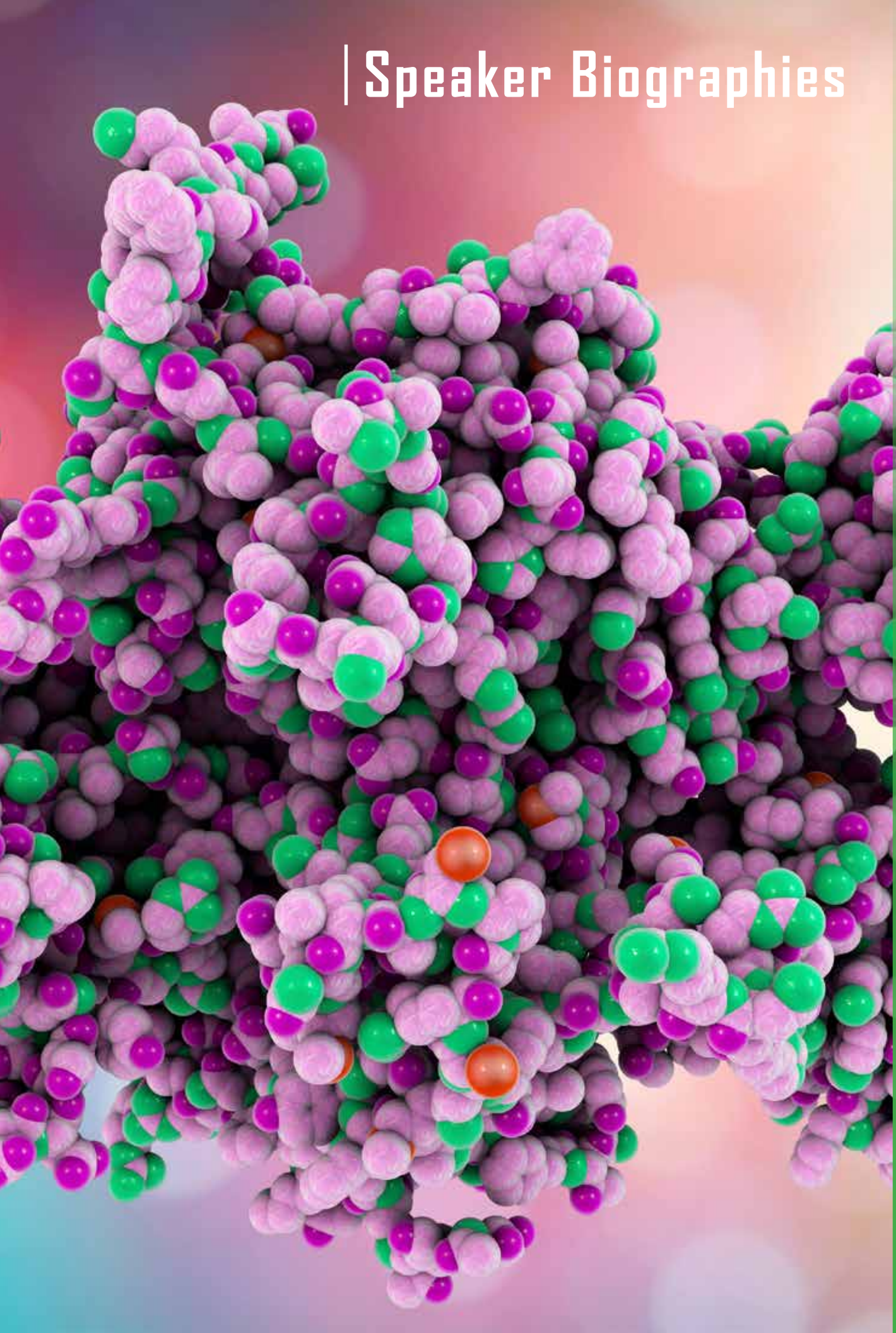
To say that Waleed was a unique and unforgettable individual is an understatement. Anyone who spent any time with him would applaud the logo on the sweatshirt that he is wearing in the attached photo. "Waleed. The Man. The Myth. The Legend."

Waleed was an exceptional peptide chemist and leaves behind a large body of publications and patents from his research endeavors. He had an immense belief in the power and potential of peptide therapeutics for providing life-changing medical treatments. His irrepressible personality, passion and advocacy for peptide science, generosity in sharing his vast knowledge, and his forthright style drew and endeared him to people he met. It was always clear to those who knew him that he expected high standards from his scientific colleagues. He welcomed and joyfully plunged into robust scientific debates, was honest in sharing his opinions and scientific advice and celebrated advances in his research field.

When he retired to live in Del Mar, California he was cajoled by a friend to get a dog. He often commented on the fact that he had no idea that one little French bulldog could so completely steal his heart. Buddy was his constant companion who enthusiastically greeted visitors to his home. He continued to be actively engaged in research, serving as a scientific advisor to Pharma and biotech companies. He generously gave his time to the Peptide Therapeutics Foundation, playing a major role on the program committee each year. He rarely missed a meeting and never failed to ask challenging scientific questions at the peptide symposia. Above all, he mentored young scientists and supported the careers of promising academic researchers in the peptide field.

There is no doubt he would want each of us to honor his legacy by following his example and giving generously of our time and intellect to ensure that the field of peptide research continues to advance.

Speaker Biographies



Archita Agrawal, Ph.D.
Postdoctoral Researcher, Salk Institute for Biological Studies
The Structure-function of the Endocrine FGFs: Nature Repeats Itself

Archita Agrawal is a postdoctoral fellow at the Salk Institute for Biological Studies. Her thesis work focused on defining the molecular basis of interaction between endocrine FGF-proteins (FGF19, 21, 23) and their receptor complexes. The work showed that relatively short C-terminal peptides of 25-amino acids of either FGF19 or FGF21 can competitively antagonize the cellular signaling of each hormone, and enabled the design of higher potency FGF21-agonist. Her work led to the discovery that the C-terminus of FGF23 contains two distinct Klotho-interacting sites. Dr. Agrawal's current research work is aimed at uncovering the bioactive microproteins of the human genome that are encoded by unannotated small-open reading frames in disease models such as cancer, in Prof. Alan Saghatelian's laboratory. She received her Ph.D. at Indiana University under the mentorship of Prof. Richard DiMarchi.

Annelise Barron, Ph.D.
Associate Professor of Bioengineering, Stanford University, Schools of Medicine and Engineering
One Ellipsoid to Rule Them All — Cathelicidin-mimicking Peptoids that are Antiviral, Antibacterial & Antifungal and Well-tolerated in the Airways

Annelise E. Barron is the W.M. Keck Associate Professor of Bioengineering at Stanford University.

The broad theme of the Barron lab is the study and biomimicry of natural host defense peptides (antimicrobial peptides). We study the molecular biophysics and mechanisms of LL-37—a centrally important human host defense peptide—and its involvement in Alzheimer's dementia (via LL-37 dysregulation and degradation by pathogen virulence factors). Alzheimer's dementia can be caused by (or at least, accompanied by) polymicrobial cerebral infections, a phenomenon receiving renewed attention given recent discoveries. We are also working to develop biostable peptoid mimics of LL-37 as therapeutics that can combat antibiotic-resistant infections, especially cerebral infections, ear infections, and sinus / lung infections.

Dr. Barron is a chemical and biological engineer. She was trained in chemical engineering at the University of Washington (B.S.) and U.C. Berkeley (Ph.D., under the mentorship of Prof. Harvey W. Blanch), and was a Pharmaceutical Chemistry postdoc with Prof. Ken A. Dill (UCSF) and Dr. Ronald N. Zuckermann (Chiron Corp.). She has served on the faculty at Stanford since 2007, and prior to that, served on the Chemical & Biological Engineering faculty of Northwestern University in Evanston, IL for 10 years (1997-2007). Dr. Barron has been awarded the NIH Pioneer Award (2020), the Oskar Fischer Award (2022), the Presidential Early Career Award for Scientists & Engineers (PECASE) through NIH / NHGRI (1999), the Beckman Young Investigator Award (1999), and the Camille Dreyfus Teacher-Scholar Award (1998), among other awards. Dr. Barron was the youngest scientist ever to serve on the Scientific Advisory Committee to the Director of the NIH, under Dr. Elias Zerhouni. She has more than 177 publications and a current H-index of 50 (Web of Science, All Databases), and serves on the advisory boards of several biotechnology companies.

Weibo Cai, Ph.D.
Vilas Distinguished Achievement Professor of Radiology, Medical Physics, Materials Science & Engineering, Pharmaceutical Sciences, University of Wisconsin-Madison
Harnessing the Power of Molecular Imaging for Precision Medicine: Radiolabeled Peptides, Antibodies, and Everything in Between

Weibo Cai is a Vilas Distinguished Achievement Professor of Radiology, Medical Physics, Materials Science & Engineering, and Pharmaceutical Sciences at the University of Wisconsin - Madison. He received a BS degree in Chemistry from Nanjing University, China (1995) and a PhD degree in Chemistry from the University of California San Diego (2004; Mentor: Murray Goodman). Between 2005 and 2008, Dr. Cai did his post-doctoral research in the Molecular Imaging Program at Stanford University (Mentor: Xiaoyuan Chen). In February 2008, Dr. Cai joined the University of Wisconsin - Madison as a Biomedical Engineering Cluster Hire, and was promoted to Associate Professor with Tenure in 2014, and Full Professor in 2018. Dr. Cai's research at UW-Madison (http://mi.wisc.edu) is primarily focused on molecular imaging and nanobiotechnology.

Dr. Cai has authored >380 peer-reviewed articles (total citation: >36,000 times; h-index: 100), edited 3 books, and given >300 talks. Dr. Cai has received many awards, including the European Association of Nuclear Medicine (EANM) Springer Prize (2011 & 2013), American Cancer Society Research Scholar (2013-2017), EANM Annual Congress Plenary Lecturer (2016), Fellow of AIMBE (2018), Fellow of SNMMI (2019), Fellow of RSC (2021), SNMMI Radiopharmaceutical Sciences Council's Michael J. Welch Award (2022), Journal of Nanobiotechnology (JNB) Trailblazer Award (BMC/Springer Nature, 2022), among others.

Dr. Cai has served on the Editorial Board of >20 scientific journals, and participated in many grant review panels (USA, Canada, and >10 European countries, etc.). He is currently the Editor-in-Chief of Journal of Nanobiotechnology (IF 9.429) and Associate Editor of European Journal of Nuclear Medicine and Molecular Imaging (IF 10.057). Dr. Cai is an active member of several scientific societies and he has served on various committees. What Dr. Cai is most proud of is that his trainees at UW - Madison have received ~150 awards to date, and more than 15 of his trainees have started independent research groups at world-class universities. Lastly, Dr. Cai has also served various roles for several industrial corporations.

Eunhee Choi, Ph.D.
Assistant Professor, Department of Pathology and Cell Biology, Columbia University
Activation of the Insulin Receptor by Insulin and Non-insulin Peptide: Application in Human Disease

Dr. Eunhee Choi completed a postdoctoral fellowship at the UT Southwestern Medical Center under the supervision of Dr. Hongtao Yu. She worked on the function of cell division regulators and discovered an unexpected connection between cell division regulators and insulin signaling. Now she is an assistant professor in Columbia University, where her research focuses on the function, regulation, and mechanism of insulin receptor signaling. Recently her team discovered that insulin and insulin mimetics activate insulin receptor in different ways, providing insights into the development of new strategies for the treatment of insulin resistance.

Nick Cox, Ph.D.
Director, Peptide Therapeutics Foundation; Senior Director of Chemical Biology, Novo Nordisk
Closing Remarks

Dr. Cox is the Senior Director of Chemical Biology at Novo Nordisk's R&D hub in the greater Boston area, where his team drives peptide, protein, and other therapeutic discovery efforts targeting chronic conditions including obesity, diabetes, cardiovascular disease, and rare blood and endocrine disorders. Prior to joining Novo Nordisk, he completed his training as a Postdoctoral Scholar in Stanford's ChEM-H institute (2014-2016) under the mentorship of Prof. Chaitan Khosla and Dr. Mark Smith, serving as chemistry lead on numerous projects in early-stage drug discovery. Dr. Cox received his Ph.D. in Chemistry from the University of Washington (2013) in the laboratory of Prof. Gojko Lalic, where he studied organic methodology and transition metal catalysis.

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
Welcoming 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 cochaired the 22nd American Peptide Symposium and the GRC on Biology and Chemistry of Peptides. He has published over 195 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, the RSC MedImmune Protein and Peptide Science Award and the Akabori Memorial Award from the Japanese Peptide Society. 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.

Ruth Gimeno, Ph.D.
Group Vice President, Diabetes, Obesity and Cardiometabolic Research, Eli Lilly and Company
Peptide Therapeutics for the Treatment of Obesity and Type 2 Diabetes

Dr. Gimeno leads research and early clinical development in diabetes, obesity and cardiometabolic disorders for Eli Lilly and Company with responsibility from project initiation through the end of Phase 2. Dr. Gimeno received undergraduate training in medicine at the Julius-Maximilians-University in Würzburg, Germany, and obtained a Ph.D. in biology from the Massachusetts Institute of Technology in Cambridge, USA. Prior to joining Lilly in 2011, Dr. Gimeno led research teams focused on metabolic disease drug discovery at Millennium Pharmaceuticals, Wyeth and Pfizer. Under Dr. Gimeno's leadership, Lilly has built a strong pipeline of innovative molecules in diabetes, obesity and cardiovascular disease, which includes tirzepatide, a dual GIP and GLP-1 receptor agonist, and retatrutide, the first triple GIP, GLP-1 and Glucagon receptor agonist to enter Phase 3 clinical development. Dr. Gimeno has coauthored more than 50 publications and is coinventor of several patents. In 2022, Dr. Gimeno was named as one of the top 20 women leading biopharma R&D by *Endpoints News*.

Ken Herrmann, MD, MBA
Department Chair, Nuclear Medicine, Universitätsklinikum Essen, Germany
Radionuclide Theranostics: Current State and Future Perspectives

Ken Herrmann currently acts as Chair of the Department of Nuclear Medicine at the Universitätsklinikum Essen in Germany, Chair of the EANM Oncology & Theranostics Committee, and serves as a Section Editor of the Journal of Nuclear Medicine. Ken's earlier career found him as a Visiting Assistant Professor promoted to Associate Professor in the Ahmanson Translational Imaging Division of the Department of Molecular and Medical Pharmacology at the University of California Los Angeles in addition to holding his position as Vice Chair of the Department of Nuclear Medicine at the Universitätsklinikum Würzburg. Ken holds a Doctorate Degree from Humboldt Universität Berlin and completed his residency in Nuclear Medicine at Klinikum rechts der Isar, Technische Universität München in addition to his MBA, which he received from the Universität Zürich, Switzerland in 2011.

Christopher P. Holmes, Ph.D.
VP of Chemical and Pharmaceutical Development, Apellis
Pegcetacoplan: the CMC Journey through Development to FDA Approval

Chris obtained his Ph.D. in organic chemistry at UC Berkeley where we worked on developing novel synthetic methodologies for natural products. During his post-doc at Stanford University, he worked in bioorganic chemistry designing, synthesizing, and testing new inhibitors targeting the branched-chain amino acid pathway as a source for new herbicides. After a stint at Chevron Chemical Co. in the agricultural chemicals doing work on novel fungicides, he moved to the Affymax Research Institute where he played a key role in establishing the company as a leader in combinatorial chemistry. After Glaxo-Welcome purchased Affymax, Chris led a team of scientists in the high throughput synthesis, Hit Identification, and Hits-to-Leads groups providing novel lead compounds to the broader Glaxo-Welcome organization. Highlights included the creation of more than 2.5 million small molecules and over 100 different encoded small molecule libraries. After being spun out of Glaxo-Welcome, Affymax continued as an independent company focusing on peptide therapeutics in hematology, neuroprotection, oncology, and immune-mediated disease programs. Chris led a chemistry department of medicinal, analytical, and computational chemists. Chris oversaw the scale-up, analytical development, transfer to multiple CMOs, regulatory filings, and overall development of Omontys® (peginesatide), the first synthetic PEGylated peptide approved by the FDA for the treatment of anemia. Chris then joined Regado Biosciences as the VP of Chemical and Pharmaceutical Development groups working on Revolixys™ Kit (a two component mixture of a PEGylated RNA aptamer pegnivacogin and its active RNA control agent anivamersen). Chris ran a successful CMC consulting company for several years serving clients in early-stage research primarily focused on peptides, conjugated peptides, and small molecules. He later joined Apellis Pharmaceuticals as VP of CMC operations where he oversaw CMC aspects in the development of pegcetacoplan, a PEGylated synthetic peptide that blocks Complement C3. Pegcetacoplan has been recently approved as EMPAVELI™ for the treatment of PNH and as SYFOVRE™ for the treatment of Geographic Atrophy. Chris is a named inventor on 32 patents and has co-authored over 50 articles in peer-reviewed journals.

Arthur A. Levin, Ph.D.
Avidity Board Member and Distinguished Scientist & Strategic Leader, Avidity Biosciences, Inc.
Advances in Oligonucleotide Delivery to Muscle Using Antibody Oligonucleotide Conjugates (AOCs): Transferrin Receptor 1 (TfR1) Mediated Uptake of AOCs for the Treatment of Muscular Dystrophies

Arthur Levin, a founding member of Avidity, serves as Distinguished Scientist and Strategic Leader at Avidity Biosciences and is a member of the Board of Directors. He previously held the position of Chief Scientific Officer at Avidity. Dr. Levin is a key opinion leader in the RNA therapeutics field who has led teams responsible for the development of many oligonucleotides. Prior to joining Avidity, Dr. Levin was the Executive Vice President of Research and Development at miRagen Therapeutics and held senior drug development roles at Ionis (formerly Isis) Pharmaceuticals and Santaris Pharma.

Dr. Levin has played key roles in the development of numerous oligonucleotides including the first approved antisense drugs and the first microRNA-targeted therapeutic in clinical trials. He has a combined four decades of experience in all aspects of drug development from discovery through

drug registration, both in large pharma and biotech companies. Dr. Levin has published more than 100 scientific articles and several of the most cited reviews in the field. He is on the scientific advisory boards and Boards of Directors of multiple institutions. Art received a doctorate in toxicology from the University of Rochester, and a bachelor's degree in biology from Muhlenberg College.

John D. Lewis, Ph.D.
Founder, CEO of Entos Pharmaceuticals
Safe and Effective Delivery of Nucleic Acids Using Proteolipid Vehicles Formulated with Fusion-Associated Small Transmembrane Proteins

Dr. John Lewis is a scientist, academic, and serial entrepreneur. His research interests include novel nanotechnology, nanoparticle drug delivery technologies, and imaging related to infectious diseases and chronic diseases such as aging and cancer. He also pioneered intravital imaging in the in vivo study of tumor cell invasion and metastasis to discover key targets for cancer therapeutics.

As an academic, Dr. Lewis is a Professor and the Bird Dogs Chair in Translational Oncology at the University of Alberta and the founding member of the Alberta Prostate Cancer Research Initiative. The chair is designated to translate scientific discoveries into clinical applications to improve the diagnosis, treatment, survival, and quality of life of people with cancer.

John Lewis is the founder, CEO, or CSO of multiple spin-off companies developing treatments for cancer, infectious diseases, and age-related diseases, and the CEO of Entos Pharmaceuticals, a clinical-stage biotechnology company developing genetic medicines using the Fusogenix PLV drug delivery system.

Dr. Lewis trained at The Scripps Research Institute and received a Ph.D. in biochemistry from the University of Victoria.

Jiandie Lin, Ph.D.
Bradley M. Patten Collegiate Professor of Life Sciences, Professor of Cell & Developmental Biology, Research Professor in the Life Sciences Institute, University of Michigan
Neuregulin 4 (NRG4) as a New Hormonal Therapy for NASH-related Liver Cancer

Dr. Jiandie Lin is Bradley M. Patten Collegiate Professor of Life Sciences, Professor of Cell & Developmental Biology, and Research Professor in the Life Sciences Institute at the University of Michigan.

Dr. Lin's laboratory investigates the fundamental biology of metabolic signaling and disease pathogenesis and develops new therapeutic strategies targeting metabolic disorders. His lab has published research work in Nature, Nature Medicine, Molecular Cell, Cell Metabolism, and JCI.

Dr. Lin is a fellow of American Association for the Advancement of Science (AAAS) and recipient of the 2020 American Diabetes Association Outstanding Scientific Achievement Award. He served as President of the Chinese American Diabetes Association (CADA) (2015-2018) and Chair of the Nutrition, Metabolism, Health and Disease (NMHD) study section (2020-2022).

Jinsha Liu, Ph.D.
Advisor, Eli Lilly and Company
Establishing a Novel Platform for Discovery of CPPs that Localize in Cytoplasm

Jinsha Liu received her bachelor's degree in Biosciences from Northeast Normal University in China and her master's degree in cancer biology at California State Fresno in the US. She continued her graduate training in neuroscience at Sanford Burnham Prebys Medical Discovery institute in La Jolla, CA, during which she focused on investigating the roles of T-cadherin (CDH13) and adiponectin in modulating synaptic plasticity to enable associative memory functions in mouse hippocampus. Jinsha joined Eli Lilly and company in 2018 as a postdoctoral scientist with the goal of establishing a novel discovery platform to identify cell-penetrating peptides, called NNJA platform. She is now an advisor at Lilly and continuing her work on optimizing the NNJA platform and extending the platform applications for delivery of therapeutic cargos.

Thomas Lutz, Ph.D.
Professor in Veterinary Physiology; Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich
Amylin: State of the Union

I am a trained veterinarian and did my Ph.D. at the University of Queensland, Australia, on the pathophysiology of feline diabetes mellitus. For the last 30 years, my interest in research at the University of Zurich has been to understand the physiological control of eating and metabolism, and the dysfunction of this system in obesity, type 2 diabetes mellitus and other metabolic diseases. My group contributed to a large extent to today's knowledge on the mechanisms of action of the pancreatic hormone amylin in metabolic control. We identified key brain regions that mediate amylin's physiological effects. Next to my research on the amylinergic control of metabolism, we are investigating the mechanisms underlying the effects of Roux-en-Y gastric bypass surgery and the pathophysiology of diabetes mellitus in cats.

David Maggs, MD, FRCP
Advance Therapeutics, UT Health San Antonio, TX
The Interplay of Insulin and Glucagon: From Physiology to Pharmacology

Dr. Maggs/David is a life science industry executive and physician who has over 30 years of academic and industry experience. He first graduated from Guys Hospital (London, UK), completed postgraduate studies at University of Nottingham, and held fellowship and faculty appointments at Yale School of Medicine (New Haven, CT). He is a Fellow of the Royal College of Physicians (London, UK) and currently holds an adjunct faculty position at UT Health Science Center, San Antonio (TX). His industry experience includes leadership positions at Warner Lambert, Pfizer, Amylin Pharmaceuticals, GI Dynamics, Fractyl Laboratories and more recently Becton Dickinson. His past clinical experience involved the care of patients with diabetes and his academic interests have been in the field of cardiometabolic disease where he is well published. During his industry career, he has worked directly on numerous pharmaceutical and device platforms, from early inception through to commercialization, from rare conditions through to broad population-level diseases. He is currently CEO of Abvance Therapeutics, an early-stage company that is exploring the interplay of the two islet hormones insulin and glucagon and how this interplay could be leveraged to design a novel pharmaceutical for the treatment of diabetes.

Adam Mezo, Ph.D.
President, Peptide Therapeutics Foundation; Executive Scientific Director, Discovery Biology Research, Neurocrine Biosciences, Inc.
Opening Remarks

Dr. Adam Mezo has worked in the pharmaceutical industry for over 20 years with a focus on the discovery of novel peptide, small molecule and protein therapeutics.

Dr. Adam Mezo is currently Executive Scientific Director, Discovery Biology Research at Neurocrine Biosciences, Inc in San Diego. In his current role at Neurocrine, he is focused on the discovery of novel peptide therapeutics for a range of unmet medical needs. Prior to this role, he led teams of chemists, biochemists and drug hunters at the Ferring Research Institute, Eli Lilly, Biogen Idec and Syntonix. He has worked in various therapeutics areas, including diabetes, hemophilia, immunology and reproductive and women's health. Although peptides are his focus, he has also led teams in other modalities including small molecules and proteins as projects and priorities dictate. Dr. Mezo has over 50 published manuscripts and conference presentations, along with over 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.

Lucas Siow
Co-Founder and CEO of ProteinQure Inc.
Computational Design of Novel Peptides for SORT1 and Their Use in Oncology

Lucas is the CEO and one of the co-founders of ProteinQure. His background is in applied math and statistics (UC Berkeley and University of Pennsylvania), with an MBA from the University of Toronto. He has worked in numerous industries as a data scientist and management consulting before co-founding ProteinQure. ProteinQure is a five year old startup with 20 employees based in downtown Toronto. ProteinQure is the only computational platform combining AI and molecular dynamics to design exotic peptides and their conjugates. They have worked with three of the top 25 pharma companies in the world, and have multiple projects successfully demonstrating in-vivo success. They utilize non-canonical amino acids and complex scaffolds to engineer potency, stability, specificity and other drug like properties.

Dietrich A. Stephan, Ph.D.
Founder & CEO, NeuBase Therapeutics
Stealth Editing™ - A Novel Non-Immunogenic In Vivo Gene Editing Technology

Dietrich is a visionary in the field of human genetics, driven by his mission to reduce suffering and death caused by various diseases. He is a firm believer in the potential to diagnose and repair the human body, much like other machines, albeit with added complexity. Dietrich's career spans the translational continuum, from basic research to commercial development of healthcare solutions. As a researcher, Dietrich has served as a professor and chairman of the Department of Human Genetics at the University of Pittsburgh and had academic affiliations with Harvard Medical School, Johns Hopkins University, and Children's National Medical Center. His groundbreaking research has identified the genetic basis of numerous rare and common diseases, conceptualized novel genomics technologies, and fostered large, multi-institutional collaborations with significant funding from the National Institutes of Health. His work has been published in top-tier peer-reviewed journals such as the New England Journal of Medicine, Science, Nature, Cell, and the Proceedings of the National Academy of Sciences. Dietrich's translational efforts include his tenure as Deputy Director for Discovery Research at the Translational Genomics Research Institute (TGen), where he established the pioneering Neurogenomics Division, among the first and largest genomics efforts applied to neurological and mental health disorders. During this time, his team identified a major process in the brain that controls learning and memory and is developing therapies based on this understanding that improve cognition, powered the Autism Genome Project that has led to new diagnostic tests and therapy development, led the genomics portion of the Alzheimer's Disease Neuroimaging Consortium and was the first to commercialize APOE4 testing for healthy individuals to understand their risks of future disease, and conducted extensive whole-genome analysis of sporadic ALS which identified new mechanisms and new therapies that are in development, among other "big science" projects that are opening up new vistas of hope for patients. Additionally, Dietrich has played pivotal roles in founding and building public-private translational institutes across the country, including the New York Genome Center and a translational institute in the Washington DC region.

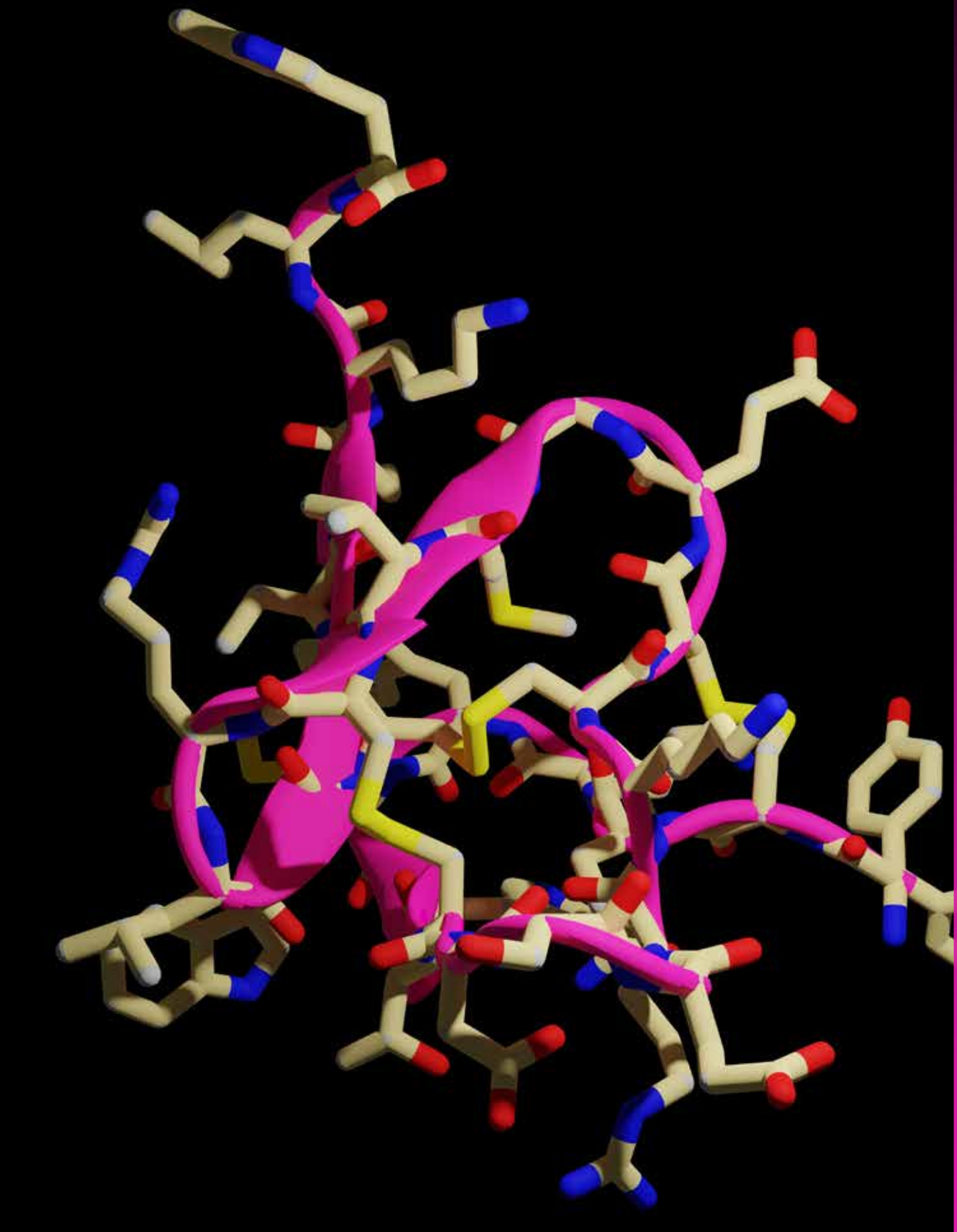
His efforts have resulted in numerous molecular diagnostics, targeted therapeutics, job creation, and the growth of bio clusters. As a serial entrepreneur, Dietrich has founded, co-founded, advised, and led numerous biotechnology companies, to fulfill his personal mandate to bring diagnostics and therapeutics to millions of patients worldwide. These include Navigenics, Guardant Health, Pendulum, Peptilogics, and NeuBase. A dedicated mentor, Dietrich's trainees now lead academic programs and companies across the country. He has received numerous accolades for his work and has been featured in media outlets such as the Wall Street Journal. Dietrich holds a B.Sc. from Carnegie Mellon University, a Ph.D. from the University of Pittsburgh, and completed his fellowship at the National Human Genome Research Institute of the National Institutes of Health.

Jonathan Young, Ph.D., J.D.
Co-Founder and Chief Operating Officer, Akero Therapeutics, Inc.
Development of Efruxifermin, an FGF21 Analog, for Treatment of Nonalcoholic Steatohepatitis (NASH)

Jonathan Young is Co-Founder and Chief Operating Officer of Akero. Prior to co-founding Akero, Jonathan held roles as a Venture Partner at Apple Tree Partners (ATP) and General Counsel and Vice President of Policy/Advocacy at Braeburn Pharmaceuticals, Inc., an ATP portfolio company. He previously worked as Partner and General Counsel of FoxKiser, a Washington, D.C. law firm specializing in regulatory approval and life cycle management for pharmaceutical and biotechnology companies. Jonathan is an advocate for policies that empower patients and people with disabilities to reach their life goals. In 2009, he was appointed by President Barack Obama, and confirmed by the U.S. Senate, to serve as Chairman of the National Council on Disability, an independent federal agency advising the President and Congress on disability policy; he previously coordinated White House disability policy for President Bill Clinton. He currently serves on the board of directors of the MedStar Health Research Institute. Jonathan holds a Ph.D. in American history from the University of North Carolina at Chapel Hill, and a J.D. from Yale Law School.

Notes

Speaker Abstracts



The Structure-function of the Endocrine FGFs: Nature Repeats Itself

Archita Agrawal, Ph.D.
Postdoctoral Researcher
Salk Institute for Biological Studies

Endocrine Fibroblast Growth Factors (FGF19, FGF21 and FGF23) are members of the FGF-family that contain FGF-structural core and a unique C-terminal sequence element to support their individual physiological functions. FGF19 and FGF21 primarily function at liver, adipose and pancreas to regulate glucose, lipid and bile acid metabolism. These proteins represent clinical candidates for treatment of diabetes, obesity and fatty liver diseases. Their unique C-terminal sequence enables their high affinity interaction with a co-receptor Klotho-□ for FGF19 and FGF21, or Klotho for FGF23 and allows to form a tripartite receptor complex with one of the FGF-receptors (FGFR1-4). We found that short C-terminal peptides (25-aa) of FGF19 and FGF21 can effectively antagonize native hormones' cellular signaling. The in-depth structure-activity relationship studies led to the engineering of higher potency FGF21-agonist. While structurally related, FGF23 has a distinct biological function at kidney and parathyroid gland to regulate phosphate and vitamin-D metabolism. The C-terminus of FGF23 is three times the size of FGF19/21 and our results show that it possesses two KL-interaction sites. The inactivation of either one of the sites diminishes the activity of peptide antagonist, or correspondingly the protein-agonist while inactivation of both sites is deleterious to FGF23-activity. Collectively, the results presented here enhance our biochemical understanding of endocrine FGFs and have led to the discovery of novel peptide antagonists.

One Ellipsoid to Rule Them All — Cathelicidin-mimicking Peptoids that are Antiviral, Antibacterial & Antifungal and Well-tolerated in the Airways

Annelise E. Barron, Ph.D.
Associate Professor of Bioengineering
Stanford University, Schools of Medicine and Engineering

Viral infections, such as those caused by SARS-CoV-2 and Influenza A, affect millions of people each year. Few antiviral drugs can effectively treat these infections. The standard approach in the development of antiviral drugs involves the identification of a unique viral target, followed by the design of an agent that addresses that target. Antimicrobial peptides (AMPs) represent a novel source of potential antiviral drugs. AMPs can inactivate numerous different enveloped viruses through disruption of their viral envelopes. Yet the clinical development of AMPs as antimicrobial therapeutics has been hampered by a number of factors, especially their enzymatically labile structure as peptides. We report the antiviral potential of peptoid mimics of AMPs (sequence-specific N-substituted glycine oligomers). These peptoids have the advantage of being insensitive to proteases, and exhibit increased bioavailability. Our results demonstrate that several peptoids exhibit potent in vitro antiviral activity against SARS-CoV-2 and Influenza virus when incubated prior to infection, and also have promising in vivo activity. Thus, they have direct effects on the viral structures that render the viral particles non-infective. Visualization by cryo-EM shows viral envelope disruption similar to what is observed in AMP activity against these viruses. Even at 50X we observe no cytotoxicity against primary cultures of epithelial cells. Results suggest a biomimetic mechanism, likely due to the differences between the phospholipid head group makeup of viral envelopes and host cell membranes, thus underscoring the potential of this class of molecules as safe and effective broad-spectrum antiviral agents. Furthermore, in recent work we have found some of the same peptoids are effective in killing both bacterial and fungal pathogens that commonly co-occur in pneumonia in ICU patients, and to sterilize biofilms. We discuss how and why differing molecular features between ten different peptoid candidates may affect both antimicrobial activity and selectivity, specifically, the self-assembly of the most effective peptoids into discrete micellar

structures such as ellipsoidal micelles comprising ~100 peptoid molecules per micelle. Remarkably, some of these same peptoids with broad-spectrum activity against respiratory viruses are also active against a broad array of pathogenic bacterial and fungal organisms, offering the possibility of a truly novel therapeutic approach to treating polymicrobial infections of the airways.

Harnessing the Power of Molecular Imaging for Precision Medicine: Radiolabeled Peptides, Antibodies, and Everything in Between

Weibo Cai, Ph.D.
Vilas Distinguished Achievement Professor of Radiology, Medical Physics, Materials Science & Engineering, Pharmaceutical Sciences, University of Wisconsin - Madison

The Molecular Imaging and Nanotechnology Laboratory at the University of Wisconsin - Madison (http://mi.wisc.edu/) is mainly focused on three areas: 1) development of multimodality molecular imaging agents; 2) nanobiotechnology and its biomedical applications; and 3) molecular therapy of cancer and various other diseases. In this talk, I will present our representative work on molecular imaging, image-guided drug delivery, and targeted therapy in various animal models (cancer and other diseases) with radiolabeled peptides, proteins, antibodies, and antibody fragments. Some of these have been translated into clinical investigation. The primary imaging techniques used in these studies are positron emission tomography (PET), photoacoustic tomography (PAT), optical imaging, and magnetic resonance imaging (MRI). Some representative molecular targets that we have investigated are CD105 (i.e. endoglin), PD-1/PD-L1, CTLA-4, CD146, VEGFR, integrin $\alpha_v\beta_3$, among others.

References:

1. Wei W, Rosenkrans ZT, Liu J, Huang G, Luo Q-Y, Cai W. ImmunoPET: Concept, design, and applications. Chemical Reviews, 2020, 120, 3787-3851.

2. Wei W, Younis MH, Lan X, Liu J, Cai W. Single-domain antibody theranostics on the horizon. Journal of Nuclear Medicine, 2022, 63, 1475-1479. 13.

3. Luo H, Hernandez R, Hong H, Graves SA, Yang Y, England CG, Theuer CP, Nickles RJ, Cai W. Noninvasive brain cancer imaging with a bispecific antibody fragment, generated via click chemistry. Proceedings of the National Academy of Sciences of the United States of America. 2015, 112, 12806-12811.

4. Yang Y, Hernandez R, Rao J, Yin L, Qu Y, Wu J, England CG, Graves SA, Lewis CM, Wang P, Meyerand ME, Nickles RJ, Bian XW, Cai W. Targeting CD146 with a 64Cu-labeled antibody enables in vivo immunoPET imaging of high-grade gliomas. Proceedings of the National Academy of Sciences of the United States of America, 2015, 112, E6525-E6534.

5. Luo H, England CG, Shi S, Graves SA, Hernandez R, Liu B, Theuer CP, Wong HC, Nickles RJ, Cai W. Dual targeting of tissue factor and CD105 for preclinical PET imaging of pancreatic cancer. Clinical Cancer Research, 2016, 22, 3821-3830.

6. Ehlerding EB, Sun L, Lan X, Zeng D, Cai W. Dual-targeted molecular imaging of cancer. Journal of Nuclear Medicine, 2018, 59, 390-395.

Activation of the Insulin Receptor by Insulin and Non-insulin Peptide: Application in Human Disease

Eunhee Choi, Ph.D.
Assistant Professor
Department of Pathology and Cell Biology
Columbia University

Defects in insulin receptor (IR) signaling cause metabolic diseases, including diabetes. Furthermore, genetic mutations of the IR can result in severe insulin resistance syndromes with limited treatment options. A selective IR agonist without sequence homology to insulin, S597, activates IR and mimics insulin's glycemic control effects. Our recent study demonstrated that S597 activates IR as efficiently as native insulin but via an alternative mechanism. In contrast to the maximum four insulin molecules that bind to two distinct IR sites and induce a compact T-shaped IR, two S597 molecules induce an extended T-shaped IR. S597 simultaneously binds to two protomers of IR and stabilizes a structurally distinct active IR dimer, triggering insulin-like signaling. Strikingly, S597 fully activates IR mutants that disrupt insulin binding or destabilize the insulin-mediated active IR conformation. Furthermore, S597 induces biased IR signaling in different tissues, and a minor change in its sequence can partially recover the selective IR signaling. Our structural and functional studies demonstrate the potential of structure-based drug design for developing insulin mimetics that target insulin resistance.

Peptide Therapeutics for the Treatment of Obesity and Type 2 Diabetes

Ruth E. Gimeno, Ph.D.
Group Vice President
Diabetes, Obesity and Cardiometabolic Research
Eli Lilly & Company

Obesity and type 2 diabetes (T2D) are complex metabolic disorders that affect millions of people worldwide and result in significant morbidity and mortality. An imbalance between nutrient intake and nutrient utilization in obesity leads to increased fat mass, ectopic fat deposition in tissues such as liver, pancreas, heart and muscle, and chronic inflammation. The resulting insulin resistance leads to beta-cell failure and ultimately T2D. Peptide hormones, such as glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), glucagon, peptide YY (PYY) and amylin, orchestrate the body's response to nutrients. They increase insulin secretion in response to elevated blood glucose (GLP-1 and GIP), increase amino acid and lipid catabolism as well as hepatic glucose output (glucagon), decrease the rate at which nutrients are being absorbed by decreasing gastric emptying (GLP-1 and amylin) and increase satiety after eating (GLP-1, GIP, amylin and PYY), thereby resulting in decreased appetite. GLP-1 analogs, e.g., exenatide, liraglutide and dulaglutide, were initially developed as glucose-lowering therapeutics for the treatment of T2D. As more potent and long-acting molecules became available and as titration regimens that minimized gastrointestinal side effects were developed, it became clear that GLP-1 analogs not only lower glucose, but are also effective agents to lower body weight with beneficial effects on complications and comorbidities of T2D, such as cardiovascular disease and fatty liver disease. Two GLP-1 analogs, liraglutide and semaglutide, are currently approved for chronic weight management in obesity. More recently, multifunctional peptide agonists that combine two (GIP and GLP-1; GLP-1 and glucagon) or three (GIP, GLP-1 and glucagon) pharmacological activities were demonstrated to show weight loss and in some cases glucose lowering that exceeds what can be achieved with single GLP-1 receptor agonists, initiating a new wave of peptide therapeutic efforts.

Tirzepatide is a GIP and GLP-1 receptor agonist that is approved for type 2 diabetes and under regulatory review for chronic weight management. The GLP-1 and glucagon receptor agonist survodutide, and the triple GIP, GLP-1 and glucagon receptor agonist retatrutide, are in Phase 3 clinical development. A second approach to peptide multi-pharmacology has been to combine individual peptide analogs as a single injection. A combination of semaglutide with an amylin and calcitonin receptor agonist, cagrilintide, is currently in Phase 3 clinical development for obesity and T2D, and combinations of PYY with semaglutide or tirzepatide are being tested in early phase clinical studies. This talk will review key events leading to the development of peptide therapeutics for T2D and obesity, and provide an overview and perspective of current and future efforts that leverage naturally occurring peptide hormones for the treatment of obesity, T2D and related disorders.

Radionuclide Theranostics: Current State and Future Perspectives

Ken Herrmann, MD, MBA
Department Chair, Nuclear Medicine
Universitätsklinikum Essen, Germany

Radionuclide theranostics involves radioactively labelled molecules binding to tumor overexpressed targets. The two recent FDA approvals of Lutathera (targeting somatostatin 2 receptor) and Pluvicto (targeting the prostate specific membrane antigen) triggered a wide interest in radionuclide theranostics. This talk will give a short introduction to the field of radionuclide theranostics, summarize currently approved therapies and discuss new radionuclide theranostic concepts in clinical translation. Whereas radionuclide theranostics may not only involve peptides and small molecules they have been so far the most successful.

Pegcetacoplan: the CMC Journey through Development to FDA Approval

Christopher P. Holmes, Ph.D.
VP of Chemical and Pharmaceutical Development
Apellis

Inhibition of Complement proteins in humans, once believed to be undruggable targets and off-limits to medicinal chemistry efforts, has turned out to be a rich source of new drugs spanning the range of injectables, to long-acting oligonucleotides, to orally-available small molecules. Pegcetacoplan is a synthetic, injectable PEGylated peptide designed to inhibit Complement protein C3 and C3b. Highlights of the development of pegcetacoplan will be presented from a CMC perspective and will include some of the challenges we experienced along the way. A brief review of some of the pivotal clinical trial results will be summarized that led to the approval of pegcetacoplan as both EMPAVELI™ for the treatment of PNH in 2021 and as SYFOVRE™ for the treatment of Geographic Atrophy in 2023.

Advances in Oligonucleotide Delivery to Muscle Using Antibody Oligonucleotide Conjugates (AOCs): Transferrin Receptor 1 (TfR1) Mediated Uptake of AOCs for the Treatment of Muscular Dystrophies

Arthur A. Levin, Ph.D.
Avidity Board Member
Distinguished Scientist & Strategic Leader
Avidity Biosciences, San Diego, CA

The promise of oligonucleotide therapeutics is to use Watson-Crick-Franklin base-pairing rules to design drugs directly based on genomic information. Until recently, that promise has remained elusive because of cell barriers to oligonucleotide uptake. Receptor-mediated uptake via bioconjugation oligonucleotides has changed that. GalNAc conjugated oligos bind to the ASGPR on hepatocytes and are internalized. AOCs utilize monoclonal antibodies to cell surface proteins which are internalized in order to facilitate the functional delivery of oligonucleotide therapeutics into a broad range of cell and tissue types. AOCs combine the selective binding of antibodies and the specificity of oligonucleotides and facilitate receptor-mediated uptake in cells such as muscle. Oligonucleotides conjugated to monoclonal antibodies to TfR1 demonstrate disease-modifying activity in skeletal and cardiac muscle. Non-clinical pharmacology and toxicology data support the advancement of AOCs from the lab to the clinical setting and presently there are AOCs in clinical trials for myotonic dystrophy type 1, facioscapulohumeral dystrophy and Duchene muscular dystrophy. The molecular pharmacology, pharmacokinetics and safety profile of AOCs will be presented including recent clinical proof of concept for the technology.

Safe and Effective Delivery of Nucleic Acids Using Proteolipid Vehicles Formulated with Fusion-Associated Small Transmembrane Proteins

John D. Lewis, Ph.D.
Founder, CEO
Entos Pharmaceuticals

Genetic medicines hold great promise to treat a wide array of diseases, yet success in the clinic has been hindered by limitations in the tolerability, scalability, and immunogenicity of current delivery platforms. We sought to overcome these limitations by combining aspects from viral and non-viral platforms to develop a proteolipid vehicle (PLV) that incorporates fusion-associated small transmembrane (FAST) proteins from fusogenic orthoreoviruses into a well-tolerated lipid formulation using a scalable microfluidic mixing approach. We screened a library of FAST recombinants to identify a chimeric FAST protein with enhanced membrane fusion activity. A series of lipid formulations incorporating the chimeric FAST protein were optimized for high nucleic acid encapsulation, charge neutralization, and improved tolerability in vitro and in vivo. FAST-PLVs administered systemically in mouse and non-human primate models demonstrated broad biodistribution and significantly improved intracellular delivery and expression of messenger RNA (mRNA) and plasmid DNA (pDNA). At high local or systemic doses FAST-PLVs showed low immunogenicity and maintained activity upon repeat dosing over extended periods. We utilized FAST-PLVs to deliver a pDNA follistatin gene therapy in vivo that increased circulating levels of follistatin, resulting in significantly increased muscle mass and grip strength. The activity and safety profile of FAST-PLVs make them a promising platform for redosable gene therapies and genetic medicines.

Neuregulin 4 (NRG4) as a New Hormonal Therapy for NASH-related Liver Cancer

Jiandie Lin, Ph.D.
Bradley M. Patten Collegiate Professor of Life Sciences
Professor of Cell & Developmental Biology
Research Professor in the Life Sciences Institute
University of Michigan

Hormones released by peripheral metabolic tissues play an important role in the regulation of nutrient metabolism, energy homeostasis, and disease pathogenesis. Neuregulin 4 (NRG4) is a small peptide hormone secreted primarily by adipose tissue and acts on the ERBB3/4 receptor tyrosine kinases. Adipose NRG4 expression is downregulated in mouse and human obesity. Moreover, reduced plasma NRG4 levels have been linked to fatty liver disease in humans. The causative role of NRG4 in metabolic disease has been demonstrated by gain- and loss-of-function mouse studies. Genetic ablation of NRG4 signaling sensitizes mice to diet-induced metabolic disorders, including insulin resistance and NASH, whereas transgenic restoration of NRG4 expression in adipose tissue exerts protective effects. Beyond its beneficial effects on metabolic parameters, NRG4 signaling serves as a hormonal checkpoint that restrains the tumor-prone liver immune microenvironment in NASH and suppresses the development of NASH-associated liver cancer. In a therapeutic setting, recombinant NRG4-Fc fusion protein exhibited remarkable potency in suppressing HCC and prolonged survival in treated mice. These findings pave the way for therapeutic intervention of liver cancer by targeting the NRG4 hormonal checkpoint.

Establishing a Novel Platform for Discovery of CPPs that Localize in Cytoplasm

Jinsha Liu, Ph.D.
Advisor
Eli Lilly and Company

Two main challenges, targeted and cytosolic delivery, have hindered development of small interference RNA (siRNA) in the clinic. To overcome both, we have established a novel platform based on phage display, called NNJA Platform. In this approach, a lysosomal cathepsin substrate is engineered within the flexible loops of minor coat protein, PIII, which displays a unique random sequence at its N-terminus. NNJA peptides (on phage) that bind to an internalizing cell expressed proteins should localize to the cytoplasm. That is because phage internalization and subsequent localization to lysosome will result in cleavage of PIII, rendering phage non-infective. Such phage will be eliminated from the selected pool and only peptide-phage that escapes lysosomes will advance to the next round. Here, we describe proof of concept studies that include selection of NNJA library against three different cell types. Cytosolic localization of NNJA peptides on phage was confirmed using confocal microscopy. More importantly, conjugation of siHPRT to monomeric or multimeric NNJA peptides resulted in significant reduction in HPRT mRNA levels in various cell types without any cytotoxicity. Application of NNJA platform is now being extended for cytosolic delivery of additional therapeutic cargos.

Amylin: State of the Union

Thomas Lutz, Ph.D.
Professor in Veterinary Physiology
Institute of Veterinary Physiology
Vetsuisse Faculty
University of Zurich

The mature 37 amino acid peptide amylin is derived from the IAPP (islet amyloid polypeptide) gene and is produced in pancreatic beta-cells and – in much lower amounts – in other tissues, like the stomach, spinal ganglia and in the brain. Amylin is characterized by an interesting dichotomy because amylin has to propensity to aggregate into fibrils in some species, but on the other hand also to the physiological control of metabolism (probably in all species). These dichotomous roles seem to be "functionally independent" in that some amylin forms (e.g., human, feline) aggregate into oligomers and eventually mature amyloid fibrils; this process is probably independent of the cell membrane bound amylin receptor (AMY) because aggregation is initiated intracellularly. On the other hand, the soluble monomeric form of mature amylin activates the AMY in the brain to produce hormonal effects on glucose metabolism (inhibition of glucagon secretion, control of gastric emptying) and nutrient intake (induction of satiation), hence beneficial weight-lowering and anti-diabetic effects. The AMY is a specific heterodimer receptor that consists of the calcitonin receptor core protein (CTR) and one of several receptor activity modifying proteins (RAMP). The mechanisms underlying amylin's metabolic effects and the responsible AMY subtypes have been amply investigated in recent years and are the basis for the development of amylin agonists that are already in clinical use for the treatment of diabetes mellitus or that are in clinical development as weight lowering drugs, respectively. Similarities and differences between amylin and its receptor agonists will be discussed.

The Interplay of Insulin and Glucagon: From Physiology to Pharmacology

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Advance Therapeutics
UT Health San Antonio TX

The physiological roles of the islet hormones, insulin and glucagon, have been well described over the last century. The glucose lowering property of insulin has been leveraged as now a cornerstone treatment of the many millions of subjects affected with diabetes around the world. The opposing glucose elevating property of glucagon has now become a standard as a rescue treatment for emergent hypoglycemia, ironically caused, in the majority of subjects, by pharmacological use of insulin. Despite the extraordinary life-saving attribute of insulin to treat excessive hyperglycemia, its narrow therapeutic window and non-physiological mode of administration often results in problematic hypoglycemia and this is a major limitation of insulin as a standard treatment for the average patient with diabetes. The majority of insulin-treated subjects are unable to regulate glycemia to near-normal levels through insulin use because of fear of hypoglycemia. There are numerous approaches being explored to better refine the glucoregulatory properties of insulin and leveraging the opposing actions of glucagon as a companion pharmacology is one such approach. Over the last decade or more, there has been a stronger appreciation of the wider metabolic actions of both insulin and glucagon beyond their opposing actions on ambient glycemia: effects are being described on lipid and protein metabolism, energy expenditure and body weight control. There has also been an emerging understanding of a more nuanced relationship between the two sister islet cells, the beta cell and alpha cell, and how that influences the interplay of their main secretory products, insulin and glucagon respectively. One such observation has elucidated that, during the

diurnal secretion of the two hormones from the pancreatic islets, they operate under glycemia-dependent conditions, meaning insulin dominates glucagon action when plasma glucose is on the rise and the opposite occurs when plasma glucose falls. This relationship is being explored to develop a novel pharmaceutical approach that may allow a more refined modality to manage the excessive fluctuations in glycemia observed in subjects with diabetes, with the companion aspiration that this approach may confer a broader metabolic benefit.

Computational Design of Novel Peptides for SORT1 and Their Use in Oncology

Lucas Siow
Co-Founder and CEO
ProteinQure Inc.

Sortilin (SORT1) is a member of the vacuolar protein sorting 10 protein (Vps10p) family that functions as a receptor regulating peptide and protein trafficking between the plasma membrane, lysosomes, and trans-golgi network. As a cell surface receptor, SORT1 is able to mediate efficient endocytosis of extracellular ligands to the lysosomal compartment. Numerous reports have identified enriched SORT1 expression in a variety of tumor types, including triple-negative breast cancer (TNBC), a subtype of breast cancer associated with aggressive clinical behavior and poor disease outcomes. We sought to exploit SORT1-dependent internalization of peptides as a platform for rapid and specific chemotherapy delivery into TNBC cells. Using PQStudio (our proprietary computation-enabled design capabilities), we generated high affinity SORT1 targeting peptides that exhibit efficient receptor dependent internalization and lysosomal localization. Alternative computational approaches such as AlphaFold2 failed to recapitulate the peptide design. Peptide drug conjugates (PDCs) were generated via a linkage strategy that combines our designed peptides to the antimitotic agent monomethyl auristatin E (MMAE). Our PDC molecules exhibit potent tumor regression in a MDA-MB-231 TNBC cell derived xenograft model, thereby highlighting the potential of SORT1-engaging PDCs as an efficacious targeted chemotherapeutic delivery strategy.

Stealth Editing™ - A Novel Non-Immunogenic In Vivo Gene Editing Technology

Dietrich A. Stephan, Ph.D.
Founder & CEO
NeuBase Therapeutics

NeuBase Therapeutics is a biotechnology company developing Stealth Editors™ to perform in vivo gene editing without triggering the immune system. The biotech company is at the forefront of a new wave of gene editing, engineering an approach predicated on synthetic peptide-nucleic acid (PNA) chemistry to directly "drug the genome" and address disease at its base level. Most diseases are undruggable with biologic or small molecule approaches, leaving millions of patients with limited options. Precision genetic medicines represent a new class of therapies that target the DNA and RNA mutations that drive disease. Nearly all diseases are caused by genetic changes, underscoring the critical importance of new medicines that can "drug the genome" for the future of health. Unlike other editing technologies on the market, the company's editing mechanism doesn't use the CRISPR/Cas9 bacterial protein and edits genes using a nuclease-free approach. NeuBase believes its technology can potentially address up to ~90% of all known human mutations, which could reshape the gene editing landscape in the coming years.

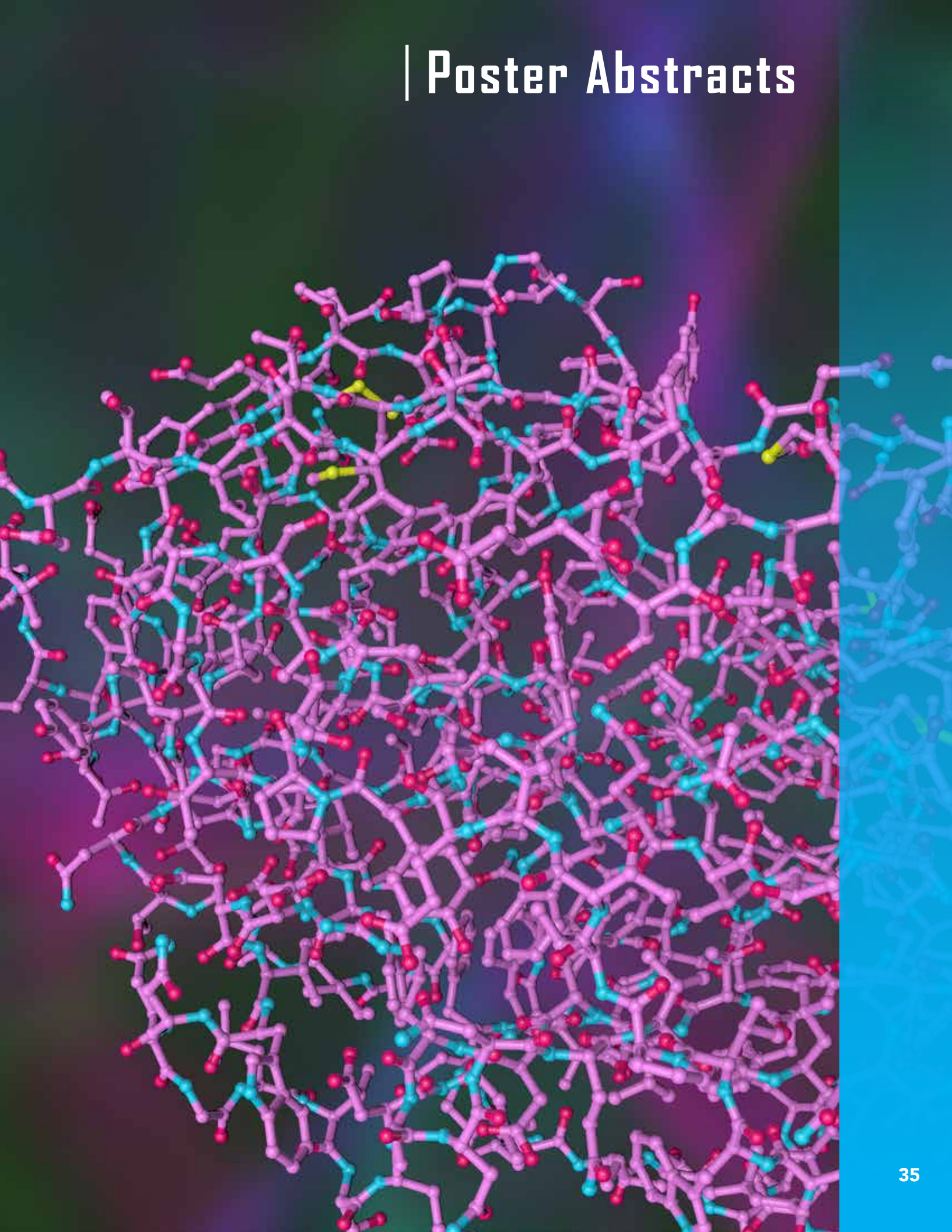
Development of Efruxifermin, an FGF21 Analog, for Treatment of Nonalcoholic Steatohepatitis (NASH)

Jonathan M. Young, Ph.D., J.D.
Co-Founder and Chief Operating Officer
Akero Therapeutics, Inc.

Fibroblast Growth Factor 21 (FGF21) alleviates cellular stress and regulates whole-body metabolism by exerting both paracrine and endocrine effects through activation of a co-receptor complex of β-klotho and one of its cognate FGF receptors (FGFRs), FGFR1c, FGFR2c or FGFR3c. Early preclinical data showed considerable promise for FGF21 in treating numerous diseases, including Type 2 diabetes mellitus (T2D) and nonalcoholic steatohepatitis (NASH). Translating this potential into a therapeutic has required extending the half-life of endogenous FGF21, which is estimated to be 1-2 hours. Clinical studies with several FGF21 analogs employing distinct half-life extension approaches failed to deliver expected efficacy and safety profiles in humans, despite showing substantial improvements in lipoproteins and on some other endpoints. Efruxifermin (EFX) is a fusion protein comprised of a human IgG1 Fc domain linked to a pair of human FGF21 moieties, each of which features three natural amino acid substitutions to prevent degradation and aggregation, resulting in balanced agonism of all three FGF receptors and a half-life of about 3 days. Results from the Phase 2a BALANCED, Phase 2b HARMONY, and Phase 2b SYMMETRY studies evaluating EFX for the treatment of pre-cirrhotic NASH (fibrosis stage 1 to 3) and cirrhosis due to NASH (fibrosis stage 4, compensated) point to a potentially differentiated clinical profile for EFX. Key observations include rapid fibrosis regression and NASH resolution as well as improvements in non-invasive markers of liver injury and fibrosis, glycemic control, lipoproteins, and body weight, and a favorable safety and tolerability profile. EFX's unique potential to be an important NASH therapeutic, if approved, will be presented. Attention will be given to the broader NASH development landscape, with a particular focus on glucagon-like peptide-1 (GLP-1) receptor agonists and dual/triple agonists of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) and/or glucagon.

Notes

Poster Abstracts



P01 | Novel Thiocarbazate Building Blocks: A Universal Synthetic Platform for Azapeptide Production

Ahmad Altiti, Mingzhu He, Kai Fan Cheng, Sonya VanPatten, Umair Ahmed, Ibrahim T Mughrabi, Stavros Zanos, Yousef Al-Abed
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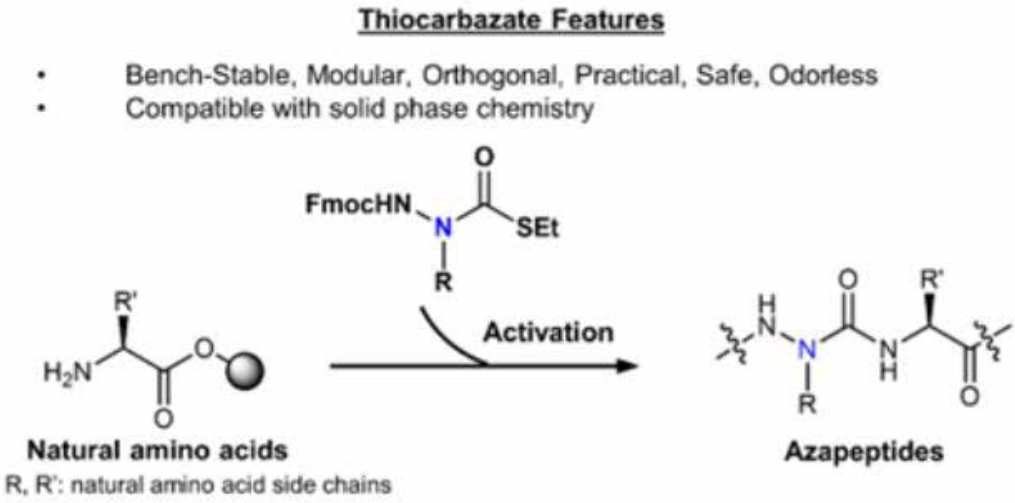
Peptides, polymers of amino acids, have a growing role as therapeutics. Their rapid degradation by proteases, however, is an inherent limitation and chemical modifications to native peptides have been used to overcome this weakness. In this work1, we describe a functionalized thiocarbazate scaffold as a novel surrogate of natural amino acids, that, once activated, can replace selected amino acid(s) in a peptide sequence using standard peptide synthetic methods and machinery. This methodology facilitates custom editing (replacing targeted amino acid(s) with aza-surrogates) of peptides, and has potential to mitigate protease sensitivity, thereby extending half-life/bioavailability while at the same time typically preserving structural features and biological activities. The convenience of this original azapeptide synthesis platform is demonstrated in several bioactive peptides. This novel, bench-stable thiocarbazate-based synthetic platform offers a robust and universal approach to optimize both new and existing peptide-based therapeutics.



1. Altiti, A.; He, M.; VanPatten, S.; Cheng, K. F.; Ahmed, U.; Chiu, P. Y.; Mughrabi, I. T.; Jabari, B. A.; Burch, R. M.; Manogue, K. R.; Tracey, K. J.; Diamond, B.; Metz, C. N.; Yang, H.; Hudson, L. K.; Zanos, S.; Son, M.; Sherry, B.; Coleman, T. R.; Al-Abed, Y., Thiocarbazate building blocks enable the construction of azapeptides for rapid development of therapeutic candidates. Nature Communications 2022, 13 (1), 7127.

P02 | CorTS 1 Derived Antibacterial Peptides: A Promising Therapy for Bacterial Keratitis

Aditi Arora[1], **Sushmita G Shah**[2] **Archana Chugh**[*1]
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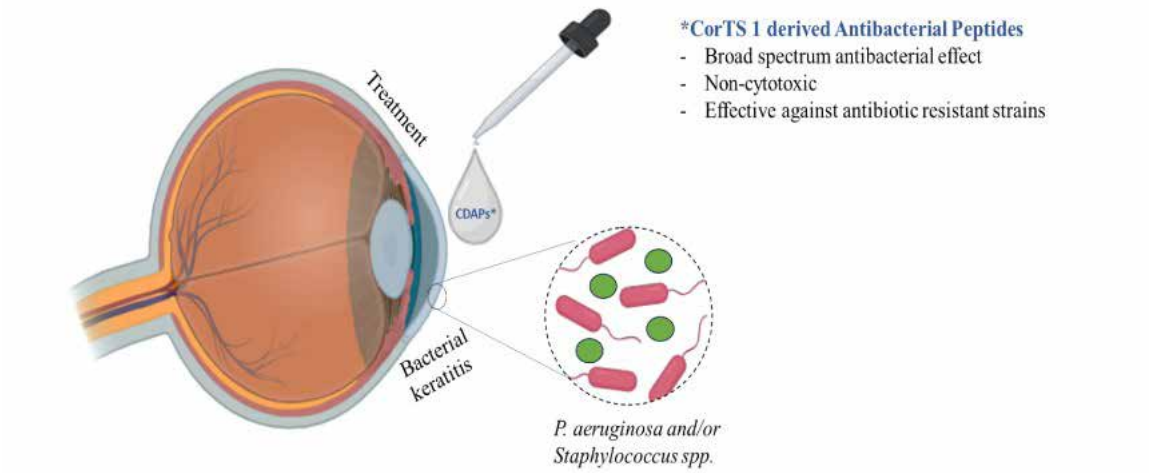
Bacterial keratitis (BK) is one of the leading causes of visual impairment and corneal blindness. *Pseudomonas aeruginosa* and *Staphylococcus spp.* are the predominantly reported etiological agents for BK. Significant resistance to classical antibiotics has been reported in these ocular pathogens. Antimicrobial peptides are emerging as a suitable alternative for treatment of such drug resistant infections. Previously, our group has reported a novel corneal targeting membrane active peptide CorTS 1 (Corneal Targeting Sequence 1) for efficient ocular therapy. It demonstrated potent antibacterial effect against methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. However, antibacterial activity of CorTS 1 against gram negative pathogens was found to be very weak. In the present study, derivatives of CorTS 1 have been designed with the aim of enhancing the potency and spectrum of antibacterial activity of the peptide. Among the designed peptides, CorTS 1 Derived Antibacterial Peptide 2 (CDAP-2), a highly α -helical peptide, showed remarkable bactericidal activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus, S. epidermidis*, MRSA and *Pseudomonas aeruginosa* with no cytotoxicity to human corneal epithelial cells. Additionally, we are investigating the anti-biofilm effect and mechanism of action of CDAPs on *P. aeruginosa.*

Keywords: Bacterial keratitis, ocular targeting, antimicrobial action, CorTS 1



1. Shankar, S., Shah, S.G., Yadav, S. and Chugh, A., 2021. Novel corneal targeting cell penetrating peptide as an efficient nanocarrier with an effective antimicrobial activity. European Journal of Pharmaceutics and Biopharmaceutics, 166, pp.216-226 (patent application no.: 202111002997);
*Amino acid sequences of CDAPs are under patent

P03 | Towards New Amanitin Analogs: Accessing (2S,3R,4R)-Dihydroxy-isoleucine and its Analogs for Incorporation into α-Amanitin

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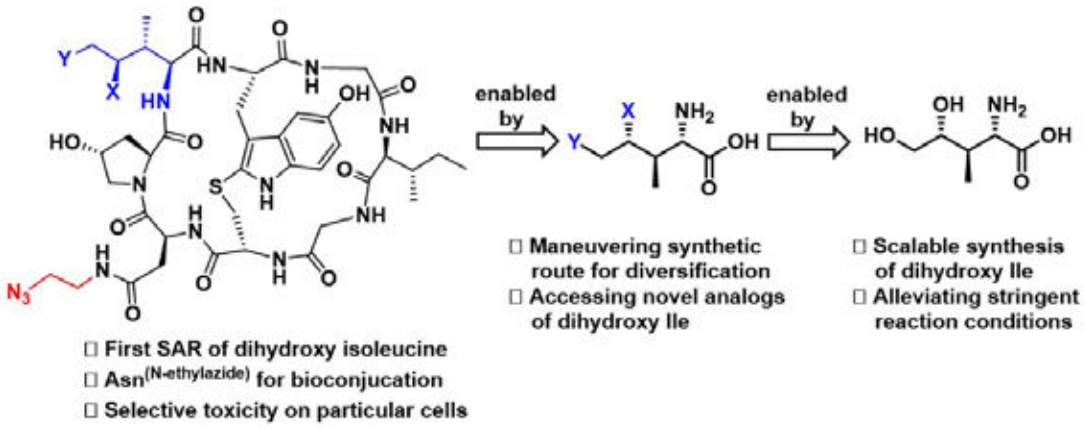
α -Amanitin is an extremely toxic bicyclic octapeptide extracted from the death-cap mushroom, Amanita phalloides. It is an allosteric, selective, and potent inhibitor of RNA polymerase II, the enzyme indispensable for cellular function and homeostasis. With the appealing properties of stability, potency, and a unique mechanism of action, α-amanitin has shown formidable promise as a payload in antibody–drug conjugates for cancer treatment. α-Amanitin includes numerous synthetic hurdles with di-hydroxy isoleucine being one of the prominent synthetic challenges. It is a chiral, oxidized non-canonical amino acid indispensable for toxicity. It is a high functional density molecule containing a carboxylic acid, an amine, diols, and β-branched methyl on a five-carbon linear main chain, thereby making the synthesis extensive and arduous. Herein we address the intricacy and challenges of synthesizing di-hydroxy isoleucine in gram-scale quantities, with a rationale of truncating synthetic complexity. We have synthesized di-hydroxy isoleucine, thereby enhancing the application of α-amanitin in cancer therapy. Furthermore, assimilating the synthetic route gave us access to 10 novel analogs. All of them were incorporated in the amanitin core and subsequently, we developed a structure-activity relationship (SAR) study around di-hydroxy isoleucine which is distinctive and the first of its kind. The cytotoxicity of new amanitin analogs was investigated on four different cell lines exhibiting encouraging results for further potential applications.



P04 | Quantitative Proteomics, Immunopeptidomics and PRMs Assays Enabled the Discovery and Validation of Peptide (neo)epitopes in the NASH Liver of Mice Fed High Fat Diet

Cristina C Clement Ph.D.[1], Rajesh K. Soni[2] and Laura Santambrogio[1] MD Ph.D.
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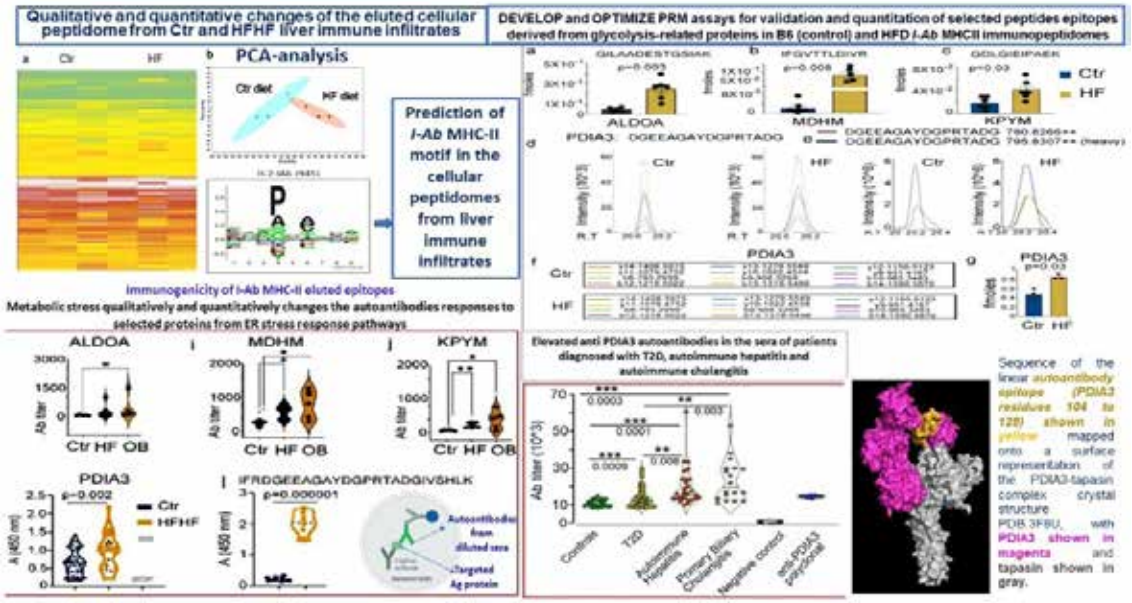
The redox environment-driven lipotoxicity and glucotoxicity induce tissue damage and wires an MHC-class-II (I-Ab) ligandome displayed by dendritic cells (DC) which is enriched in peptides (neo) epitopes derived from proteins involved in cellular metabolism, oxidative phosphorylation, and responses to oxidative stress. To reveal the mechanisms underlying the metabolic redox stress induced changes in the MHC-II ligandome and to further assess the potential immunogenicity of the new displayed epitopes we developed proteomics and immunopeptidomic profiling platforms consisting of a combination of DIA nano LC/MS analysis with the search against the DDA spectral libraries containing the proteomics and peptidomics data from DCs harvested from the C57Bl6 mice undergoing normal or HF/HF diet and Ob/Ob mice. In addition, we purified the immune cells infiltrates in the NASH liver of mice fed a normal and HF/HF diet and extracted their total cellular peptidomes (MW<10kDa) using a mild acid (MA) extraction method. The DIA/DDA analysis of the cellular peptidomes lead to the identification of epitopes derived from the glycolytic pathway (such as MDH, ALDOA, PKM) and ER-stress response, such as PDIA3. A peptide epitope of the PDIA3 protein (DGEEAGAYDGPRTADG) was selected for synthesis and absolute quantification in the total I-Ab eluted immunopeptidomes from mice kept on different diet regime using parallel-reaction monitoring (PRM) and stable-isotype labeling. Additionally, we performed ELISA assays against a linear B cell PDIA3 epitope (IFRDGEEAGAYDGPRTADGIVSHLK) on sera collected from mice fed an HFHF versus a control diet and quantified increased autoantibodies toward PDIA3 protein in the former over the latter experimental conditions.



P05 | Peptides Derived from the RRWQWRMKKLG Sequence: Evaluation of Antibacterial Activity Against *Escherichia coli* and *Staphylococcus Aureus* ATCC Strains

Kelin Cuero-Amu[1], Zuly Jenny Rivera[1,2] y Javier Eduardo García[1,3]
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The high incidence of infections and resistant bacteria is a threat to global public health. Lactoferrin and its derivative bovine lactoferricin are antimicrobial peptides (PAMs) which stand out due to their antimicrobial, anticancer and immunomodulatory action. In this study, the antibacterial effect of synthetic peptides derived from the LfcinB (20-30) sequence: RRWQWRMKKLG was evaluated against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 strains. It was found that ^{26}[F]-LfcinB (20-27)$_2$, LfcinB (20-25)$_2$, ^{2}[Nal]-LfcinB (20-30) and ^{26}[F]-LfcinB (20-30)$_2$ presented minimal inhibitory concentrations (MICs) of 9, 11, 15 and 15 µM respectively, against *E. coli* ATCC 25922 and low hemolytic activity. The molecules ^{26}[Nal]-LfcinB (20-30)$_2$ and ^{26}[Bpa]-LfcinB (20-30)$_2$ presented a MIC of 14 µM against *S. aureus* ATCC 29213, however, the high hemolytic activity found infers low selectivity and a possible cytotoxic effect by these peptides. Additionally, the effect of these peptides was evaluated against clinical isolates finding MICs of 9 and 14 uM for *E. coli* and *S. aureus* respectively.

Keywords: antimicrobial peptide, *E. coli, S. aureus,* hemolytic effect

P06 | Better Stability Through Stapling

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Equillium, Inc. La Jolla, CA

EQ102 is a PEGylated peptide modeled off the D-helix of the IL-2 family of cytokines. It binds directly to CD132 and specifically inhibits the signaling of IL-15 and IL-21. Celiac Disease is a gluten-triggered autoimmune disease affecting the small intestine that is driven by IL-15 / IL-21 signaling. Since peptides are inherently labile in the gastrointestinal tract, we worked to improve proteolytic stability of EQ102 for oral drug delivery.

Hydrocarbon stapling is a recognized method to confer protease resistance to peptides. A 'staple walk' was performed to determine the optimal position of hydrocarbon staples that imparted resistance to proteases and maintained functional activity. After establishing the optimized positions of the hydrocarbon staples, the amino acids contributing to the binding of CD132 were optimized via *in silico* modelling. Utilizing a known crystal structure of IL-15 interacting with CD132, the residues responsible for the interaction were mutated to generate a list of candidate mutations to enhance binding.

The enhanced molecule is termed EQ302. It was administered to mice via oral gavage in a formulation designed to enhance permeability of the intestine. Harvested small intestine issue contained significant amounts of EQ302. To demonstrate efficacy of the pepptde, a cytokine challenge model was used in mice using human IL-15. Mice express endogenous Interferon-gamma (IFNγ) when dosed with human IL-15. Inhibiton of IFNγ expression was observed in mice dosed with EQ302 prior to cytokine challenge. This data illustrates that new characteristics can be added to a peptide improving its capability to be delivered orally.

P07 | Cyclic Peptides as Inhibitors to Thrombosis Initiation: A Comparison of Synthetic and Genetically Modified Expressed Peptide Scaffolds

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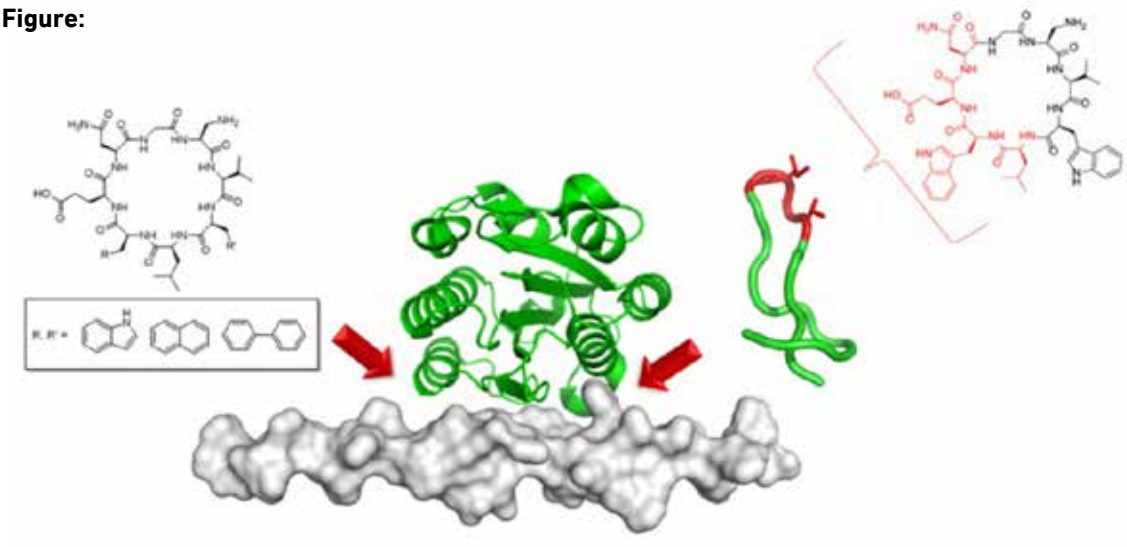
Peptides excel in their potential as safe, tolerable and efficacious therapeutics, especially when arranged into stabilizing macrocycles. The interaction between blood protein von Willebrand Factor (vWF) and collagen is a potential target for peptide drug design. The binding of these proteins initiates thrombosis, to which cardiovascular events such as heart attacks and strokes attribute their devastating effects. We have previously targeted this interaction using our stable head-to-tail cyclized peptides with moderate potency from in vitro assays. Here we describe the application of our strategy in the development of new, more potent peptides. First, we explain changes in the composition of our synthetic cyclic peptides to include unnatural aromatic amino acids. Then, we discuss grafting key amino acids from our successful first generation cyclic peptides onto a lasso peptide scaffold. Lasso peptides are bacterial natural products with a threaded macrocycle structure and looped macrolactam ring created by an isopeptide bond between the N-terminus and an acidic side chain. We test the potency of each peptide using our fluorescence-linked immunosorbent assay, which uses anti-vWF antibodies to detect the quantity of vWF bound to collagen-coated plates in the presence of our peptides. We also test the stability of our peptides against a panel of proteases, thus emulating cellular conditions. Thus far, the changes to the amino acid sequence and their scaffold presentation have shown improvements to the in vitro efficacy of these peptides as inhibitors to the vWF-collagen interaction while maintaining proteolytic stability. Our studies inform future advances in active cyclic peptide drugs.

Figure:



P08 | Toward an All-in-One Automated Peptide Purification and Synthesis Solution

D. Gonzales[1], R. Zitterbart[2], M. Muthyala[2], S. Lüdtke[2], D. Sarma[2]

[1]Gyros Protein Technologies, Tucson, USA
[2]Gyros Protein Technologies, Teltow, Germany

Introduction

Rapid parallel production of high-quality synthetic peptide sequences by solid-phase peptide synthesis (SPPS) and subsequent purification is a challenging yet essential pursuit. Especially synthetic neoantigen peptides with medium to long lengths used in cancer immunotherapies need to be manufactured rapidly and with high purity. However, conventional batch-synthesis is slow and purification with sequential highpressure liquid chromatography (HPLC) offers only low throughput, leading to extended production times and potential product loss requiring repeated synthesis. Shorter and more reliable production timelines are essential to ensure the timely delivery of lifesaving personalized medicine to patients. This study presents a PurePep® solution to improve neoantigen production for cancer therapy. The integrated approach includes induction heating (IH) for rapid automated peptide synthesis followed by automated orthogonal PurePep® EasyClean (PEC) purification[1] on the PurePep® Chorus synthesizer.

Synthesis with induction heating (IH)

We chose a set of 17 neoantigen peptides (P01–P17) with varying hydrophobicity and length, including six sequences (P12–P17) from the literature that are severely challenging to synthesize and purify (Tab. 1).[2,3] Synthesis (0.1 mmol) steps included:

- Coupling: DIC/Oxyma 3 min at 90°C
- Capping: Ac_2O/Pyr. 2 min at 50°C
- Fmoc-deprotection: DMF/Pip 1 min at 90°C

*Tab. 1: Sequences of neoantigen peptides used in this study. P12–P17 were reported to be particular difficult to be synthesized and purified. *synthesis performed at room temperature.*

ID	Sequence²	Source	Length	C-Terminus
P01*	GWVKPIIGHHAVGDQYRAT	[2]	20	Amide
P02*	TLYEQEIEV	[2]	9	Amide
P03*	SLLNQPKAV	[2]	9	Amide
P04*	ALAVLSNYDA	[2]	10	Amide
P05*	VTEKNGYYF	[2]	10	Amide
P06*	MQENNTPGTYLLSVSARD	[2]	19	Amide
P07	ETPTPTIVHPGSLPLHLG	[2]	19	Amide
P08	ENLKQNDISAEFTYQTKDA	[2]	19	Amide
P09	NYNDRYDEI	[2]	9	Amide
P10	TYPPLLVAF	[2]	10	Amide
P11	EIGWSYTGALNQKN	[2]	14	Amide
P12	TLQIRGRERFEMYRELNEALELK	[3]	25	Acid
P13	DWGGQHHGLREVLAAALPASCLWGA	[3]	25	Acid
P14	YSLDSSGNQNLYAMYQLSHFQSISYL	[3]	23	Acid
P15	TMLVSSLRDHFPDLPLHHTHDTS	[3]	28	Acid
P16	SNLDITPDCPRWRAWWGGFLCGA	[3]	24	Acid
P17	FTLQIRGRERFEMYRELNEALELKD	[3]	25	Acid

Automated PEC purification

- PEC-Linker coupling with Oxyma/DMF for 15 min at 60°C
- TFA cleavage at RT for 2 h (83:5:5:5:2 TFA/H2O/PhSH/EDT/TIS)
- PEC purification at RT with one-size-fits-all protocol for 5.2h

Tab. 2: Gained amounts and UV_{214nm} purities after synthesis and after PEC purification of all analyzed neoantigen peptides.

ID	Crude yield / mg	Crude purity / %	Yield after PEC / mg	PEC-grade purity / %
P01*	218	87	65	85
P02*	118	99	33	92
P03*	138	87	52	96
P04*	141	95	48	85
P05*	115	95	37	96
P06*	135	38	12	80
P07	113	80	68	85
P08	98	22	38	70
P09	127	48	47	79
P10	54	99	28	96
P11	124	58	47	88
P12	130	92	35	89
P13	41	52	15	80
P14	48	10	19	72
P15	76	57	37	74
P16	59	47	23	83
P17	97	18	22	79
Mean	108	60	37	84

Polish with HPLC

- Knauer Semi-prep. HPLC, C18 column 15 x 2 cm column
- 2 mL injection loop, 10–60% MeCN in 30 min

Tab. 3: Reported and gained UV_{214nm} purities (in %) after using Induction Heating-SPPS (IH), IH + PEC, IH + HPLC and IH + PEC + HPLC. Green indicate higher purities to heated Flow-SPPS + HPLC.[3]

ID	Batch-SPPS + HPLC[3] / %	Flow-SPPS + HPLC[3] / %	IH-SPPS crude / %	IH-SPPS + HPLC / %	IH-SPPS + PEC / %	IH-SPPS + PEC + HPLC / %
P12	failed	92	65	87	89	94
P13	failed	89	59	80	80	96
P14	failed	53	18	50	72	98
P15	93	99	18	79	74	93
P16	failed	45	46	72	83	94
P17	failed	58	60	72	78	94
Mean	-	69	44	73	79	94

Advantages of PurePep solutions

Fast synthesis on PurePep Chorus

- PurePep Chorus (Fig. 1 A) combines induction heated peptide synthesis (IH-SPPS), automated cleavage and purification in one instrument
- 17 min synthesis cycle time with induction heating (Fig. 1 B) resulting in 2.5 – 7.4 h total time per set of 5–6 peptides (incl. capping)
- IH-SPPS enabled the synthesis that failed using batch synthesis (Tab. 3; IH-SPPS crude)

Automated purification on PurePep Chorus:

- Orthogonal PEC purification with the PEC Auto Kit (Fig. 1 C), yielding superior PEC-grade mean purity than HPLC of 84% (Tab. 2).
- PEC method (Fig. 2) allows peptide dissolution in DMSO or HFIP for reliable processing from SPPS to purification.

- Automated purification of 6 peptides "ready to collect" (Fig. 1 D) in 5.2 h with only 36 minutes total hands-on time.

- HPLC polish after PEC is highly reliable (Tab. 3; IH + PEC + HPLC), yielding >90% final purity including reported difficult sequences.

Fig. 1: A: PurePep Chorus synthesizer. B: Induction heating of reaction vials. C: Content of the PEC Auto Kit. D: Set-up of RVs filled with 40 mL Agarose and collection of purified peptide in the collection vials on the left.

PurePep EasyClean (PEC) in detail

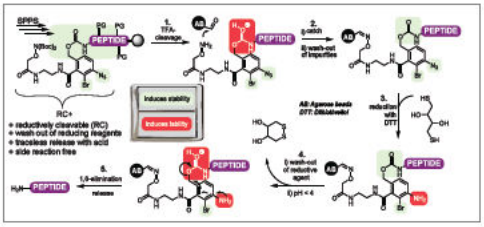


Fig. 2: Chemical steps of the PEC technology used in the PEC Auto Kit, utilizing the universal linker system and its reduction-triggered safety release.

References

1. R. Zitterbart et al. *Chem Sci* 2021, 12, 2389
2. N. Hilf et al. *Nature* 2019, 565, 240
3. N. L. Truex, B. Pentelute et al. *Sci Rep* 2020, 10, 723

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P09 | Myristic Acid-Trans-Activator of Transcription Dual Conjugation of Protein Kinase C Beta II Peptide Inhibitor is More Potent than Myristic Acid Conjugation in Attenuating Superoxide Release in Isolated Rat Polymorphonuclear Leukocytes

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Introduction
Protein kinase C beta II (PKCβII) activation promotes polymorphonuclear (PMN) superoxide (SO) production via NADPH oxidase (NOX-2). Previously, myristic acid conjugated PKCβII inhibitor (myr-PKCβII-) significantly attenuated phorbol 12-myristate 13-acetate (PMA)-induced PMN SO release compared to controls. We hypothesize that conjugation of myr combined with trans-activator of transcription (Tat; [myr-Tat- PKCβII-]) would enhance PMN SO release attenuation compared to myr- PKCβII-.

Methods:
We tested the effects of myr-Tat-PKCβII-; N-myr-Tat-CC-SLNPEWNET) on PMN SO release compared to myr-PKCβII-, scrambled myr-tat-PKCβII- (myr-Tat-PKCβII- scram), unconjugated PKCβII-, and 0.5% dimethyl sulfoxide (DMSO) vehicle control group. Rat PMNs were incubated for 15 min at 37oC with myr-PKCβII- (20μM) or myr-tat-PKCβII- (2μM, 5μM, 7.5μM, 10μM, 20μM), or myr-Tat-PKCβII-scram (2μM, 5μM, 7.5μM, 10μM, and 20μM). PMN SO release was calculated by the change in absorbance (550 nm) over 390 sec via ferricytochrome c reduction after PMA stimulation (100nM). Data were analyzed with ANOVA Fisher's PLSD post-hoc analysis.

Results:
Myr-Tat-PKCβII-: 5μM (n=15, 0.392±0.04), 7.5μM (n=11, 0.397±0.05), 10μM (n=8, 0.211±0.05,) and 20μM (n=6, 0.121±0.02) significantly attenuated PMN SO release compared to control (n=103, 0.496±0.02, all $p<0.05$), whereas myr-PKCβII- was only significant at 20μM (n=6, 0.303±0.02, $p<0.05$) compared to DMSO vehicle control. Intracellular delivery of myr-Tat-PKCβII-2μM (n=9, 0.436±0.06) and all concentrations of myr-Tat-PKCβII-scram were not significantly different from DMSO vehicle controls.

Conclusion:
Results suggest myr-Tat dual conjugation is superior to myr-conjugation alone at intracellular delivery of peptide cargo. Future studies will investigate the concentration-dependent effects of PKCβII translocation to membrane targets, using immunocytochemistry and western blot analysis.

P10 | O-Acyl Isopeptide Prodrugs of Teixobactin Derivatives

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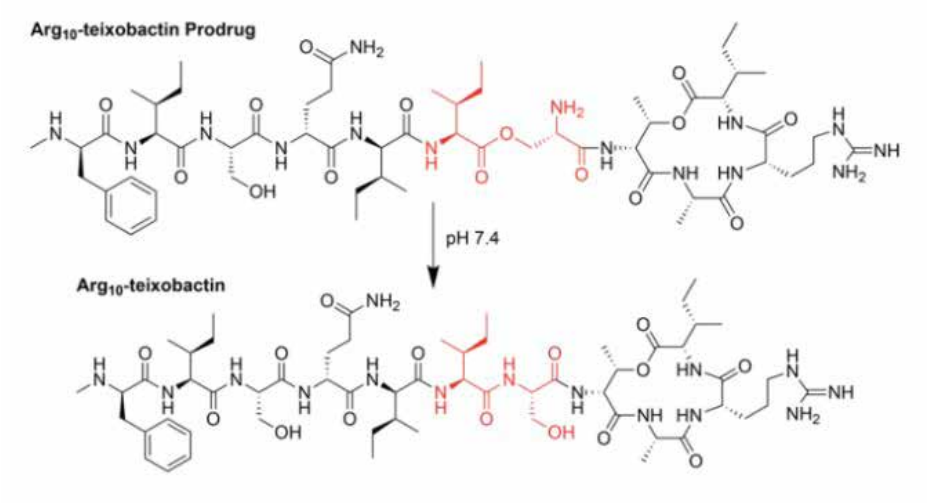
The antibiotic teixobactin is a promising drug candidate against drug-resistant pathogens, such as
MRSA and VRE, but forms insoluble gels that may limit intravenous administration. O-Acyl isopeptide prodrug analogues of teixobactin circumvent the problem of gel formation while retaining antibiotic activity. The teixobactin prodrug analogues contain ester linkages 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. Upon exposure to physiological pH, the prodrug analogues undergo clean conversion to the corresponding amides, with half-lives between 13 and 115 min, with a 3 to 5-fold increase at 37 °C. Prodrug analogues containing lysine, arginine, or leucine at position 10 exhibit good antibiotic activity against a variety of Gram-positive bacteria while exhibiting little or no cytotoxicity or hemolytic activity. A mouse thigh infection model against MRSA demonstrates *in vivo* efficacy. Because *O*-acyl isopeptide prodrug analogues of teixobactin exhibit clean conversion to the corresponding teixobactin analogues with reduced propensity to form gels, it is anticipated that teixobactin prodrugs will be superior to teixobactin as drug candidates.



References:
1. **Jones, C. R.;** Guaglianone, G.; Lai, G. H.; Nowick, J. S. Chem. Sci., **2022,** 13, 13110–13116.

P11 | Capability Enablement in Oral Peptide Drug Discovery: Design and Synthesis Approaches in Macrocyclic Peptide Programs Utilizing High Throughput Experimentation

Bing Li, Anilkumar Nair, Yuriy Slutskyy, Dennis Feng, Michael Lo, Christopher W. Plummer, Kuanchang Chen, Fa-Xiang Ding, Hubert Josien, Yan Guo, Jennifer Johnston, Sookhee Ha, Milana Maletic, Shawn Walsh, Subharekha Raghavan, Abbas Walji, Blair Wood
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Macrocyclic peptides attract much attention in modern drug discovery research as a versatile therapeutic modality because of their ability to target traditionally undruggable protein surface interactions. To date, the majority of the clinically approved cyclic peptides are derived from natural products. In the coming years, the position of cyclic peptides in the pharmaceutical industry is expected to outperform small molecules. Orally available and cell permeable peptides will be the focus, which would demand enormous capability enablement to design and synthesize de novo peptides in the desired property space. At Merck, in a modality agnostic drug discovery approach, we have established many industries leading capabilities for rapid progression of SAR and lead optimization in cyclic peptide therapeutics. This poster will discuss late-stage functionalization strategies on fully elaborated cyclic peptides, thereby allowing rapid access to novel chemical space and accelerating SAR studies.

P12 | High Performance Machine Learning on Small Peptide Datasets

Lily Lindmeier, Ewa Lis
Koliber Biosciences, Inc.

The public release of DALL-E in 2021 and ChatGPT in 2023 has ushered in a new era in Artificial Intelligence enabling humans to work along AI to generate images and text. The use of these tools is fundamentally changing the way humans create by providing a variety of starting points, accelerating the process, and eliminating tedious tasks. The awe-inspiring success of ChatGPT is based on novel model architectures (transformers) as well as an unsupervised training approach with masked tokens that leverages vast unlabeled datasets. Similarly to natural language, transformers can be trained on amino acid sequence data enabling development of models that capture an evolutionary understanding of peptides and proteins.

This presentation demonstrates the progression of the Koliber AI peptide / protein platform towards minimizing dataset sizes required to train machine learning models. A suite of applications was explored including anti-microbial and immune-modulating peptides. The models were trained on a wide variety of peptide datasets including cyclic peptides and peptides with non-canonical amino acids. Examples are shown that demonstrate zero shot / de novo predictions of substitutions that enhance enzyme function via increase in activity and broadening of substrate specificity.

P13 | Implementation and Optimization of the Counterion Change of Peptides and Evaluation of the Effect of the Counterion on Biological Activity

Amalia Giselle Lopez-Sanchez[1], Kelin Johana Cuero-Amu[2], Andrea Carolina Barragan-Cardenas[2], Ricardo Fierro-Medina[1], Zuly Jenny Rivera-Monroy[1] and Javier Eduardo García-Castañeda[1]

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In the search for new more selective therapeutic agents with fewer side effects, antimicrobial peptides (AMPs) have been studied as a promising alternative. Among the most promising AMPs is Bovine Lactoferricin (LfcinB), whose sequence is in the N-terminal region of the Bovine Lactoferrin (BLF) protein. Synthetic peptides, monomeric and dimeric; LfcinB derivatives have shown antibacterial and antifungal activity and cytotoxic effect against different types of cancer.

One of the most widely used methodologies to obtain LfcinB analogues is solid phase peptide synthesis using the Fmoc/tBu (SPPS-Fmoc/tBu) strategy, in which the peptide is removed from the resin by adding acid trifluoroacetic acid (TFA) in excess, forming a salt that has trifluoroacetate as a counterion. The counterion of the peptides is a relevant factor since it can generate changes in the conformation and affect the activity of the peptides *in vitro* and *in vivo* studies; and the most accepted presentations for the manufacture of pharms are salts with acetate or hydrochloride as counterion.

To contribute to the future development of a broad-spectrum drug, in this work the exchange of the counterion trifluoroacetate for hydrochloride was implemented and optimized in two peptides with anticancer activity. These were characterized by FT-IR, RP-HPLC, LC-MS and NMR, evidencing that their chromatographic and spectroscopic properties are maintained. Additionally, it was found that the antibacterial and anticancer activity of the peptide was no affected.

P14 | Amphipathic Hybrid Cell Penetrating Peptide-Doxorubicin Conjugate as a Strategy to Overcome Multi-Drug Resistance and Decreased Cardiotoxicity

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Doxorubicin (DOX) is a potent anthracycline chemotherapeutic agent that effectively treats breast cancer, leukemia, and lymphoma malignancies. However, its clinical application is hindered by its inherent dose-limiting cardiotoxicity and acquired resistance. We have previously shown that peptides containing hydrophobic and positively charged residues are efficient molecular transporters of DOX. Herein, we report an amphipathic hybrid cell-penetrating peptide [R5W4] KRRWWRLWRRLWRβA. DOX was conjugated via a glutarate linker with the peptide to afford the peptide-DOX conjugate. Antiproliferative assays were performed on a panel of cancerous cell lines using the Peptide-DOX conjugate (PDC) and corresponding physical mixtures of the peptide and DOX to evaluate the effectiveness of the synthesized PDC compared to the free drug. [R_5W_4]K-RRWWRLWRRLWRβADOX conjugate demonstrated higher cytotoxicity against cancer cells at 4 μM and 8 μM than the free DOX at 5 μM. After 72 h incubation, the conjugate

inhibited ovarian adenocarcinoma (SK-OV-3) cell viability by 63% and breast cancer cell lines (MDAMB-231 and MCF-7) by 74% and 66%, respectively; in comparison to the free drug at 5 μM, the following inhibition percentages were observed: (SK-OV-3, 30%), (MDAMB-231,30%), and (MCF-7, 40%). Furthermore, the PDC showed significantly lower toxicity towards healthy myocardial cells (H9C2) with cell viability of 80% compared to 60% for the free DOX, while exhibiting higher activity against DOX-resistant cells (MES-SA/MX2) with only 13% cell viability compared to 84% for the free DOX treatment. Mechanistic studies revealed that direct translocation and clathrinmediated endocytosis are responsible for the transport of Doxorubicin.

P15 | The Role of Uncoupled Endothelial Nitric Oxide Synthase in Ischemia-Reperfusion Injury: Lessons Learned from Protein Kinase C Epsilon Peptide Modulation

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Introduction
Ischemia-reperfusion (I/R) injury is characterized by increased reactive oxygen species (ROS) during reperfusion. Uncoupled endothelial nitric oxide synthase (eNOS) is a major source of ROS in myocardial I/R, hindlimb I/R and cerebral I/R. During reperfusion, activation of protein kinase C epsilon (PKCε) enhances ROS via uncoupled eNOS due to an increased dihydrobiopterin/ tetrahydrobiopterin ratio. Inhibiting uncoupled eNOS using myristic acid conjugated-PKCε peptide inhibitor (N-Myr-EAVSLKPT [Myr-PKCε-]) attenuates ROS. This study examined the effects of Myr-PKCε- in kidney I/R. We hypothesized that Myr-PKCε- will exert renal-protective effects by attenuating PKCε translocation in kidney I/R compared to scrambled peptide (N-Myr-LSETKPAV [Myr-PKCε-scram]).

Methods
Renal pedicles of anesthetized male C57BL/6J mice (25–30g) were clamped bilaterally for 19 mins. One minute before unclamping, 1.6 mg/kg Myr-PKCε- (n=6) or Myr-PKCε-scram (n=7) was administered i.v. Glomerular filtration rate (GFR; μl/min) and serum creatinine (Cr; mg/dL) were measured at baseline, 24hrs, 72hrs, and 96hrs post-injury. Immunohistochemistry (IHC) staining of samples was used to detect cell membrane localization of PKCε using AperioImageScope. Data was analyzed using Student's t-test.

Results
Myr-PKCε- significantly improved GFR and reduced Cr throughout reperfusion compared to Myr-PKCε-scram (both, $p<0.05$). Myr-PKCε- restored final GFR (96hrs.) to 52% and Cr to 54% vs. Myr-PKCε-scram, that recovered to 29% (GFR) and 18% (Cr) of initial baseline (~222±15 μl/min and ~0.07±0.01 mg/dL), respectively. Myr-PKCε- (1.77x108±3.14x107) significantly decreased the number of positive signals in IHC compared to Myr-PKCε-scram (3.58x10-1±5.03x10-2) ($p<0.05$).

Conclusion
Results suggest Myr-PKCε- improved renal function following kidney I/R and attenuated PKCε localization in tubular epithelium compared to Myr-PKCε-scram.

P16 | A Novel Cell Permeable Protein Kinase C Beta II Peptide Inhibitor Elicits Potent Cardioprotective effects When Given during Reperfusion in Rat Ex-Vivo and Porcine In-Vivo Ischemia-Reperfusion Injury

James Ramsarran[1], Logan Clair[1], Juliet Melnik[1], Desmond Boakye Tanoh[2], Jennifer Dang[1], Taurai Augustin[1], Tameka Dean[1], Qian Chen[1], Robert Barsotti[1], and Lindon Young[1,2]

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Introduction

Myocardial reperfusion (R) injury is induced after blood flow restoration following myocardial ischemia (I). During R, protein kinase C beta II (PKCβII) induces reactive oxygen species production. Dual conjugation of PKCβII peptide inhibitor (PKCβII-) with myristic acid (myr) and trans-activator of transcription (Tat) (myr-Tat-CC-SLNPEWNET [myr-Tat-PKCβII-]) enhances intracellular delivery. We hypothesize 20ng/kg myr-Tat-PKCβII- (in vivo) and 100 pM (ex vivo) would exert cardioprotective effects in both myocardial I/R injury (MIR) models by reducing infarct size and improving post-reperfusion cardiac function compared to controls.

Methods

Ex vivo: We tested 100nM – 10fmol myr-Tat-PKCβII- in rat MIR. Isolated hearts from anesthetized male SD rats underwent global I (30-min)/R(50-min). Myr-Tat-PKCβII- given during first 5min of R. DP/dt max was measured and infarct size was determined. Data analyzed by ANOVA using Fisher's PLSD test.

In vivo: Regional I (1 hr)/R (3 hrs) induced in male Yorkshire pigs. At R, myr-Tat-PKCβII- or scrambled control peptide 20ng/kg was given by intra-arterial bolus. Ejection fraction (EF) was calculated and infarct size was determined. Data analyzed using Student's t-test.

Results

Ex-vivo: Myr-Tat-PKCβII- (100nM – 100fmol; 10-14±3%; n=3-6) significantly reduced infarct size compared to controls (21±3%; n=21; $p<0.05$). DP/dt max significantly improved (100pM-1pM; 961±274; n=5-6) compared to 100pM scrambled myr-Tat-PKCβII- (386±86, n=4; $p<0.05$).

In-vivo: Myr-Tat-PKCβII- 20ng/kg significantly restored EF to within 1.4 ± 0.7% of baseline and reduced infarct size to (10.0±2.8%; n=4) compared to scrambled myr-Tat-PKCβII- (6.4 ± 2.1%; 28.5±8.3%; n=6; $p<0.05$).

Conclusion

Results suggest utilization of 20ng/kg in future studies to evaluate infarct size and cardiac function in a 12-week porcine survival study.

P17 | Melanocortin Receptor 4 Agonist PL8905 in Combination with Glucagon-like Peptide 1 Produces Synergistic Weight Loss, Reduced Food Intake, and Greater Glucose Control in Diet-induced Obese Rats

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Palatin Technologies, Inc., Cranbury, NJ

Introduction
The melanocortin pathway regulates energy balance. Studies investigated the effect of PL8905, a novel selective melanocortin receptor 4 (MC4R) agonist in combination with glucagon-like peptide 1 (GLP-1) in diet-induced obese (DIO) rats.

Methods
Subcutaneous treatment with PL8905 0.3, 1, and 3 mg/kg alone and in combination with continuous infusion of GLP-1 1 mg/kg/day was investigated in DIO rats (n=100). Randomization/infusion pump implantation occurred on day 0. On days 5-9 animals were dosed with PL8905 BID, infused with GLP-1, or both. Body weight was measured on days 0–9; glucose levels on days −2 and 10.

Results
GLP-1 alone resulted in a slight increase in body weight at day 9 (normalized to day 5) in line with vehicle-treated rats. PL8905 alone produced significant declines of ~1.6%–3.4% ($P<0.01$ vs vehicle). PL8905 combined with GLP-1 produced greater declines of ~2.9%–5.1% ($P<0.01$). PL8905/GLP-1 groups showed significant ($P<0.01$) reduction of blood glucose levels compared to PL8905 doses alone when compared with baseline. There were trends but no significant glucose effect for PL8905 or GLP-1 alone. Feed intake in PL8905 groups and PL8905/GLP-1 combination groups decreased vs vehicle, although it recovered on day 9 for PL8905 groups.

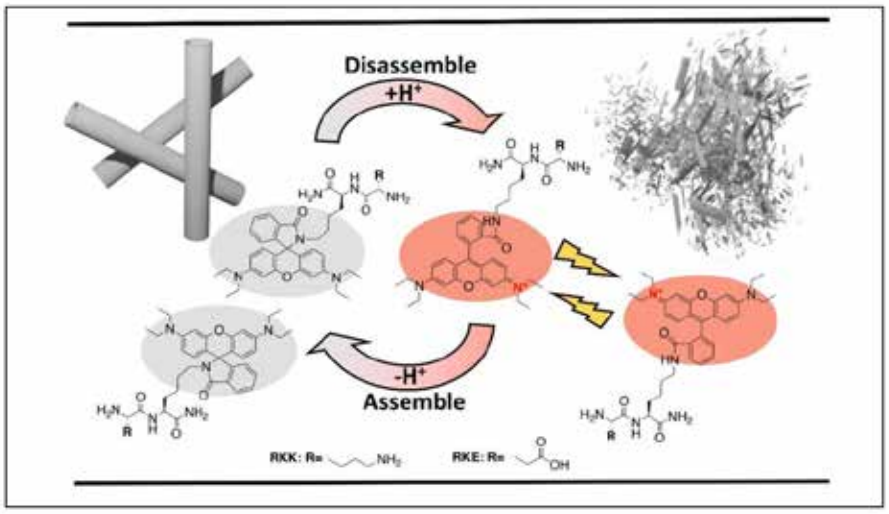
Conclusion
1 mg/kg/day GLP-1 had little effect on food intake, body weight, or blood glucose in DIO rats. However, when combined with PL8905, rats showed a significantly greater weight loss and glucose control than PL8905 monotherapy or vehicle. Combination treatment with MC4R agonists and GLP-1 agonists may be a more effective therapy for diabetes and obesity.

P18 | Exploring the Potential of Rhodamine-dipeptide Nanomaterials for Tracking the Assembly State within the Cell

Sagarika Taneja, Ziyuan Meng, Jon R. Parquette

Cancer nanotherapeutics have offered several advantages over traditional medicines in terms of better cellular uptake, selectivity, enhanced bioavailability, and higher therapeutic indices. One persisting challenge is to figure out the sub-cellular organelle at which the disassembly of nanomaterials occurs. Our lab has developed two Rhodamine based pH-responsive selfassembled probes, Rho-KK and Rho-KE (Rho=Rhodamine, K=lysine, E=glutamic acid). The objective is to validate if the developed probes can enter the HT-29 cells as self-assembled nanotubes. Rhodamine amide derivatives undergo acid/metal ion induced ring opening of its closed ring, non-fluorescent spirolactam form to open-ring, fluorescent amide form at low pH. Attachment of a β-sheet forming dipeptide motif enables the system to assemble into nanotubes. Rho-KK/Rho-KE can exist either in monomeric, open-ring state (λ_{max} 580 nm) at low pH (4.1/4.2) or in the assembled nanotubular state which exhibits aggregation induced fluorescence (λ_{max} 460 nm) at pH> (5.8/6.3). The structural changes as a function of pH have been validated using UVVis, CD, and fluorescence spectroscopy. Between pH (4.1-5.8/4.2-6.2) for Rho-KK/Rho-KE, the transition from fluorescent open-ring Rhodamine form to monomeric, non-fluorescent spirolactam form occurs. The hypothesis is that these probes upon entering the cancer cells would emit

fluorescence corresponding to their location within the cells as intracellular organelles have different pH values, from the early (pH 6.3) to the late endosome (pH 5.5) and the lysosome (pH 4.7). Relationship between pH and nanotube/monomer state of probes can be verified by recording the emitted wavelength and simultaneously visualizing their location within the cell using confocal microscopy.

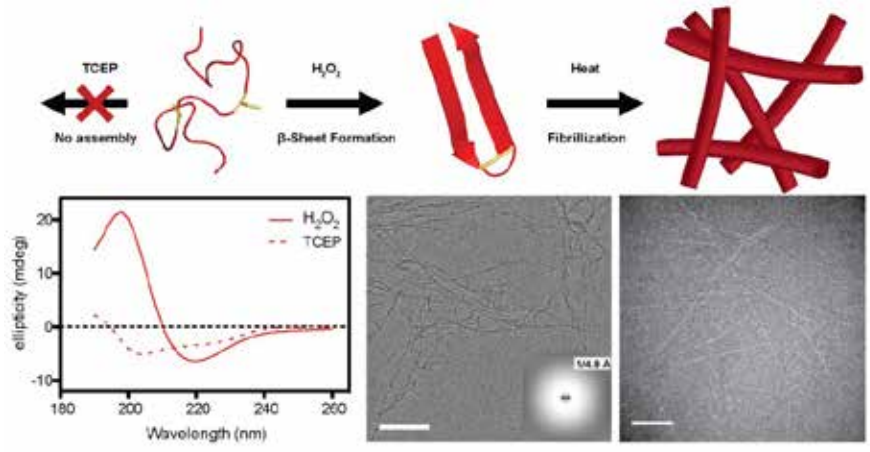


P19 | Sequence-Programmable Order-Disorder Transitions in Disulfide Stapled Supramolecular Peptide Nanofibers

Zachary Urbach, Naomy Marrufo, Khawla Mustafa, Quynh Theresa Huong Do, Jully Nguyen Le, Yuanming Song, Rafael Umeda, Ali Mohraz, Ruqian Wu, Doug Tobias, Zhibin Guan, Allon Hochbaum

University of California, Irvine, Irvine, CA 92697

Disulfide staples can alter molecular structure and toggle different protein conformations. It is important to understand how staples can affect the way proteins and peptide interact with inter- and intra-molecularly. Herein we designed a staple structure within a peptide that can gate a β-sheet that assembles into amyloid fibers. Under oxidative conditions the fibers form when the disulfide bridge is formed and under reducing conditions fibers do not form. We explore through kinetic fluorescence tests the evolution of these structures and suggest an auto-catalytic pathway for formation that is triggered by initial structure formation. This structure inducing auto-catalysis leads to rapid fiber formation and could give key insights into sequence dependent motifs that trigger such fibrillization behavior in other systems.



P20 | Supramolecular Assembly of a Macrocyclic β-hairpin Peptide Derived from Aβ in Aqueous Solution

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The Aβ peptide assembles into oligomers and fibrils that are central to the pathology of Alzheimer's disease (AD). Despite mounting evidence suggesting that oligomeric species are neurotoxic, there are few high-resolution models of Aβ oligomer assembly in aqueous solution. To address the need for oligomer models, the assembly of macrocyclic peptides containing amyloidogenic fragments from the central and Cterminal regions of Aβ was studied using solution phase NMR. Our laboratory previously reported the assembly of peptide 1a into cytotoxic hexamers. However, peptide 1a does not assemble into distinct oligomers that can be characterized by NMR. In this study, we mutated Phe20 to cyclohexylalanine (Cha) to stabilize peptide 1b oligomer formation and studied its supramolecular assembly. SDS-PAGE shows that peptide 1b assembles into hexamers. X-ray crystallography reveals that peptide 1b also crystallizes into hexamers that are almost identical to those observed from peptide 1a. The SDS-PAGE and crystal structure prove that the Cha mutation does not disrupt hexamer formation. Furthermore, peptide 1b adopts a β-hairpin conformation, is soluble at millimolar concentrations, and shows concentration dependent oligomerization. Surprisingly, peptide 1b oligomerizes into a putative dodecamer in solution phase NMR conditions. Characterizing an oligomer of peptides derived from Aβ in aqueous solution should provide additional insight into Aβ oligomer formation.

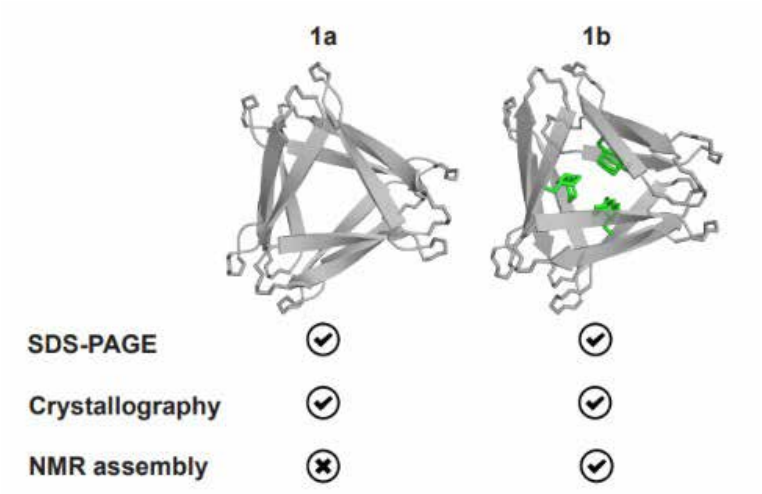

Figure 1.
Cartoon illustrations of peptide **1a** and **1b** hexamers. Mutated residues for peptide **1b** are shown in green.

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