Symposium Sponsors

PEPTIDE THERAPEUTICS FOUNDATION

Dear Colleagues,

Welcome to the 7th Annual Peptide Therapeutics Symposium. This year's Symposium continues the tradition of focusing on the discovery and development of peptide-based drug candidates through state-of-the-art lectures, cutting-edge poster presentations, and informal scientific networking.

The Symposium opens with a session on innovations in Peptide Chemistry followed by a discussion on the State of Pharmaceutical Innovation and the basis for transformational change in the traditional pharmaceutical-biotech R&D model. The evening concludes with a Poster Session and Opening Reception in an environment conducive to networking and non-structured personal interactions.

We convene on Friday morning with two keynote lectures by Professors William Degrado and Matthias Tschöp, respectively highlighting the forefront of molecular design and combinational pharmacology. Subsequent presentations detail the status of breakthrough therapeutics that span development from regulatory approval to early clinical studies. The program also emphasizes novel approaches to address the inherent physiochemical and pharmaceutical limitations of peptides. This includes new methods to extend duration of action, improve therapeutic index and facilitate non-injectable administration.

As in previous years the program, the venue and social time have been designed to support networking among an intimate, but diverse spectrum of experts that constitute the field of peptide therapeutics.

We look forward to welcoming you to this 7th meeting of the Peptide Therapeutics Foundation and your contribution to the Symposium.

Sincerely,

Richard D. DiMarchi
Symposium Chair & Chairman of the Board, Peptide Therapeutics Foundation

Sponsors, Peptide Therapeutics Foundation

Amylin Pharmaceuticals
CovX
Ferring Research Institute
Ipsen
PolyPeptide Group
Roche
Zydus Cadila

Amylin Pharmaceuticals

Amylin Pharmaceuticals LLC is a wholly owned subsidiary of Bristol Myers Squibb, and is committed to improving lives through the discovery, development and commercialization of innovative medicines.

The company was founded in 1987 on the discovery of a hormone, amylin, produced by the same beta cells of the pancreas that make insulin. Since then, Amylin has built a strong foundation on research and development. Amylin's scientists are primarily focused on investigating the potential utility of new peptide hormone candidates. The company has amassed significant research and clinical expertise in metabolic medicine including the areas of diabetes, obesity and cardiovascular disease. By "Challenging Science," Amylin challenges conventional thinking to create innovative approaches to the discovery, development and commercialization of novel therapies for metabolic diseases. Amylin's approach and dedication are rooted in the belief that they will be "Changing Lives" for millions of people - not only with the drugs currently in late-stage development, but also with their pipeline of future therapies. In August 2012, the company was acquired by Bristol Myers Squibb.

CovX

CovX is the peptide therapeutic R&D unit of Pfizer, responsible for the internal peptide research pipeline, from discovery to clinical proof-of-concept. CovX is located in San Diego, one of the most vibrant biopharmaceutical clusters in the country. Initially established in 2002 as a start-up biotech, CovX developed "CovX-Bodies," unique and proprietary biotherapeutics which combine the therapeutic potential of peptides and antibodies. Acquired by Pfizer in 2008, CovX has retained its entrepreneurial spirit, autonomy and agility, while gaining access to the commitment, resources and funding of the largest biopharmaceutical company in the world. Building upon and beyond its existing peptide expertise and proprietary technology platform, CovX is aiming at developing the most versatile and comprehensive peptide drug discovery capability in the industry in terms of molecular target drugability, targeting and delivery, and duration of action. CovX research focuses on significant unmet medical needs and unique target product profiles addressable only by peptides in the fields of Oncology, Cardiovascular Disease & Metabolism, Immunology & Inflammation and Neuroscience.

Ferring Research Institute

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

Ferring Pharmaceuticals (Ferring) is a private, research-driven specialty biopharmaceutical company active in global markets. The company identifies, develops and markets innovative products in the fields of endocrinology, gastroenterology, infertility, obstetrics, urology and osteoarthritis. In recent years Ferring has expanded beyond its traditional European base: with over 4,500 employees worldwide, it operates subsidiaries in over 50 countries and makes its products available in more than 90 countries. The company has emerged as a world leader with one of the largest peptide therapeutics portfolios in the industry. As part of its commitment to developing innovative products to treat diseases with high unmet medical need, Ferring invests heavily in its research infrastructure both in terms of people and technology.

Ipsen

Ipsen (Euronext: IPN; ADR: IPSEY) is a global specialty-driven pharmaceutical company with total sales exceeding €1.1 billion in 2011. Ipsen's ambition is to become a leader in specialty healthcare solutions for targeted debilitating diseases. Its development strategy is supported by four franchises: neurology / Dysport®, endocrinology / Somatuline®, uro-oncology / Decapeptyl® and hemophilia. Moreover, the Group has an active policy of partnerships. R&D is focused on innovative and differentiated technology-driven platforms, peptides and toxins. In 2011, R&D expenditure totaled more than €250 million, above 21% of Group sales. The Group has total worldwide staff of close to 4,500 employees.

The PolyPeptide Group

The PolyPeptide Group is a privately held group of six companies that employs 420 staff worldwide. The PolyPeptide Group focuses exclusively on the manufacture of peptides and related substances and is a leading provider of custom and generic GMP-grade peptides for a range of pharmaceutical and biotechnology applications. With corporate roots that began in the 1950s, the Group was formally launched in 1996. Today, it operates a growing international network of peptide manufacturing facilities. Its world-class chemists and support personnel offer an unparalleled range of services for clients of every size and at every stage of product development. The PolyPeptide Group has been pre-approval inspected by the FDA over fifteen times as well as by other Regulatory Authorities. Altogether, the Group 25 approved APIs. More information about PolyPeptide Group is available at www.PolyPeptide.com.

In addition to large-scale GMP manufacturing, the PolyPeptide Group offers a wide range of other peptide services including radiolabelling, organic synthesis, cosmetic peptides and small-scale custom synthesis. It also has an extensive catalog of peptides and building blocks. The Group's customers range from emerging pharmaceutical companies and biotech organizations through to Big Pharma. The remaining business is primarily linked to the sale of peptide generics, including Calcitonin, Deslorelin, Gonadorelin, Leuprolide, Octreotide, hPTH (1-34), Somatostatin, Triptorelin and Arg-Vasopressin.

Roche

Headquartered in Basel, Switzerland, Roche is a leader in research-focused healthcare with combined strengths in pharmaceuticals and diagnostics. Roche is the world's largest biotech company with truly differentiated medicines in oncology, virology, inflammation, metabolism and CNS. Roche is also the world leader in in-vitro diagnostics, tissue-based cancer diagnostics and a pioneer in diabetes management. Roche's personalized healthcare strategy aims at providing medicines and diagnostic tools that enable tangible improvements in the health, quality of life and survival of patients. In 2011, Roche had over 80,000 employees worldwide and invested over 8 billion Swiss francs in R&D. The Group posted sales of 42.5 billion Swiss francs. Genentech, United States, is a wholly owned member of the Roche Group. Roche has a majority stake in Chugai Pharmaceutical, Japan. For more information: www.roche.com.

Zydus Cadila

Zydus Cadila is an innovative global pharmaceutical company that discovers, develops, manufactures and markets a broad range of healthcare products. The group's operations range from API to formulations, animal health and wellness products. Headquartered in the city of Ahmedabad in India, the group has global operations in four continents spread across USA, Europe, Japan, Brazil, South Africa and 25 other emerging markets.

In its mission to create healthier communities globally, Zydus Cadila delivers wide ranging healthcare solutions and value to its customers. With over 15,000 employees worldwide, a world-class research and development centre dedicated to discovery research and nine state-of-the-art manufacturing plants, the group is dedicated to improving people's lives.

From a turnover of Rs. 250 crores in 1995, the group posted revenues of Rs. 5200 crores in FY12. The group had posted a turnover of Rs. 4600 crores in FY 11, making it a billion dollar company. The group aims to be a leading global healthcare provider with a robust product pipeline; achieve sales of over $3 bn by 2015 and be a research-based pharmaceutical company by 2020.

Thursday, October 25th, 2012

2:00 p.m. – 6:00 p.m.
Registration Check-in
Frederic de Hoffmann Auditorium Reception Area, Lower Level

2:30 p.m. – 5:45 p.m.
7th Annual Peptide Therapeutics Symposium
Frederic de Hoffmann Auditorium

2:30 p.m. – 3:00 p.m.
Opening Remarks
Pierre Rivière, Ph.D.
Director and President, Peptide Therapeutics Foundation
Chief Scientific Officer, CovX
Senior Vice President, Pfizer Worldwide Research & Development

3:00 p.m. – 5:00 p.m.
OPENING SESSION: PEPTIDE CHEMISTRY

Moderator
Hans-Joachim Böhm, Ph.D.
Director, Peptide Therapeutics Foundation
Global Head of Chemistry, Roche

3:00 p.m. – 3:40 p.m.
Chemoselective Strategies for the Synthesis of Complex Assemblies of Peptide
Philip Dawson, Ph.D.
Associate Professor of Cell Biology & Chemistry, The Scripps Research Institute

3:40 p.m. – 4:05 p.m.
Cell-penetrating Peptides with Intrinsic Biological Activity
Francesca Milletti, Ph.D.
Head, Cheminformatics & Statistics, Roche Pharmaceuticals

4:05 p.m. – 4:30 p.m.
Peptide Macrocyclization Enabled by Amphoteric Molecules
Andrei K. Yudin, Ph.D.
Professor, Davenport Research Laboratories, Chemistry Department, University of Toronto

4:30 p.m. – 5:00 p.m.
Break
Frederic de Hoffmann Auditorium Reception Area, Lower Level

5:00 p.m. – 5:45 p.m.
INDUSTRY LECTURE DISCUSSION:

Moderator
Pankaj Patel
Director, Peptide Therapeutics Foundation
Chairman and Managing Director, Zydus Cadila

The State of Pharmaceutical Innovation
Bernard Munos, MBA
Founder, InnoThink Center for Research in Biomedical Innovation

5:45 p.m. – 7:30 p.m.
Poster Session & Opening Reception
Frederic de Hoffmann Auditorium Reception Area, Lower Level

Friday, October 26th, 2012

7:00 a.m. – 6:00 p.m.

7th Annual Peptide Therapeutics Symposium
Frederic de Hoffmann Auditorium

7:00 a.m. – 12:00 p.m.

Registration Check-in
Frederic de Hoffmann Auditorium Reception Area, Lower Level

7:00 a.m. – 8:00 a.m.

Breakfast & Poster Viewing
Frederic de Hoffmann Auditorium Reception Area, Lower Level

8:00 a.m. – 8:15 a.m.

Welcoming Remarks
Adrienne Day, Ph.D.
Secretary and Treasurer, Peptide Therapeutics Foundation
Director, Business Development, Ferring Research Institute

8:15 a.m. – 9:00 a.m.

KEYNOTE LECTURE

Moderator
Waleed Danho, Ph.D.
Distinguished Research Leader (Retired), Hoffmann-LaRoche, Inc.

Design of Therapeutics that Act on Membranes and Membrane Proteins
Bill DeGrado, Ph.D.
Professor, Department of Pharmaceutical Chemistry; Investigator, Cardiovascular Research Institute, University of California, San Francisco

9:00 a.m. – 9:45 a.m.

KEYNOTE LECTURE

Moderator
Richard DiMarchi, Ph.D.
Symposium Chair and Chairman of the Board, Peptide Therapeutics Foundation
Standiford H. Cox Distinguished Professor of Chemistry, Jill & Jack Gill Chair in Biomolecular Sciences, Department of Chemistry, Indiana University

Gut-peptide Based Poly-pharmacy for the Treatment of Obesity & Diabetes
Matthias Tschöp, M.D.
Alexander-von-Humboldt Professor; Director, Institute for Diabetes and Obesity, German Center for Diabetes Research, Helmholtz Center Munich

9:45 a.m. – 10:15 a.m.

Beverage Break & Poster Viewing
Frederic de Hoffmann Auditorium Reception Area, Lower Level

Friday, October 26th, 2012 continued

10:15 a.m. – 12:00 p.m.

SESSION 1: PEPTIDE THERAPEUTICS

Moderator
Jesse Dong, Ph.D.
Director, Peptide Therapeutics Foundation
Vice President, Compound Discovery, Ipsen

10:15 a.m. – 11:00 a.m.

Omontys: Beating the Odds in the Journey of a Drug from Bench to Bedside
Anne-Marie Duliege, M.D., M.S.
Chief Medical Officer, Affymax, Inc.

11:00 a.m. – 11:30 p.m.

Identification and Characterization of KAI-4169, A Novel Peptide for the Treatment of Secondary Hyperparathyroidism
Gregory M. Bell, M.D.
Senior Vice President, Development, KAI Pharmaceuticals

11:30 a.m. – 12:00 p.m.

Stapled Peptide Drugs: Translation to the Clinic
Tomi K. Sawyer, Ph.D.
Chief Scientific Officer and Senior-Vice President, Drug Discovery and Innovative Technologies, Aileron Therapeutics

12:00 p.m. – 1:15 p.m.

Lunch & Poster Viewing
Frederic de Hoffmann Auditorium Reception Area, Lower Level

1:15 p.m. – 3:00 p.m.

SESSION 2: PEPTIDE THERAPEUTICS

Moderator
Soumitra Ghosh, Ph.D.
Director, Peptide Therapeutics Foundation
Senior Director, Research, Amylin Pharmaceuticals, Inc.

1:15 p.m. – 1:45 p.m.

LY2605541, a Novel Long-acting Basal Insulin
Mel Prince, M.D.
Senior Medical Director, Lilly Diabetes, Eli Lilly and Company

1:45 p.m. – 2:10 p.m.

NMDA Receptor Modulating Peptides with Therapeutic Potential: From Monoclonal Antibodies to the Creation and Development of GLYX-13
Joseph R. Moskal, Ph.D.
Professor and Director, The Falk Center for Molecular Therapeutics, McCormick School of Engineering and Applied Sciences, Dept. of Biomedical Engineering, Northwestern University

Friday, October 26th, 2012 continued

2:10 p.m. – 2:35 p.m.

XEP-018: A New Myorelaxant Peptide Lead Compound from the Venom of the Cone Snail *Conus consors*
Reto Stöcklin, Ph.D.
President & CEO, Research & Development, Atheris Laboratories

2:35 p.m. – 3:00 p.m.

XTENylation Offers a Biodegradable Alternative to PEGylation
Volker Schellenberger, Ph.D.
Chief Scientific Officer, Amunix, Inc.

3:00 p.m. – 3:30 p.m.

Beverage Break & Poster Viewing
Frederic de Hoffmann Auditorium Reception Area, Lower Level

3:30 p.m. – 4:50 p.m.

SESSION 3: PEPTIDE PHARMACEUTICS

Moderator
Claudio Schteingart, Ph.D.
Director, Peptide Therapeutics Foundation
Vice President, Science & Technology – Research, Ferring Research Institute

3:30 p.m. – 4:00 p.m.

Tunable Half-life Extension of Therapeutics by Controlled Chemical Release from Macromolecular Conjugates
Daniel V. Santi, M.D., Ph.D.
President, ProLynx LLC; Professor, University of California, San Francisco

4:00 p.m. – 4:25 p.m.

Development of Modified Human Serum Albumins for Peptide and Protein Half-life Extension: Towards Monthly Dosing
Mark Perkins, Ph.D.
Customer Solutions Manager, Novozymes Biopharma UK Ltd.

4:25 p.m. – 4:50 p.m.

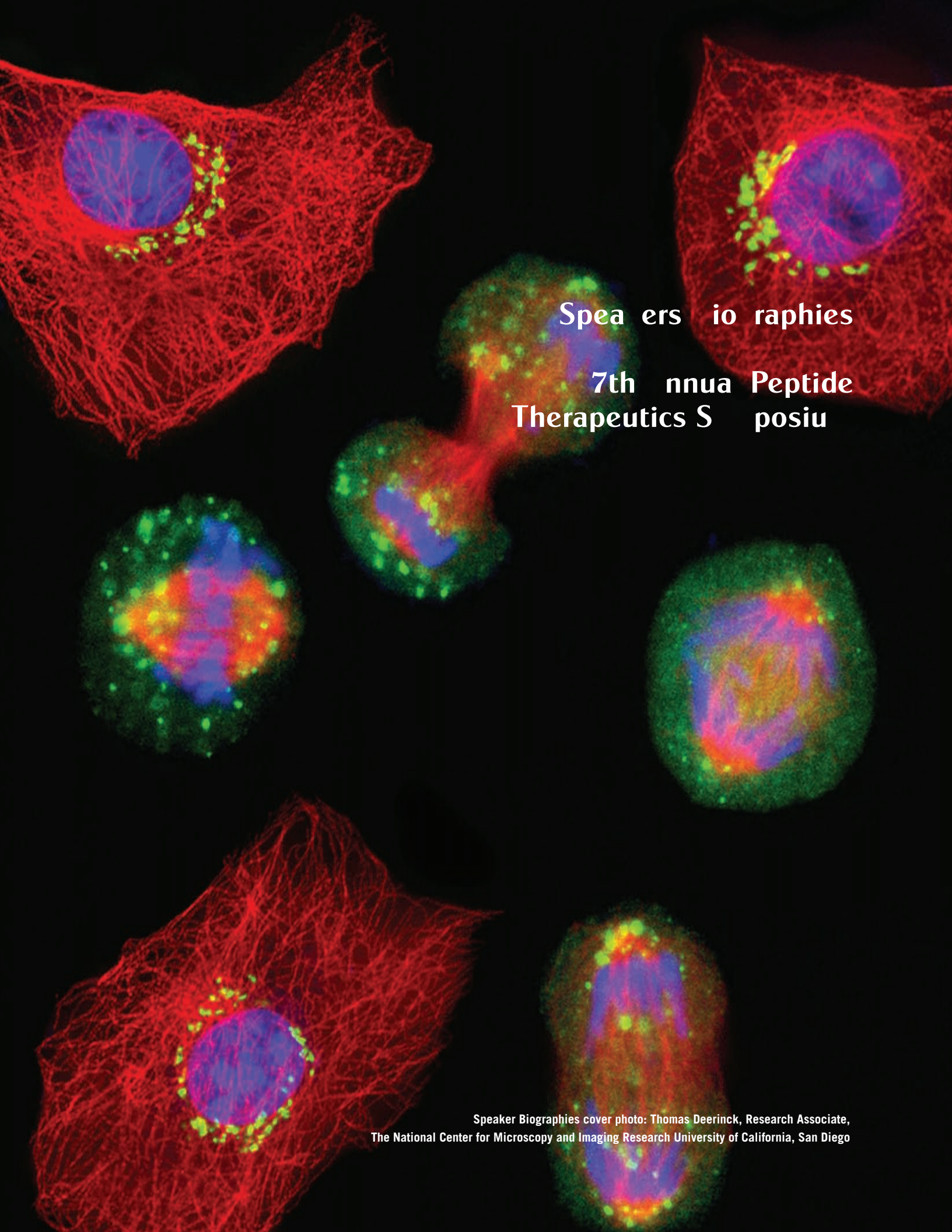
Clinical Development of a Novel PTH Analog for the Treatment of Osteoporosis with an Optimized Oral Tablet Formulation
Nozer Mehta, Ph.D.
Vice President, Research and Development, Unigene Laboratories, Inc.

4:50 p.m. – 5:00 p.m.

Closing Remarks
Jane Salik, Ph.D.
Director, Peptide Therapeutics Foundation
CEO, PolyPeptide Group

5:00 p.m. – 6:00 p.m.

Networking Reception
Frederic de Hoffmann Auditorium Reception Area, Lower Level



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Speaker Biographies cover photo: Thomas Deerinck, Research Associate,
The National Center for Microscopy and Imaging Research University of California, San Diego

Gregory M. Bell, M.D. | Senior Vice President, Development, KAI Pharmaceuticals
Identification and Characterization of KAI-4169, A Novel Peptide for the Treatment of Secondary Hyperparathyriodism

Gregory Bell, M.D. earned his medical degree from Cornell University Medical College and completed his training in Internal Medicine and Rheumatology at Brown University and the University of California, San Francisco (UCSF), respectively. Dr. Bell was a member of the UCSF Department of Medicine from 1991 through 1996 and his research focused on basic mechanisms of lymphocyte activation.

In 1996 Dr. Bell joined Merck & Co., Inc and, in 1999, became the National Arthritis Medical Director in the Medical and Scientific Affairs department of the U.S. Human Health Division. While at Merck, Dr. Bell contributed to several products that received regulatory approval including Vioxx®, Etoricoxib® and Cancidas®. Dr. Bell later joined Abgenix, Inc. and became Vice President of Clinical Development, Biometrics and Clinical Operations with responsibilities across all clinical programs including Vectibix® before joining KAI Pharmaceuticals in 2005. Dr. Bell is currently Senior Vice President of Development and Chief Medical Officer at KAI Pharmaceuticals and is an assistant professor of Clinical Medicine at UCSF. Dr. Bell is a member of the American College of Rheumatology (ACR) and has served on several committees of the ACR.

Philip Dawson, Ph.D. | Associate Professor of Cell Biology and Chemistry, The Scripps Research Institute
Chemoselective Strategies for the Synthesis of Complex Assemblies of Peptide

Philip Dawson is an Associate Professor of Cell Biology and Chemistry at The Scripps Research Institute (TSRI) in La Jolla, CA. He received an A.B. in Chemistry from Washington University in St. Louis in1992 and a Ph.D. in 1996. from The Scripps Research Institute in La Jolla, CA where he was a charter member of the graduate program in Macromolecular and Cellular Structure and Chemistry under the guidance of Steve Kent. Following postdoctoral work at the California Institute of Technology with Harry Gray and Tom Meade in 1997, he returned to TSRI as a member of the departments of Chemistry and Cell Biology. He was elected to the Council of the American Peptide Society and was co-chair of the 22nd American Peptide Symposium in 2011 and will co-chair the 2016 Gordon Research Conference. He has published over 100 papers and reviews and has been honored with the Alfred P. Sloan Foundation fellowship, the Vincent du Vigneaud Award from the American Peptide Society and the Max Bergmann Gold Medal. His research focuses on the development of new synthetic tools for protein chemistry and their application in the areas of nanotechnology, HIV vaccine development, targeted imaging and protein boiphysics.

Professor Dawson is an expert in the area of chemoselective ligation and was an inventor of the widely used native chemical ligation approach for the synthesis and semisynthesis of proteins. His laboratory has developed new approaches for linking unprotected peptides toother biological macromolecules in aqueous solution including the use of mercaptobenzyl ligation auxiliaries and the development of aniline catalysis for oxime and hydrazone ligation reactions. The Dawson lab has used these methods to synthesize a variety of proteins to study protein folding and topology, enzymatic catalysis, homogeneous glycoproteins and domains involved in thrombosis. His current research focuses on the development of new methods for bioconjugation and the efficient synthesis of C-terminally functionalized peptides using both Boc and Fmoc approaches. His laboratory utilizes these methods for the development of HIV vaccine candidates and the application of nanoparticles to biological systems and therapies for lysosomal storage diseases.

Adrienne Day, Ph.D. | Secretary and Treasurer, Peptide Therapeutics Foundation; Director, Business Development, Ferring Research Institute
Welcoming Remarks

Dr. Adrienne Day is the Director of Business Development for Ferring Research Institute. She has more than 15 years of experience in the biotechnology and biopharmaceutical industries, and has worked in the non-profit, for-profit and startup environments.

Prior to joining Ferring Dr. Day ran a successful consulting practice. She has previously served as Vice President of Business Development at the Burnham Institute for Medical Research, Vice President of Business Development Conforma Therapeutics, Senior Director of Business Development at Molecumetics Ltd., Associate Director of Corporate Development at Ligand Pharmaceuticals. She was Ligand Pharmaceuticals' first Project Manager, and began her biotechnology career at Invitrogen Corporation where she held various positions.

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

Bill DeGrado, Ph.D. | Professor, Department of Pharmaceutical Chemistry; Investigator, Cardiovascular Research Institute, University of California, San Francisco
Design of Therapeutics that Act on Membranes and Membrane Proteins

Bill DeGrado earned his Ph.D. from University of Chicago in Chemistry in 1981 and started his career as a Research Chemist at DuPont Central Research & Development Department. Dr. DeGrado continued his work at DuPont in various capacities including Research Leader, Research Fellow and concluding as the Senior Director of the DuPont Merck Pharmaceutical Company, Medicinal Chemistry Department from 1994-1995. Following his work with DuPont, Dr. DeGrado began his work in education at UPenn School of Medicine where he held a Professor position in the Department of Biochemistry and Biophysics from 1996-2011. In 2012 he joined the faculty of University of California, San Francisco (UCSF) in the Department of Pharmaceutical Chemistry and also became an Investigator for the Cardiovascular Research Institute at UCSF.

Dr. DeGrado has received numerous awards including the Du Vigneaud Award for Young Investigators in Peptide Research, the Protein Society Young Investigator Award, the Eli Lilly Award in Biological Chemistry, the DuPont Merck Summit Award, the Merrifield Award, Peptide Society, the Ralph F. Hirschmann Award in Peptide Chemistry (American Chemical Society) and the Makineni Award (APS). He is a Fellow of the Association for the Advancement of Science and the AAAS (Advancing Science, Serving Society) as well as a Member of the National Academy of Sciences (U.S.A.).

Richard DiMarchi, Ph.D. | Symposium Chair & Chairman of the Board, Peptide Therapeutics Foundation; Standiford H. Cox Distinguished Professor of Chemistry Jill & Jack Gill Chair in Biomolecular Sciences, Department of Chemistry, Indiana University
Chairman

Dr. DiMarchi contributions in peptide & protein sciences consists of three decades of work in academia, the pharmaceutical industry and biotechnology companies. He is the Cox Distinguished Professor of Biochemistry and Gill Chair in Biomolecular Sciences at Indiana University. His current research is focused on developing macromolecules with enhanced therapeutic properties through biochemical optimization with non-natural amino acids, an approach termed chemical-biotechnology. He is a co-founder of Ambrx, Inc. and Marcadia Biotech. He is a scientific advisor to Ferring, Merck, Roche and three venture funds; 5AM, TMP, and Twilight.

Dr. DiMarchi is a retired Group Vice President at Eli Lilly & Company where for more than two decades he provided leadership in biotechnology, endocrine research and product development. He is readily recognized for discovery and development of rDNA-derived Humalog® (LisPro-human insulin). This designer insulin represents the first demonstration that structurally altered rDNA-derived biosynthetic proteins can improve pharmacological performance without increasing the risk of an abnormal immunological response. As scientist and manager, Dr. DiMarchi also significantly contributed to the commercial development of Humulin®, Humatrope®, Xigris®, rGlucagon®, Evista®, and Forteo®.

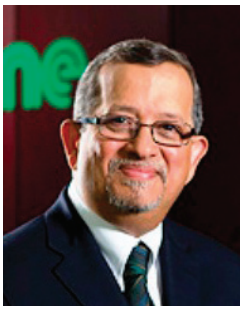
Dr. DiMarchi is the recipient of numerous awards including the 2005 AAPS Career Research Achievement Award in Biotechnology, the 2006 ACS Barnes Award for Leadership in Chemical Research Management, the 2006 ACS Esselen Award for Chemistry in the Service of Public Interest, the 2007 Carothers Award for Excellence in Polymer Sciences, the 2009 Watanabe Award for Life Sciences Research, and the 2011 Merrifield Award for Career Contributions in Peptide Sciences.

Anne-Marie Duliege, M.D., M.S. | Chief Medical Officer, Affymax, Inc.
Omontys: Beating the Odds in the Journey of a Drug from Bench to Bedside

Dr. Duliege has over 20 years of scientific and leadership experience in the pharmaceutical industry. She joined Affymax in 2004 as VP of clinical, regulatory and medical affairs. She was instrumental in raising capital and taking the company public She was promoted to Chief Medical Officer in 2007. Under her leadership, her team completed an extensive development program and obtained FDA's approval for the company's lead product, OMONTYS®. As a member of the executive team, Dr. Duliege is responsible for the clinical and non-clinical research of the company and helps set company strategies, contributes to investor relations, and supports business development activities.

Prior to joining Affymax, Dr. Duliege was a Senior Medical Director at Chiron Corporation with a record of significant accomplishments in executing Phase 1 through 4 clinical studies in pulmonary medicine, infectious disease and immunology. She held positions of Senior Research Physician at Genentech, Inc., working on HIV treatment and vaccine, and epidemiologist at the National Institute of Science & Medical Research in Paris.

Dr. Duliege received her MD degree and her certification in Pediatrics from Paris Medical School; she also has an M.S. in Epidemiology from Harvard School of Public Health and an M.S. in Biostatistics from Paris Medical School. She continues to practice medicine as an Adjunct Clinical Assistant Professor at Stanford's School of Medicine and Lucile Packard Children's Hospital. She serves on the board of non-profit organizations focused on the prevention of HIV. She is personally committed to supporting public health efforts in developing countries.



Nozer Mehta, Ph.D. | Vice President, Research and Development, Unigene Laboratories, Inc.

Clinical Development of a Novel PTH Analog for the Treatment of Osteoporosis with an Optimized Oral Tablet Formulation

Dr. Nozer Mehta received a Master's Degree from the University of Mumbai, India, and a Doctorat d'Université (equivalent to the Ph.D.) with Honors from the Université Louis Pasteur in Strasbourg, France. He has worked as a staff scientist at the Cancer Research Institute in Mumbai and as a research assistant professor at the University of Nebraska in Lincoln. Dr. Mehta joined Unigene Laboratories, Inc. as a Senior Scientist in 1982. He has played a key role in developing Unigene's therapeutic programs as well as the platform technologies for recombinant expression and alternate delivery routes for peptide drugs. He is an inventor on several key patents on Unigene's technologies and programs. His current position at Unigene is Vice President, Research and Development.

Francesca Milletti, Ph.D. | Head, Cheminformatics & Statistics, Roche Pharmaceuticals

Cell-penentrating Peptides with Intrinsic Biological Activity

Francesca Milletti leads the Cheminformatics and Statistics group at Roche (Nutley, NJ, USA). She joined Roche in 2010 after a postdoctoral fellowship at Novartis, Basel. Before that, she was a visiting student at the University of California, San Francisco (2008). She received her Ph.D. and undergraduate degree in chemistry from the University of Perugia, Italy, where she developed computational tools for pKa prediction and tautomer enumeration. Her research focuses on novel computational methods for drug discovery with a special interest on cell-penetrating peptides.

Joseph R. Moskal, Ph.D. | Professor and Director, The Falk Center for Molecular Therapeutics, McCormick School of Engineering and Applied Sciences, Dept. of Biomedical Engineering, Northwestern University

NMDA Receptor Modulating Peptides with Therapeutic Potential: From Monoclonal Antibodies to the Creation and Development of GLYX-13

Joseph Moskal received his Ph.D. in biochemistry from the University of Notre Dame in 1977. Following this he was a Staff Fellow in the Laboratory of Biochemical Genetics, under the direction of Marshall Nirenberg at the National Institutes of Health. He next joined the Laboratory of Cell Biology, National Institute of Mental Health directed by Michael Brownstein and Julius Axelrod as a Senior Staff Fellow. From there he joined the faculty of the Departments of Neurosurgery and Neuroscience at The Albert Einstein College of Medicine, Bronx, NY where he was also the Director of the Neurosurgery Laboratories. He moved to Chicago in 1990 with an appointment in the Departments of Cell Biology and Biomedical Engineering at The Feinberg School of Medicine and McCormick School of Engineering and Applied Sciences at Northwestern University, respectively. At that time he also was the Director of the Chicago Institute for Neurosurgery and Neuroresearch. Presently he is a Professor in the Dept. of Biomedical Engineering, Director of the Falk Center for Molecular Therapeutics and is the Founder and Chief Science Officer of Naurex, Inc. He has published over one hundred papers in peer reviewed journals as well as a number of invited reviews and book chapters. He also holds eleven patents. His recent work has focused on the creation and development of neuroactive peptides with therapeutic potential in central nervous system disorders. One of these neuropeptides, GLYX-13, has been shown to have significant efficacy for treatment resistant depression and is now in Phase II clinical development.

Bernard Munos, MBA | Founder, InnoThink Center for Research in Biomedical Innovation
The State of Pharmaceutical Innovation

Bernard Munos is the founder of InnoThink, a consultancy that focuses on pharmaceutical innovation — specifically, where it comes from and how to get more of it. He was previously an advisor for corporate strategy at Eli Lilly, where he focused on disruptive innovation and the radical redesign of R&D. His research has been published in Nature and Science, and he was recently profiled by Forbes magazine. This year, the popular industry newsletter FiercePharma named him one of the 25 most influential people in biopharma today.

Munos received his M.B.A. from Stanford University, and holds graduate degrees in agricultural economics and animal science from the University of California, Davis, and the Paris Institute of Technology for Life, Food and Environmental Science.

Munos has presented his findings at numerous meetings sponsored by the National Academies, the Institute of Medicine, the OECD, the President's Cancer Panel, The NIH Leadership Forum, the World Health Organization, the US Patent & Trademark Office, Genome Canada, the American Chemical Society, as well as leading universities, think-tanks, and foundations in the US, Europe, the Mideast and Asia. He is a non-executive Director of Glenmark Pharmaceuticals, and advises numerous companies, government-funded research organizations, and disease foundations on how to become better innovators.

Mark Perkins, Ph.D. | Customer Solutions Manager, Novozymes Biopharma UK Ltd.
Development of Modified Human Serum Albumins for Peptide and Protein Half-life Extension: Towards Monthly Dosing

Mark Perkins is a Customer Solution Manager for Novozymes Biopharma UK where he works with partners who are evaluating Novozymes recombinant human albumin and associated half-life extension technologies. Mark has been working in the pharmaceutical industry for over seven years and has previously held positions as a materials specialist with Vectura, a speciality inhaled drug development company and as project manager with Molecular Profiles. Mark holds a Ph.D. in pharmaceutical sciences from the University of Nottingham,

Mel Prince, M.D. | Senior Medical Director, Lilly Diabetes, Eli Lilly and Company
LY2605541, a Novel Long-acting Basal Insulin

Dr. Prince joined Eli Lilly and Company as a Senior Clinical Research Physician in September, 1998 after a distinguished career in academic internal medicine and endocrinology. After receiving his MD degree and completing post-doctoral training in Internal Medicine and Endocrinology at the University of Texas Medical Branch in Galveston (UTMB), Dr. Prince completed a research fellowship in Endocrinology at the University of Colorado Health Sciences Center under the direction of Dr. Jerrold Olefsky. He was on the staff at UTMB, the Oregon Health Sciences University, the University of California, San Diego and most recently, the Indiana University School of Medicine. At Indiana University, Dr. Prince was Professor of Medicine and served as the Director of the Endocrinology Fellowship Training Program, Principal Investigator for the Diabetes Prevention Program, Director of the Indiana Diabetes Center, Director of the Model Diabetes Unit of the NIH-funded Diabetes Research and Training Center and was an Associate Editor for the journal, Diabetes Care. At Lilly, Dr. Prince is currently a Senior Medical Director in Lilly Diabetes and is involved in the development of diabetes related compounds.

Pierre Rivière Ph.D. | Director and President, Peptide Therapeutics Foundation; Chief Scientific Officer, CovX; Senior Vice President, Pfizer Worldwide, Research & Development
Opening Remarks

Pierre Rivière is Chief Scientific Officer of CovX and Senior Vice President, Pfizer Worldwide Research & Development. He is also member of Pfizer WRD Leadership Team. With over 20 years pharmaceutical industry experience, mainly focused on peptide therapeutics, Pierre Rivière is now leading the transformation of CovX into the peptide therapeutics R&D division of Pfizer. Building upon and beyond its unique CovX bodies technology for extending half-life of macromolecules, CovX is now assembling a versatile and comprehensive peptide drug discovery capability in terms of molecular target drugability, duration of action, targeting and delivering. Before joining Pfizer, Pierre Rivière was President of the Ferring Research Institute and Senior Vice President, Research for Ferring Pharmaceuticals where he contributed to the discovery of several peptide new chemical entities, now at various stage of development, either in house or through licensees. He previously led the Gastroenterology drug discovery department of the Institut de Recherche Jouveinal. Pierre Rivière holds a Ph.D. in Biology and Physiology from the Institut National Polytechnique of Toulouse in France and has completed a post-doctoral training in the Department of Pharmacology at the University of Arizona. He is a co-founder of Cara Therapeutics, BioHeatMap, the Peptide Therapeutics Foundation and the Annual Peptide Therapeutics Symposium. He also serves as Director and President of the Peptide Therapeutics Foundation.

Jane Salik, Ph.D. | Director, Peptide Therapeutics Foundation; Chief Executive Officer, PolyPeptide Group; President and Co-Founder, PolyPeptide Laboratories Inc.
Closing Remarks

Dr. Jane Salik is the CEO of the PolyPeptide Group and President and co-founder of PolyPeptide Laboratories Inc in Torrance, California, the US subsidiary of the PolyPeptide Group. The PolyPeptide Group is a world leader in the industrial-scale manufacture of proprietary and generic GMP peptides used as Active Pharmaceutical Ingredients (APIs) by pharmaceutical, biotech and research organizations.

Dr. Salik has been involved in management, business development and sales and marketing in the peptide business for over 20 years, having served as Vice President of Sales, Marketing and Business Development at Bachem California before founding PolyPeptide Laboratories Inc. Prior to that, she was Marketing Manager at Boehringer Mannheim Biochemicals. Dr. Salik received her Ph.D. degree in biochemistry and molecular biology from The State University of New York at Stony Brook, and her B.A. degree from Lafayette College.

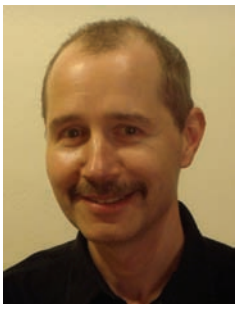
Daniel V. Santi, M.D., Ph.D. | President, ProLynx LLC; Professor, University of California, San Francisco
Tunable Half-life Extension of Therapeutics by Controlled Chemical Release from Macromolecular Conjugates

Daniel V. Santi, M.D., Ph.D., Dr. Santi received a Ph.D. in Medicinal Chemistry from SUNY in 1967 and a M.D. from UCSF in 1981. He was Assistant Professor of Chemistry at UCSB from 1968 to 1974. He joined the UCSF faculty in 1974 and was Professor of Biochemistry and Biophysics, and of Pharmaceutical Chemistry at UCSF until 2000 when he became CEO of Kosan Biosciences. He returned to UCSF in 2007 where he served as interim Director of the Clinical and Translational Science Institute and the Director of Translational Research at QB3. He became an Associate Dean of External Relationships in 2009, and has managed a successful Industry Outreach Program since that time. Dr. Santi has published over 300 scientific papers and is co-inventor on over 30 issued US patents.

Dan Santi was a member of the original Scientific Advisory Board of Chiron Corp. and has co-founded five biotechnology companies. In 1988, he founded and was Chairman of the peptide-combinatorial chemistry company Protos Corp., which merged with Chiron in 1992. He was also a co-founder of Parnassus Pharmaceuticals, and of Prospect Genomics which merged with Structural Genomix in 2001. In 1996, he co-founded Kosan Biosciences where he served as CEO and Chairman until 2006; Kosan was acquired by Bristol Myers Squibb. During his tenure as CEO the company went public (NASDAQ), and four oncology compounds were brought into clinical trials. He co-founded ProLynx LLC in 2009, where he currently serves as President.

Tomi K. Sawyer, Ph.D. | Chief Scientific Officer and Senior-Vice President, Drug Discovery and Innovative Technologies, Aileron Therapeutics
Stapled Peptide Drugs: Translation to the Clinic

Tomi is Chief Scientific Officer and Senior-Vice President, Drug Discovery and Innovative Technologies, at AILERON Therapeutics. He is concurrently is an Adjunct Professor in both the Departments of Chemistry and Biochemistry & Molecular Biology at the University of Massachusetts (Amherst), the Department of Cancer Biology at the University of Massachusetts Medical School (Worcester), and the Center for Drug Discovery at Northeastern University (Boston). Prior to joining AILERON Therapeutics, he rose through the scientific and management ranks at the Upjohn Company, Parke-Davis/Warner-Lambert, Pfizer Global R&D and ARIAD Pharmaceuticals. Tomi's multidisciplinary drug discovery track record includes contributions to clinical candidates and/or noteworthy molecular tools for several therapeutic targets, including GPCR receptors, aspartyl proteases, protein kinases, and protein–protein interaction complexes. He is credited with more than 320 scientific publications and patents, and he has given more than 170 keynote, plenary and invited lectures. Tomi has been recipient of a DuVigneaud Award (the American Peptide Society), a Kagan Lead Finding Award (Upjohn Company), a President's Distinguished Scientist Honors (Parke-Davis/ Warner-Lambert Company), Chairman's R&D Milestone Awards for Drug Discovery (ARIAD Pharmaceuticals), and a Distinguished Alumni Award (Minnesota State University–Moorhead). He is Founding Editor-in-Chief of *Chemical Biology & Drug Design* and has served on the Highlights Advisory Panel of *Nature Reviews Drug Discovery* and Editorial Advisory Boards of *Trends in the Pharmacological Sciences*, *Expert Reviews in Molecular Medicine*, *Expert Opinion on Investigational Drugs*, *Journal of Medicinal Chemistry*, *Chemistry and Biology*, *Current Medicinal Chemistry (Anti-Cancer Agents)*, *Current Organic Synthesis*, *Expert Reviews in Molecular Medicine*, *Expert Opinion on Therapeutic Patents (Oncology)*, *Drug Design and Discovery*, *Pharmaceutical Research*, *Molecular Biotechnology, and Biopolymers (Peptide Science*). Tomi received a B.Sc. degree in Chemistry at Moorhead State University (now Minnesota State University–Moorhead) and Ph.D. in Organic Chemistry at the University of Arizona.



Volker Schellenberger, Ph.D. | Chief Scientific Officer, Amunix, Inc.
XTENylation Offers a Biodegradable Alternative to PEGylation

Volker is currently Chief Scientific Officer of Amunix Inc, which he co-founded with Willem Pim Stemmer in 2006. Volker is lead inventor of Amunix' XTEN technology enabling the engineering of biotherapeutics characterized by long vivo half-life, low safety risk due to their biodegradable nature, and efficient recombinant production.

Volker has 18 years of industry experience in protein engineering and drug discovery. He served as Director of Genencor's Protein Engineering department, where he invented Combinatorial Consensus Mutagenensis, selection by micro-compartmentalization as well as mutator technology. He focused on the discovery and engineering of antibody-enzyme fusion proteins. Prior to his work on biotherapeutics, Volker led projects optimizing enzymes for industrial applications as well as microbes for metabolic pathway engineering.

Volker received his Ph.D. from Leipzig University in 1986 for studies on protease catalyzed peptide synthesis. After postdoctoral studies at the Institute for Protein Research in Pushchino (Russia) he moved to the University of Göttingen where he developed a novel method for the production of peptides from recombinant peptide-multimers. After a postdoc with Bill Rutter at the University of California, San Francisco he joined Genencor in 1994. Volker is author of over 40 scientific papers and inventor of more than 70 issued or pending patent applications. He is the recipient of the Karl Lohman award of the German Society of Biochemists.

Reto Stöcklin, Ph.D. | President & CEO, Research & Development, Atheris Laboratories
XEP-018: A New Myorelaxant Peptide Lead Compound from the Venom of the Cone Snail Conus consors

Reto Stöcklin is a scientific entrepreneur driven by innovation, and this multi-disciplinary project falls exactly within his core expertise. He is the owner and head of Atheris Laboratories, a family-owned and family-driven Swiss company he founded in Geneva in 1995, which focuses primarily on research and development in life sciences (www.atheris.com). Today, Atheris is based on a strong technology platform (mass spectrometry, bioanalytics, protein biochemistry and bioinformatics), proprietary software and databases combined to a unique expertise in exploiting bioactive molecules from venomous animals and other natural sources. Over the years, Atheris has developed a unique discovery platform that allows for extremely fast and targeted drug discovery and lead optimisation projects. Reto Stöcklin pioneered "Venomics" drug discovery and lead optimisation strategies integrating bioactivity-guided hit discovery (Melusine®, unique collections of HTS-ready pre-fractionated venoms, www.melusine.com), structure-driven hit deconvolution (venom peptidomics & proteomics, venom glands transcriptomics and genomics) coupled to biocomputing-assisted lead selection and optimisation (proprietary databases and bioinformatic tools). Dr Stöcklin also initiated and coordinates CONCO — the cone snail genome project for health (www.conco.eu). CONCO gathers 20 partners (including the prestigious J. Craig Venter Institute in Rockville, MA) and is funded by the European Commission with a budget of 10.7 million € over five years. It is the first fully integrated Venomics project, which is devoted to venomous marine snails. The genome, transcriptome and proteome of Conus consors are currently exhaustively studied. The biological activities of natural and synthetic libraries are investigated. Selected peptides are further characterised in vivo and their potential as novel biopharmaceutical drug candidates is evaluated. Additionally, the biodiversity, ecology and molecular evolution of a wide range of venomous gastropod species are studied.

Matthias H. Tschöp, M.D. | Alexander-von-Humboldt Professor; Director, Institute for Diabetes and Obesity, German Center for Diabetes Research, Helmholtz Center Munich

Gut-peptide Based Poly-pharmacy for the Treatment of Obesity & Diabetes

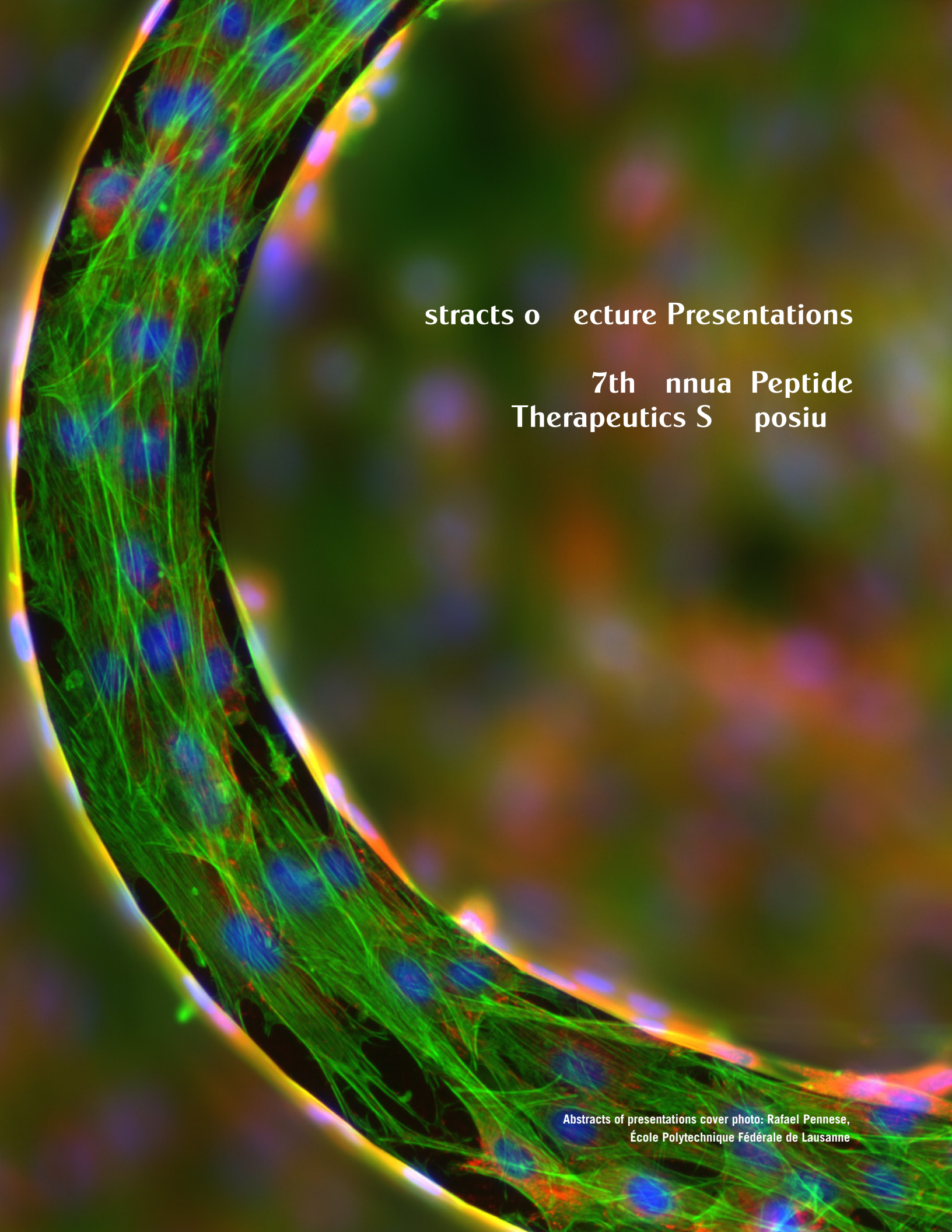
Dr. Matthias H. Tschöp, M.D., is the first and only German Physician to receive a prestigious Alexander von Humboldt Professorship, the highest-endowed German research award. Since October 2011 he has also been named the Director of the Institute of Diabetes and Obesity Research at the German Center for Environment and Health in Munich, Germany and integral component of the German National Diabetes Center (DZD). As a Professor of Medicine he chirs the Division of Metabolic Diseases at the Technical University Munich and holds a visiting professorship at Yale University.

For the last decade, Matthias H. Tschöp has been leading research operations for translational diabetes and obesity research at the University of Cincinnati. His research is mainly focusing on gut-brain communication as a key circuitry that regulates adiposity, food intake, glucose homeostasis and energy metabolism. Dr. Tschöp received his M.D. from Ludwig-Maximilians University in Munich in 1994. He then spent four years as a resident in internal medicine and as a research fellow in neuroendocrinology at the Munich University Hospital before accepting an invitation for a postdoctoral fellowship at the Eli Lilly Research Laboratories. During the following three years, he discovered the role of ghrelin in the control of food intake, metabolism and body weight. His respective publication in Nature since became one of the most cited research articles in modern metabolism research. Matthias H. Tschöp then started a research operation in 2002 at the DIfE in Potsdam and in 2003 also (in parallel) in Cincinnati. He has published more than 230 peer-reviewed articles including articles in Nature, Science, Nature Medicine, Nature Neuroscience, Nature Chemical Biology, Nature Biotechnology, Nature Methods, Journal of Clinical Investigation, PNAS, Cell Metabolism, Neuron or The Lancet. He has received numerous awards including the 2007 Scientific Achievement Award from the The Obesity Society, the 2010 Andre Mayer Award of the International Association for the Study of Obesity (IASO), the 60th Anniversary Scholar Award of the NIH/NIDDK and most recently the Outstanding Scientific Achievement Award of the American Diabetes Association (OSAA ADA 2011) and the Werner Creutzfeldt Award of the German Diabetes Association (DDG 2012). Dr. Tschöp is serving on numerous review panels including the NIH/NIDDK study section for integrated physiology of diabetes and obesity, IPOD. In 2009, he was elected into the American Society for Clinical Investigation (ASCI). He is consulting editor at Diabetes and J Clin Invest and is the founding Editor-in-Chief of the new international open access journal Molecular Metabolism. Dr. Tschöp has a substantial track record of collaborations with partners in biotechnology and the pharmaceutical industry and is a highly sought after invited speaker at international scientific conferences. He is the organizer of the 2012 international EMBO conference on Diabetes and Obesity in Heidelberg (Germany) and of the 2012 Keystone Meeting on Neural Control of Diabetes and Obesity. Most recently, Dr. Tschöp was awarded a Alliance Grant from the German Helmholtz Association, where for the next 5 years he will be coordinating efforts of more than 30 German and international research centers with a research fund of 30 Million Euro to dissect the interactions between the brain and the environment affecting metabolism.

Andrei K. Yudin, Ph.D. | Professor, Davenport Research Laboratories, Chemistry Department, University of Toronto

Peptide Macrocyclization Enabled by Amphoteric Molecules

Andrei K. Yudin received his B.Sc. degree in 1992 from Moscow State University, where he worked in the laboratory of Professor Nicolai S. Zefirov. He then received his Ph.D. degree with Professors G. K. S. Prakash and George A. Olah at the University of Southern California. In 1998, after doing postdoctoral work with K. Barry Sharpless at the Scripps Research institute, Dr. Yudin started his independent career at the University of Toronto. He received early tenure, becoming an Associate Professor in 2002, and an early promotion to the rank of a Full Professor in 2007. Since joining the Department of Chemistry, Dr. Yudin has been awarded a Cottrell Teacher-Scholar Award, Premier's Research Excellence Award, CSC Award in Combinatorial Chemistry (sponsored by Merck-Frosst, Boehringer-Ingelheim, AstraZeneca, and BiochemPharma), 2004 Amgen New Faculty Award, 2008 NSERC Accelerator Supplement Award, 2010 CSC Merck-Frosst Therapeutic Center Award, and 2010 Rutherford Medal of the Royal Society of Canada. In recognition of his work in developing biotherapeutic peptides, Dr. Yudin received a 2011 Inventor of the Year Award at the University of Toronto. In 2012 he became a Fellow of the Royal Society of Chemistry (UK) and assumed a post of Associate Editor for RSC's Organic and Biomolecular Chemistry.



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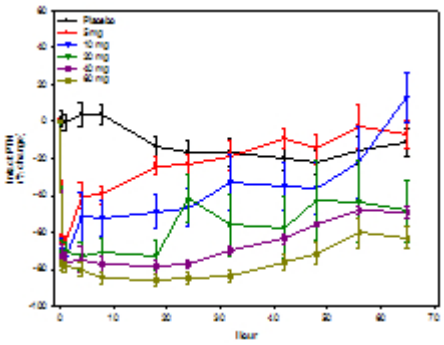
Abstracts of presentations cover photo: Rafael Pennese,
École Polytechnique Fédérale de Lausanne

Identification and Characterization of KAI-4169, A Novel Peptide for the Treatment of Secondary Hyperparathyroidism

Gregory M. Bell, M.D. I Senior Vice President, Development
KAI Pharmaceuticals
270 Littlefield Avenue, South San Francisco, CA 94080 I (650) 244-1171

Secondary hyperparathyroidism (SHPT) is a frequent and serious complication of chronic kidney disease (CKD) in which the impairment of blood and bone mineral homeostasis (calcium and phosphorus) and vitamin D metabolism lead to parathyroid gland hyperplasia and elevated parathyroid hormone (PTH) levels. Elevated PTH and phosphorus are linked to osteodystrophy, vascular calcification and increased risk for cardiovascular events, which is the leading cause of morbidity and mortality in these patients. We have identified a novel peptide agonist of the calcium-sensing receptor (CaSR), KAI-4169, that binds to and activates the CaSR and thereby inhibits secretion of PTH from the parathyroid gland. The in vivo activity of KAI-4169 was evaluated in rats and dogs where it significantly lowered plasma PTH in a dose-dependent fashion.

The initial clinical trials evaluated the safety, tolerability, PK and PD of single doses of KAI-4169 in both healthy male subjects and hemodialysis subjects. In these studies, single doses of KAI-4169 administered as an IV bolus to male subjects (0.5 – 10 mg) or to hemodialysis subjects with SHPT (5 – 60 mg) appeared to be generally safe and well-tolerated. Most treatment-emergent adverse events (TEAE) were mild or moderate in severity and there were few reports of nausea, vomiting and diarrhea, common AEs associated with the oral calcimimetics. Treatment with KAI-4169 resulted in dose-dependent reductions in iPTH in both healthy male subjects and hemodialysis subjects with SHPT. In hemodialysis subjects, treatment with KAI-4169 resulted in mean maximal reduction from baseline of 64%, 73%, 75%, 84% and 86 % at the 5, 10, 20, 40 and 60 mg dose levels, respectively (see figure).



The duration of iPTH suppression was dose-dependent with sustained reductions in serum iPTH through the 3-day interdialytic period following ≥ 20 mg single doses of KAI-4169 in hemodialysis subjects. This reduction in iPTH was associated with modest reductions in serum corrected calcium (cCa), an attenuation of the rise in serum phosphorus that occurred during the interdialytic period and dose-dependent decrease in serum fibroblast growth factor 23 (FGF23). Consistent with the changes in iPTH, beneficial effects on bone markers of bone formation and resorption were also observed. In summary, KAI-4169 is a novel, long-acting, peptide agonist of the CaSR that can be administered during hemodialysis and can provide sustained control of PTH.

Chemoselective Strategies for the Synthesis of Complex Assemblies of Peptide

Philip Dawson, Ph.D. | Associate Professor of Cell Biology and Chemistry
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 | (561) 228-2000

Chemical ligation approaches have become essential tools for the engineering of complex molecules including proteins, nucleic acids and nanoparticles. What makes these reactions so useful is their compatibility with the biological "solvent" water, and a high level of chemoselectivity that enables their application in complex molecular environments. We have worked to develop several ligation chemistries that are highly chemoselective and have sufficient ligation rates to be useful at low concentrations. In one case, the use of hydrolysis resistant thioester peptides that undergo inter- and intramolecular acyl transfer enables the total synthesis of proteins. The optimization of the ligation methodology, improved routes to the required peptide intermediates, and application of these methods to complex targets will be presented. Another challenge is the covalent assembly of macromolecules and nanoparticles. In these systems, a "native" linkage is irrelevant and the main criteria for a successful ligation methodology are fast reaction rates and high chemoselectivity. We have found that aniline catalyzed hydrazone and oxime reactions enable the controlled assembly and disassembly of macromolecular complexes in aqueous solution at micromolar concentrations. The scope of these reactions and new approaches for their catalysis will be discussed.

Design of Therapeutics that Act on Membranes and Membrane Proteins

Bill DeGrado, Ph.D. | Professor, Department of Pharmaceutical Chemistry; Investigator, Cardiovascular Research Institute
University of California, San Francisco
555 Mission Bay Boulevard, South, San Francisco, CA 94158 | (415) 476-9679

This talk will focus on the interplay of structural, biophysical and computational studies to probe the mechanism of action of molecules that act on membranes and membrane proteins. Furthermore, we use de novo molecular design to test this mechanistic understanding. Two examples will be described in which these fundamental studies have led to compounds that are progressing towards or are currently being evaluated in human clinical trials to address drug-resistant bacterial or viral infections. Computational and synthetic coarse-graining has been used to probe the key features required for the antibacterial activity of antimicrobial peptides. The mechanism of action and bacterial response to these compounds will be described. A second topic will be the mechanism of action of the M2 proton channel from influenza A virus. While the adamantane class of compounds have been used to treat influenza A virus infections for several decades, currently circulating strains of the virus are largely resistant to these drugs. Structural and molecular dynamics investigations have shown the mechanism by which protons are stabilized as they transit through the pore, leading to a new understanding of drug-inhibition as well as the development of new classes of drugs that address the problem of drug-resistance.

Omontys: Beating the Odds in the Journey of a Drug from Bench to Bedside

Anne-Marie Duliege, M.D., M.S. | Chief Medical Officer
Affymax, Inc.
4001 Miranda Avenue, Palo Alto, CA 94304 | (650) 812-8727

In March 2012, Palo Alto based Affymax received FDA approval for OMONTYS® (peginesatide) Injection, a product for the treatment of anemia due to chronic kidney disease in adult patients on dialysis. It is the first long acting ESA to come to the US market. The journey started with a selection of an erythropoiesis stimulating peptide with prolonged duration of action, and then advanced through the odyssey of extensive preclinical and clinical programs. In parallel, the company went public and entered into a worldwide partnering agreement with Takeda Pharmaceutical for the development and commercialization of OMONTYS. During that time, the regulatory environment evolved due to increasing safety concerns about the ESA class. Yet, in spite of challenging Phase 3 results, Affymax successfully went through an NDA review, resulting in approval of OMONTYS® within the 10-month PDUFA timeline, positioning the company to advance into a commercial stage. This "David vs. Goliath story" is about quality science, focus, transparency, and persistence!

Clinical Development of a Novel PTH Analog for the Treatment of Osteoporosis with an Optimized Oral Tablet Formulation

Nozer Mehta, Ph.D. | Vice President, Research and Development
Unigene Laboratories, Inc.
81 Fulton Street, Boonton, NJ 07005 | (973) 265-1100

Parathyroid hormone (PTH), when given in an intermittent and pulsatile manner, has a pronounced anabolic effect on bone. However, patient acceptance and compliance can be compromised by the necessity of daily dosing with an injectable formulation. A solid dosage enteric-coated formulation has been developed that enables oral delivery of peptides by a unique mechanism involving protease inhibition by an organic acid and enhancement of paracellular transport by an acylcarnitine. Extensive optimization has been carried out to enhance the bioavailability and consistency of peptide delivery. A PTH analog, PTH(1-31)NH2, is currently in clinical development with this oral formulation. Two phase 1 rising dose pharmacokinetic (PK) studies have been performed to evaluate the level of exposure. An initial study in postmenopausal women with tablets containing 2, 4, 6 and 8 mg of the PTH analog demonstrated a linear dose-dependent response at the three higher doses. The second study was designed to evaluate the safety as well as the inter- and intra-subject PK variability in an open label, two-period replicate dose study in 24 postmenopausal women. A single 6 mg tablet was given to each subject at period 1 and at period 2, with a 48 hour interval between each period. Blood samples were collected over a 6 hour period following dosing, and plasma PTH was quantified using a sandwich ELISA that recognizes only the intact molecule. The mean Cmax value for both periods with the 6 mg tablets was 219 pg/mL, and hence exceeded blood levels that have been shown to be anabolic with an existing injectable formulation. The PK profiles were consistent with the requirement for bone anabolic activity. There were no serious adverse events reported during the study. Based on the results from phase 1 studies, a 5 mg oral tablet dose of PTH(1-31)NH2 was tested in 97 osteoporotic women in a 24-week double blind, randomized, repeat dose parallel group phase 2 study with an open label injectable Forsteo® active control. The primary endpoint was to characterize percent change from baseline in BMD at lumbar spine (LS) after 24 weeks of once daily oral dosing. Plasma samples were collected after the first dose and at end of treatment to characterize the PK profiles. The trial met the primary endpoint with an increase of 2.2% in LS BMD with PTH(1-31)NH2 compared to baseline (p=0.004). No clinically significant hypercalcemic events or elevated urine calcium were seen in the oral PTH(1-31)NH2 arm. The PK profile for the oral tablets showed a pulsatile peak with durations of at least 1 hr but less than 5 hr which is consistent with the requirement for bone anabolic activity. The mean Cmax value for the patients receiving tablets measured at week 0 and week 24 was 295 pg/mL and 207 pg/mL, respectively. The mean Cmax for patients receiving Forsteo at both week 0 and at week 24 was 120 pg/mL. Thus the 5 mg dose resulted in higher mean Cmax values than Forsteo®.The most common adverse event in the oral PTH and placebo arms was GI pain or distress, and these events were mostly mild or moderate. Based on these positive results, further clinical development of this oral PTH analog is currently ongoing.

Cell-penetrating Peptides with Intrinsic Biological Activity

Francesca Milletti, Ph.D. | Head, Cheminformatics & Statistics
Roche Pharmaceuticals
340 Kingsland Street, Nutley, NJ 07110 | (973) 235-5000

Unlike most peptides, cell-penetrating peptides (CPPs) can target intracellular proteins and also carry other cargoes into the cell, thus offering great potential as future therapeutics. Traditionally CPPs are conjugated to a functional peptide to confer cell-penetration. However, this approach can lead to very long sequences (even more than 40 residues). This talk will illustrate examples where a CPP was engineered to carry a function and, vice versa, where a functional peptide was engineered to be also a CPP. With the help of computational models, we found key determinants for predicting cellular uptake that can help to engineer a functional peptide into a CPP. In addition, this talk will illustrate linear CPPs with unexpected high plasma stability, suggesting that some features important for cell-penetration may also be useful for prolonging the peptide half-life.

NMDA Receptor Modulating Peptides with Therapeutic Potential: From Monoclonal Antibodies to the Creation and Development of GLYX-13

Joseph R. Moskal, Ph.D. | Professor and Director
The Falk Center for Molecular Therapeutics
McCormick School of Engineering and Applied Sciences
Dept. of Biomedical Engineering
Northwestern University
1801 Maple Street, Evanston, IL 60201 | (847) 491-4802

NMDA receptor modulation has therapeutic potential for the treatment of depression. There are great unmet needs in the treatment of major depressive disorder (MDD): MDD affects approximately 10% of the adult population and is the second leading cause of global burden of disease. Human clinical studies with the NMDAR antagonist ketamine have demonstrated antidepressant effects with onset within 2 hours and durations of effect lasting several days following a single dose in patients with treatment-resistant depression and bipolar depression. These results, along with postmortem data showing that NMDAR protein expression is altered in the prefrontal cortex of depressed patients make the NMDAR a target of high interest in MDD.

GLYX-13 is glycine-site functional partial agonist (GFPA) at the NMDAR and has been shown to preferentially modulate NR2B-containing NMDARs given that the facilitation of NMDAR current by GLYX-13 at rat Schaffer collateral-CA1 synapses in vitro is completely blocked by the NR2B antagonist ifenprodil. GLYX-13 is an amidated tetrapeptide (threonine-proline-proline-threonine) derived from a hypervariable region cloned and sequenced from the monoclonal antibody, B6B21. GLYX-13 has been reported to: 1) enhance the magnitude of long-term potentiation of synaptic transmission while reducing long-term depression, 2) enhance learning in a variety of hippocampus-dependent learning tasks including trace eyeblink conditioning and the Morris water maze in both young adult and learning-impaired aging rats, 3) markedly reduce CA1 pyramidal neuronal cell death 24 hours after bilateral carotid occlusion in Mongolian gerbils when administered up to 5 hours after induction of occlusion ischemia, and 4) produce analgesic effects in the rat formalin and Bennett models of sustained pain.

Here we show that GLYX-13 produces significant, rapid acting, and long lasting antidepressant-like effects without ketamine-like side effects. Our working hypothesis is that GLYX-13 directly modulates mPFC NR2B-containing NMDARs leading to long term changes in synaptic plasticity which, in turn, modulate the long term antidepressant effects seen with GLYX-13 and other NMDAR modulators. GLYX-13 is currently in Phase II clinical development for treatment-resistant depression.

The State of Pharmaceutical Innovation

Bernard Munos, MBA | Founder
InnoThink Center for Research in Biomedical Innovation
301 Kessler Boulevard West Drive, Indianapolis, IN 46228 | (317) 721-3102

The pharmaceutical industry faces a difficult situation. It must produce more, better, and affordable innovation or face what some might call “a Kodak moment.” As companies struggle with patent cliffs and eroding revenues, there is quite a bit of confusion over what caused some of the most admired and innovative companies to experience, all at the same time, such a dramatic decline in new product output. In his talk, Munos will share insights about the dynamics of innovation and how this can be used to inspire priorities and strategies that can successfully address the industry’s triple challenge, and restore it to its coveted position.

Development of Modified Human Serum Albumins for Peptide and Protein Half-life Extension: Towards Monthly Dosing

Mark Perkins, Ph.D. | Customer Solutions Manager
Novozymes Biopharma UK Ltd.
Castle Court, 59 Castle Boulevard, NG7 1FD Nottingham, United Kingdom | +44 1159553355

Peptides are becoming increasingly attractive as drug candidates, due to their specificity, potency and relatively low toxicity. Despite these therapeutic advantages, many peptides suffer from a short plasma half-life, making delivery of an efficacious dose within an appropriate dosing regimen problematic. Numerous strategies have been employed to extend the half-life of peptides. These strategies are focused around modifying the peptide to reduce sensitivity to enzymatic degradation, increasing the hydrodynamic size of the peptide or through incorporation into a controlled release system. Attachment to recombinant human serum albumin is an attractive half-life extension platform. This strategy seeks to combine the naturally long plasma half-life of human serum albumin with the therapeutic effect of the peptide. Attachment of the peptide to the albumin molecule is achieved through either chemical conjugation of the peptide or by genetic fusion. The first part of this talk will focus on the use of conjugation and fusion to recombinant human albumin in the half-life extension of peptides, with a particular focus on those peptides currently under clinical development. In the second part of the talk I will explore the next generation of half-life extension utilising modified forms of recombinant albumin. The naturally extended half-life of albumin is regulated through a receptor mediated process called “FcRn recycling.” It is well understood that both albumin and IgG molecules bind to the FcRn receptor in a pH-dependant manner to prevent proteolysis in the endosome and facilitate recycling back into the plasma. Furthermore, a relationship between plasma half-life and affinity of IgG to this receptor has been well established. Recent work has sought to identify and modify the FcRn binding domain of albumin to allow control of the binding affinity of albumin to FcRn and ultimately alter the pharmacokinetics of the albumin molecule. This will offer flexible half-life extension that could revolutionise dosing regimens for peptide therapeutics by providing half-lives from 1 week to up to a month.

LY2605541, a Novel Long-acting Basal Insulin

Mel Prince, M.D. | Senior Medical Director, Lilly Diabetes
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The basal insulin analog LY2605541 (LY) is PEGylated insulin lispro which has a large hydrodynamic size which contributes to delaying insulin absorption and reducing clearance. In an acute study in dogs, LY demonstrated a greater effect on hepatic action and a delay in peripheral action compared with human insulin. In Phase 1 studies, LY had a duration of action of at least 36 hours, a peak to trough fluctuation <1.5, low intra-subject variability and PK properties unaffected by renal impairment. In two Phase 2 studies, LY was associated with weight loss and greater (T1DM) or similar (T2DM) improvements of glycemic control than insulin glargine (IG). In T1DM, LY was associated with a statistically significant higher overall hypoglycemia rate potentially related to the unanticipated need for lower prandial insulin doses but a statistically significant lower rate of nocturnal hypoglycemia compared with IG. In the T2DM study, there were similar overall rates of hypoglycemia, but LY treated patients had a reduced rate of nocturnal hypoglycemia compared to IG, after adjusting for baseline hypoglycemia events. In a subset of T2DM patients assessed by CGM (continuous glucose monitoring), compared to IG, LY was associated with less time spent in hypoglycemia and with lower incidence of hypoglycemia. In both studies, following LY treatment, mean ALT/AST levels statistically significantly increased from baseline and were higher than IG with mean levels remaining within the normal range. LY-treated T1DM patients had a modest increase in triglycerides (TG) and LDL-C, and decrease in HDL-C that were statistically significant from baseline and compared to IG. In LY-treated T2DM patients, TG were not significantly different from baseline, but statistically higher compared to IG with no significant difference in LDL-C or HDL-C from baseline or compared with IG. Taken together, the preclinical data and the body weight, lipid values, and liver function tests suggest that LY may have a novel mechanism of action distinct from other therapeutic insulins

Tunable Half-life Extension of Therapeutics by Controlled Chemical Release from Macromolecular Conjugates

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Conjugation to macromolecular carriers is a proven strategy for improving the pharmacokinetics of drugs, with many stable polyethylene glycol (PEG) conjugates having reached the market. Stable conjugates suffer several limitations: loss of drug potency due to conjugation, confining the drug to the extracellular space, and the requirement for a circulating conjugate. Current research is directed toward overcoming such limitations through releasable conjugates in which the drug is covalently linked to the carrier through a cleavable linker. Satisfactory linkers that provide predictable cleavage rates tunable over a wide time range that are useful for both circulating and non-circulating conjugates are not yet available. We have developed such conjugation linkers on the basis of a non-enzymatic -elimination reaction with preprogrammed, highly tunable cleavage rates. A set of modular linkers were prepared that have a succinimidyl carbonate group for attachment to an amine-containing drug or prodrug, an azido group for conjugation to the carrier, and a tunable modulator that controls the rate of -eliminative cleavage. The linkers provide predictable, tunable release rates of ligands from macromolecular conjugates both in vitro and in vivo, with half-lives spanning from a range of hours to >1 year at physiological pH. A circulating PEG conjugate achieved a 56-fold half-life extension of the 39-aa peptide exenatide.

With circulating carriers, half-life extension becomes limited by the renal elimination rate of the carrier. An approach to overcoming this constraint is to utilize non-circulating, biodegradable subcutaneous implants as drug carriers that are stable throughout the duration of drug release. We have recently used -eliminative linkers to both tether drugs to and crosslink PEG hydrogels, and demonstrate tunable drug release and hydrogel erosion rates over a very wide range. By using one -eliminative linker to tether a drug to the hydrogel, and another -eliminative linker with a longer half-life to control polymer degradation, the system can be coordinated to release the drug before the gel undergoes complete erosion. The practical utility is illustrated by a PEG hydrogel-exenatide conjugate that should allow once-a-month administration, and results indicate that the technology may serve as a generic platform for tunable ultra-long half-life extension of potent therapeutics.

Stapled Peptide Drugs: Translation to the Clinic

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Stapled Peptides are a promising breakthrough therapeutic modality for the treatment of many diseases by modulating intracellular or extracellular targets. Specifically, a–helical protein-protein interactions provide broad target space for many diseases in which traditional small-molecule or biologic strategies have significant limitations. Aileron is developing promising stapled a–helical peptides for numerous intracellular and extracellular targets and such studies have integrated drug design, structural biology, target binding, cell biology, pharmacokinetics, metabolism and in vivo efficacy. Highlights of Aileron's advancement of Stapled Peptides and translation to the clinic will be described.

XTENylation Offers a Biodegradable Alternative to PEGylation

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Amunix has developed XTEN, an unstructured protein-based polymer that mimics the biophysical properties of PEG, to extend the serum half-lives of attached therapeutics. To enable chemical conjugation of XTEN to peptides and other molecules, amino and/or thiol groups are included in its amino acid sequence at controlled locations. These reactive XTENs can be produced in large quantities by microbial fermentation, and subsequent purification yields homogenous polymer. Whereas reagent PEG is provided as a heterogenous mixture, XTEN and its conjugates are discreet molecular species observable by ESI-MS, permitting the efficient monitoring of coupling reactions. This monodisperse nature of XTEN also greatly facilitates the characterization of its derivatives by HPLC. XTENs of varying length and containing varying numbers of functional groups are available to expedite the in vitro and in vivo evaluation of XTEN-drug conjugates. XTEN has been fused to multiple approved biopharmaceuticals to extend serum half-life while retaining in vivo potency. The XTEN polypeptide is stable in serum but, unlike PEG, is readily degraded by proteases following internalization into cells, eliminating the risk of kidney vacuolation. Multiple preclinical studies have yielded no evidence of XTEN immunogenicity, and clinical evaluation of two XTENylated products is currently in progress. Thus, XTENylation is an effective method for half-life extension and provides key benefits for conjugation to pharmaceuticals.

XEP-018: A New Myorelaxant Peptide Lead Compound from the Venom of the Cone Snail *Conus consors*

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Venoms are made of hundreds of peptides optimized by Nature as highly selective and potent bioactives that already led six drugs to market. We pioneered Venomics drug discovery and lead optimization strategies integrating bioactivity-guided (unique collections of HTS-ready pre-fractionated venoms), structure-driven (venom peptidomics & proteomics, venom glands transcriptomics and genomics) coupled to biocomputing-assisted (proprietary databases and bioinformatic tools). We present the discovery of a new conotoxin from the venom of Conus consors collected in the Chesterfield Islands (New Caledonia). XEP-018, consisting of 22 amino acids folded by three disulfide bridges, shares homology with the μ-conopeptides family. Synthetic XEP-018 decreased twitch tension in mouse hemidiaphragms (IC50 = 150 nM), and displayed a higher blocking effect in mouse extensor digitorum longus muscles (IC50 = 46 nM), compared with the related m-conotoxins SIIIA, SmIIIA and PIIIA. An extensive pharmacological profiling of XEP-018 confirmed its drugability potential as inhibitor of the neuromuscular transmission. XEP-018 blocked $Na_v1.4$ (IC50 = 1.3 nM) and, to a lesser extend, the neuronal $Na_v1.2$ channels in a long-lasting manner. Cardiac $Na_v1.5$ and DRG-specific $Na_v1.8$ channels were not blocked at 1 mM. XEP-018 also blocked the a3b2 nAChR subtype (IC_{50} = 450 nM) and revealed weak activity on the a7 and a4b2 subtypes. Structure determination of XEP-018 revealed some similarities to a-conotoxins acting on nAChRs.

The most striking observations were linked to a hardly reversible effect of XEP-018 despite repetitive washings, a high selectivity and a potency that is several thousand folds higher than lidocaine related drugs. Even more surprising was the incapacity of XEP-018 to fully block $Na_v1.4$ conductance. Even at high concentrations, there was always a 5% remaining current. Being so potent and offering unprecedented long-lasting action without full blockade, XEP-018 offers opportunities for new myorelaxant biomedical applications such as dystonia by atypically acting on original physiological targets, i.e. sodium channels of unmyelinated and myelinated axons.

Gut-peptide Based Poly-pharmacy for the Treatment of Obesity & Diabetes

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Emerging insights from recent advances in metabolic diseases research suggest that one or several patterns of multiple neuro-endocrine factors are necessary for sustained modulation of body fat or metabolism set points. Gut hormones appear to reside at the core of these master–key-like signaling patterns, as indicated for example by bariatric surgery research. Over the last 7 years, we have therefore tested a large series of combination therapies based on multiple gastrointestinal and adipocyte derived signals. Balanced single molecule peptide hormone based GLP1-glucagon and GIP-GLP1 co-agonists exhibited superior body weight loss and glucose metabolism benefits in mouse models of obesity and diabetes, as compared to any established mono-agonists. Preliminary translational data indicate efficacy of GIP-GLP1 co-agonists in non-human primates. Since co-infusion of a soluble and stable glucagon mono-agonist in parallel with GIP-GLP1 co-agonist treatment provided additional benefits, a series of single molecule GIP-GLP1-glucagon tri-agonists were generated and validated. These novel tri-agonists again showed unprecedented metabolic and body weight benefits in mouse and rat models of obesity and diabetes. In a parallel approach single molecule conjugates combining a peptide (e.g. GLP1) with a steroid (e.g. estrogen) were generated to maximize metabolic benefits and minimize potential toxicity by specifically targeting a subset of estrogen receptors in GLP1-receptor carrying cells. Such peptide carrier based targeting of a specific subset of nuclear hormone receptors was successful: Administration reversed hallmarks of the metabolic syndrome in diet induced obese and insulin resistant mice without causing any detectably side effects or toxicity. The above described novel single molecule approaches to polypharmaceutical therapeutics carry the potential to open new perspectives for the treatment of metabolic diseases such as diabetes and obesity.

Peptide Macrocyclization Enabled by Amphoteric Molecules

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Peptide macrocycles are creating a prominent footprint in contemporary drug discovery programs. These molecules belong to a privileged and underexplored class of compounds with potential to interrogate extended protein surfaces. Approaches to macrocycle synthesis have been aimed at the rapid production of structurally diverse molecules. While biological selection methods such as phage display are unsurpassed in their capacity to produce extremely diverse collections for screening, these methods generally fail to address the most significant challenge facing drug discovery: production of a cell permeable, drug-like candidates with low toxicity. In addition, biological methods fail to generate the coveted medium-sized macrocycles, the most attractive molecules of this general class. On the other hand, synthetic methods aimed at solving these important challenges have lagged behind. Our novel technology offers a means to rapidly produce macrocycles equipped with structural markers that enable optimization of both cell permeability and production of diverse compounds that are differentiated on the basis of their three-dimensional shapes. Our platform is based on amphoteric aziridine aldehydes, reagents with an uncanny ability to tie up the loose ends of linear peptides into circular shapes. We have produced new classes of macrocyclic molecules in small, medium, and large size categories. We have developed a methodology that produces novel molecules that resist proteolytic cleavage and readily enter cells by way of passive membrane diffusion. Our current efforts are directed towards understanding the emergence of medicinally relevant folded structures within macrocycles and at striking the balance between hiding polar hydrogen bonds to enable cell permeation vs engaging these pivotal contacts in interaction with biological targets.

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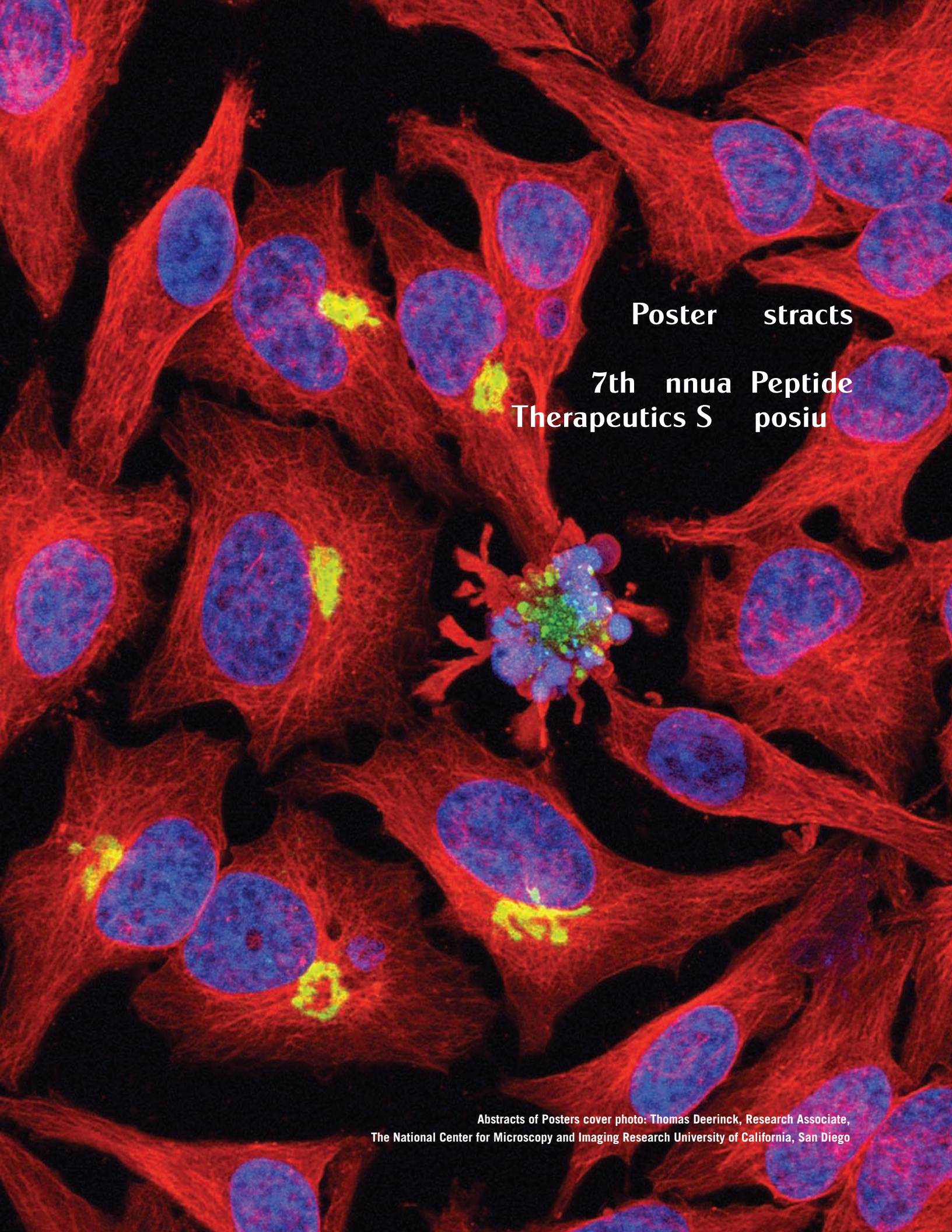

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The National Center for Microscopy and Imaging Research University of California, San Diego

P01 Convergent Branched Peptide Synthesis

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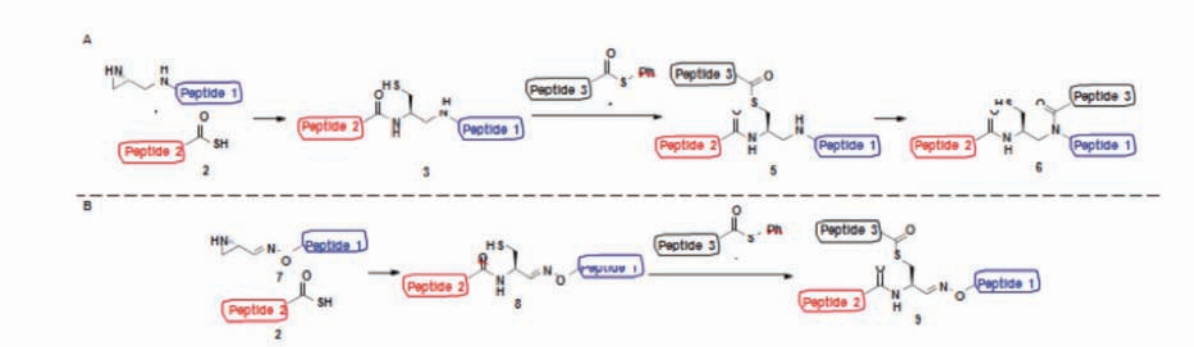
Branched peptides have been of great interest in the field of biomaterials, drug delivery and *de novo* protein synthesis. They can be used towards the construction of novel tertiary structures.[1], The stepwise construction of branched peptides is often plagued with orthogonal protecting groups. Convergent routes utilizing ligation protocols have been described in the literature, but the corresponding methods lack selectivity.[2] Utilizing dimeric aziridine aldehydes, we are able to efficiently access N-terminated aziridine peptide conjugates (1,7).[3] The aziridine-terminated peptide conjugates can be ring-opened using a Cterminal peptide thioacid resulting in a peptidomimetic backbone at a cysteine residue (3,8).[4,5] The resulting peptide can then undergo a trans-thioesterfication to give branched conjugates (5,9). Using this method we have complete control on the makeup of each branch. The two different pathways (A and B) offer added diversity at the backbone of the branched peptide. In addition, the branched thioester product of route A (5) can further be rigidified through an S-to-N acyl transfer on to the nearby amine to give a peptoid–like branching point (6). Synthetic advances towards this convergent branched peptide synthesis will be highlighted.



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P02 The Importance of Introducing Semi-Preparative Screening in Method Development - A Case Study

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Preparative HPLC differs from analytical HPLC since it needs to be performed in the nonlinear part of the adsorption isotherm in order to maximize important parameters such as column loading and productivity. In preparative HPLC method development screening is commonly performed with analytical injections in order to establish and compare selectivity in different chromatographic systems. From the screening one or more candidate phases are selected for a closer preparative study with overloaded injections. Typically, the method development is concluded by analyzing the fractions from the preparative study, to establish important final parameters, such as purity, yield and productivity. However, our case study illustrates the risk of relying only on selectivity during preparative method development.

In this case one specific chromatographic system was nearly overlooked due to low selectivity. However, when performing overloaded injections, a favorable adsorption isotherm was seen which proved beneficial for the purification. This effect was not seen during the analytical screening process and illustrates the importance of introducing semi-preparative screening in the early parts of preparative HPLC method development.

P03 Development of an SPE-LC/MS/MS Method for Quantitation of Four Synthetic Insulins in Human Plasma: Challenges of working with Large Peptides

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Insulin is perhaps one of the best known and earliest peptide therapeutics. Although biologics have historically been quantified using ligand binding assays (LBAs), over the past few years, there has been a trend toward the analysis of large molecules by LC-MS/MS. LC-MS/MS has the advantage of shorter development times, greater accuracy and precision, the ability to multiplex, and can readily distinguish between closely related insulins. Intact insulins are particularly difficult to analyze by LC-MS/MS, as MS sensitivity is low due to poor transfer into the gas phase and poor fragmentation due to the presence of multiple stabilizing disulfide bonds. In addition, insulin and its analogs suffer from non-specific binding and poor solubility, making LC and sample preparation method development difficult. A few LC-MS/MS methods exist, however most involve time-consuming and laborious immunoaffinity purification and/or multidimensional or nano-flow LC. This work provides a single, simple method for the simultaneous quantification of multiple intact insulins in human plasma, achieving LODs of 0.25 ng/mL or less.

The developed method was successfully used to quantify insulins glargine, detemir, aspart , and glulisine in human plasma. The specific SPE format allows sample concentration without evaporation, eliminating the possibility of adsorptive losses during dry-down. In addition, a novel C18 chromatographic column with a charged surface stationary phase produced peak widths for insulin that were significantly narrower than traditional C18 columns using formic acid in the mobile phase, effectively mimicking the behavior typically seen with TFA.

P04 Two Dimensional LC-SRM Assay for Trastuzumab in Human Serum

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Development of a generic, fast and automated LC-SRM method for quantification of therapeutic monoclonal antibodies in human serum using two dimensional LC-SRM with heart cutting.

Human serum samples (50 uL) were spiked with identical concentrations of trastuzumab and 13C15N-isotopically labeled peptide analogues, in the concentration range of 1-50 nM. The protein mixture was denatured, reduced, alkylated and digested with trypsin. After SPE clean-up, peptide digests were analyzed by analytical scale (2.1 mm ID) two dimensional chromatography coupled to tandem mass spectrometry (2D-LC SRM). Peptides were separated by RP chromatography at pH 10 in the first chromatographic dimension. Peptides used for trastuzumab quantification were transfered to the second chromatographic dimension undergoing an orthogonal separation at pH 2.5.

SRM parameters and chromatographic conditions were optimized to achieve the required sensitivity (1 nM or 150 ng/mL trastuzumab in serum).

The digestion protocol was optimized using two extended isotopically labeled peptide (GR_FTISADTSK and DTYIHWVR_QA) spiked in serum at the beginning of the experiment. Compared with the sensitivity of the assay in the absence of the serum matrix, the sensitivity of the single dimensional LC-SRM assay in serum was significantly reduced for both peptides (4-5 fold). After 2D chromatographic separation, the sensitivity of the LC-SRM assay was restored to 75% of the sensitivity observed in the absence of the serum digest matrix.

The quantification method developed is simple, specific, robust and has been implemented in a high-throughput (96-well plate) format. The limit of quantification (LLOQ) for trastuzumab in serum was 150 ng/mL (or 1 nM).

P05 Development of Peptidomics Assays for Mapping the Epitopes Derived from Collagen I and II Processing by Metalloproteases and Cathepsins

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In recent years several lines of research indicate the relevance of extracellular matrix remodeling in tissue homeostasis. Several enzymes, including matrix metalloproteinases (MMPs), have been described as modulators of tissue microenvironment through degradation of the extracellular matrix (in particular collagen metabolism), processing of cytokines and growth factors and processing of cellular surface receptors. The MMPs are the primary enzymes involved in collagenolysis. Peptide fragments generated by collagen processing could be presented to the immune system for generation of either tolerance or immunity. In order to reveal new potential MHC class II restricted epitopes processed by MMPs that are distinct and additional to peptides processed by endosomal proteases, we developed peptidomics assays in which the antigen processing was coupled with nano-LC ESI MS/MS sequencing of the peptides epitopes. New peptides derived from collagen-I and -II processing by the recombinant cathepsins S, B, D and L and by the late endosomal fraction isolated from dendritic cells (DCs) were sequenced and compared with the epitopes derived from the activity of pure recombinant MMPs (such as MMPs 1, 2, 3, 7, 9, 13, 14)) and from the enzymatic activity of the plasma membrane fraction isolated from the human primary DCs, leading to the generation of new peptides epitopes (with molecular weights of 800-6,000 Da). The new discovered epitopes suggest new mechanisms for the extracellular antigen processing distinct from those present in the late endosomal compartment.

MMP-2
G.ARGFPGTPGLPGV.K
A.AGRVGPPGSNGNPGPPGPPGP.S
P.PGNPGPPGPPGPPGPGIDMSA.F
F.PGLPGPSGEPGKQGAPGASGDRGPPG.P
R.GRTGPAGAAGARGNDGQPGPAGPPGPVG.P
G.AQGPPGLQGMPGERGAAGIAGPKGDRG.D
A.QLGVMQGPMGPMGPRGPPGPAGAPGPQ.G
G.NPGPPGPPGPSGKDGPKGARGDSGPPGR.A

collagen II epitopes

P06 Molecular Docking and in Silico Structure-based Design of D-Phe-Pro-D-Arg-derived Direct Thrombin Inhibitors

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New peptidic non-covalent direct thrombin inhibitors (DTIs) were designed by generating peptide lead compounds derived from the substrate sequence Phe(P3)-Pro(P2)-Arg(P1). The free energy of interaction between each ligand and thrombin was calculated with the built-in molecular mechanics force field (MMFF) provided by the docking software SCULPT (Accelrys). During the original screening, the hexapeptides [D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1¢-P2¢-P3¢-$CONH_2$] and pentapeptides [D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1¢-P2¢-CONH2] were used as scaffolds for developing the optimized final tetrapeptide lead sequence, D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1¢-CONH2. Once the lead tetrapeptide scaffold was found to have higher affinity for thrombin than the hexa and pentapeptides, based on structure-activity relationship (SAR) studies on thrombin inhibition conducted *in vitro*, new peptide candidate inhibitors were further designed as derivatives of the tetrapeptide motif D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1¢-$CONH_2$. New peptide sequences were developed by varying the P1 positions both with L and D natural or non-natural amino acids, covering a wide range of chemical structures. Trials for optimization of P3 position were further performed with un-natural amino-acids, such as D-3,3-di-Phenylalanine, trans and dihydrocinnamic acids, (L)/(D)-Tic [1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid], (L)/(D)-Thi [Thienylalanine] and D-Naphthylalanine (D-Nal). The protein template used in all molecular docking experiments was the structure of human a-thrombin in complex with the covalent inhibitor PPACK (PDB entry 1ABJ). The thrombin:peptide complex was minimized using the *SCULPT* built-in molecular mechanics force field (MMFF94). After each round of minimization, the free energy of interaction (scoring function) was assessed using both Van der Waals and electrostatic force fields. Preliminary kinetics for in vitro inhibition of alpha thrombin cleavage of the chromogenic substrate S2238 established a new structure-activity relationship (SAR) for the tetrapeptides with new unnatural aminoacids at (P3) position.

P07 Synthesis of Drug-like Macrocycles and Bridged Peptides as Modulators of Protein-Protein Interactions

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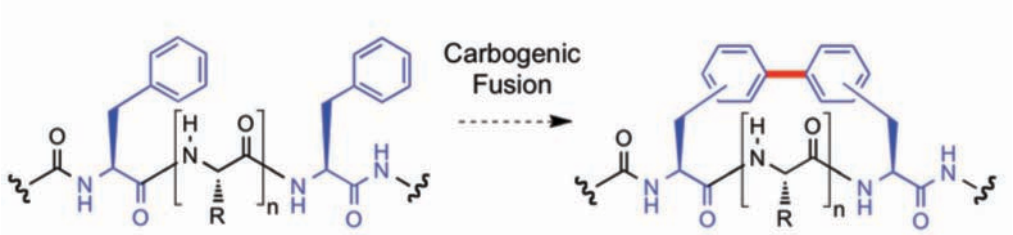
Protein-protein interactions (PPIs) represent a large and critically important sub-set of molecular interactions, integral to both extra-cellular and intra-cellular signaling pathways, typically involving large, shallow protein surface area contacts. The modulation of PPIs within cells is a largely unexploited area in drug discovery that offers huge therapeutic potential. Macrocyclic systems represent a compelling strategy for addressing intracellular PPIs, since the intrinsic conformational constraint imposed by their cyclic structure offers the prospect of improved physicochemical behavior and lower entropic losses on binding.

We will describe our results in two related areas of research:

1. Development of novel macrocyclization reactions and optimized conditions that allow efficient generation of libraries of drug-like macrocycles

2. Application of these reactions to the synthesis of side-chain to side-chain bridged macrocyclic peptides that can exhibit a stabilized bioactive conformation or secondary structure, along with reduced susceptibility to protease cleavage



P08 Branched Diketopiperazines as a Universal Scaffold for Preparation of Orally Active Small Peptide Derivatives

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Oral delivery of peptide pharmaceuticals has long been an important challenge in drug development. We have developed a new chemical platform based on branched diketopiperazines for creating orally available biologically active peptidomimetic and other “chimerical” compounds. The platform includes a diketopiperazine bio-carrier with built-in functionally active peptide fragments and bioactive molecules covalently attached via linkers. The functional groups comprising biologically active compounds have been attached to these scaffolds via biodegradable chemical linkers to produce novel chemical entities that possess multifunctional characteristics. Several families of compounds that exhibit immunostimulating, immunosuppressing, adjuvant and analgesic activity have been prepared. The platform technology we have developed allows taking a small peptide with a particular biological activity and transforming it into an orally available compound that possesses the same type of activity. Using the conventional solution method, we have synthesized a number of dually functional compounds that combine a diketopiperazine scaffold and known small molecule pharmacophores, such as fatty acids, carbohydrates, antibiotics and non-steroid anti-inflammatory molecules. This approach has been applied to molecules whose mass ranges from 300 to 1000 Daltons.

P09 Selective MC1R Agonists

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The endogenous neuropeptide hormone α-MSH has agonist activity at melanocortin (MC) receptors MC1-, 3-, 4- and 5-R and has demonstrated activity, often on par with dexamethasone, in numerous inflammatory models. Experimental evidence indicates that MC1-R agonism may be responsible for the anti-inflammatory activity of α-MSH. MC1-R agonism has demonstrated utility in preclinical disease models such as colitis, multiple sclerosis, and nephrotic syndrome.

Currently, non-selective peptides with MC1-R agonism are being evaluated in clinical trials in ulcerative colitis and acute kidney injury. In order to utilize the anti-inflammatory activity of MC1-R agonism, an ultra-selective MC1-R agonist with appropriate drug-like properties is needed.

A series of cyclic peptides have been identified with picomolar MC1-R binding affinity and functional activity. A number of the compounds are several orders of magnitude more selective for MC1-R than for MC2-, 3-, 4- or 5-R. Pharmacokinetic studies demonstrate significant in vivo stability, which should allow evaluation in vivo with once or twice daily dosing. In addition to the MC1-R hypothesis, there is literature precedent which suggests MC3-R agonism may also play a role in the reduction of inflammation. Peptides have been developed which demonstrate similar activity at both MC1-R and MC3-R. Data will be presented on selected compounds to exemplify the receptor affinity, functional activity and pharmacokinetic properties of these novel cyclic peptides.

P10 Peptide Macrocyclization via Pd-Catalyzed Chemoselective Indole C-2 Arylation

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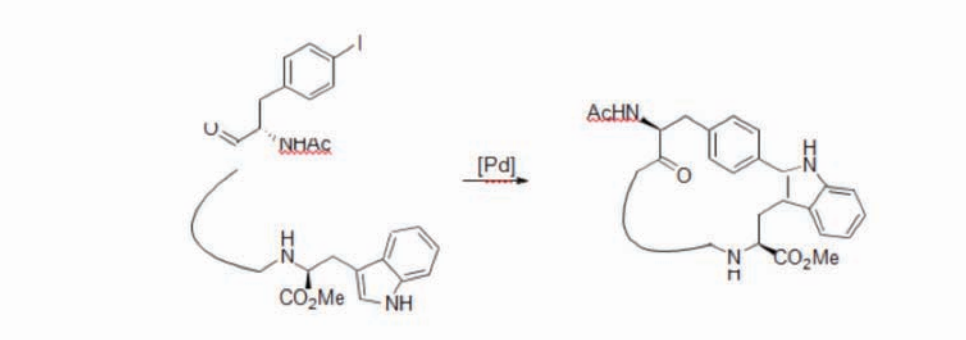
Peptide based macrocycles have found wide application, from therapeutic agents to biomaterials. Among various strategies for generating macrocyclic peptides, creation of side-chain to side-chain bridges has the advantage of not disrupting potential interaction between the N- or C-termini and the target receptor. Depending on the functional groups in the side chains, the established macrocyclization reactions have included ring-closing metathesis, amide-coupling reactions, and azide-alkyne cycloadditions. The use of transition-metal catalysts for C–C bond formation between aromatic rings constitutes an attractive way to construct biaryl-macrocycles, which represent a carbogenic fusion of naturally occurring aromatic amino acid side-chains. Here we present a macrocyclization between the side chains of tryptophan and phenylalanine derivatives through a palladium- catalyzed C–H activation reaction. This macrocyclization features a chemoselective C–H arylation of indole derivatives. The scope and the future direction of this chemistry will be discussed.



P11 A Biodegradable Hydrogel Drug Delivery System for Tunable Half life Extension

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We are developing non-circulating subcutaneous implants having independently tunable rates of drug release and biodegradation as carriers for drug delivery. Conjugation to longer-lived macromolecular carriers such as PEG is a common method for half life extension for peptide, protein, and small molecule therapeutics, yet this approach is limited by the rate of systemic clearance of the conjugates. Non-circulating drug-loaded implants provide a potential route to the goal of ultra-long half life extension, but to date it has not been possible to control and coordinate the rates of drug release and implant degradation. We have recently reported the development of self-cleaving linkers for attachment of therapeutics to macromolecular carriers. These linkers release the native unmodified drug, through a b-elimination reaction, with tunable and predictable rates ranging from hours to over one year (Santi et al., PNAS (2012) 109(16):6211-6216). By using our -eliminative linkers both to attach drugs to as well as crosslink PEG hydrogels, we can independently control the rate of drug delivery and the subsequent rate of polymer degradation over a very wide range. The system can be coordinated to completely release the drug prior to total erosion of the polymer. This technology has been applied to the generation of a PEG hydrogel-exenatide conjugate that may allow for once-a-month administration, and should serve as a generic platform for half-life extension of potent peptide, protein, and small molecule therapeutics.

P12 Photocontrol of Alzheimer´s Amyloid- β Peptide

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The aggregation of amyloid-β peptide (A β_{1-42}), causing toxicity, is a critical step in the pathogenesis of Alzheimer disease (AD). Results of AD related studies are difficult to compare because A β_{1-42} aggregation is fast and badly controllable under physiological conditions. In order to control aggregation and toxicity we designed light-switchable A β_{1-42} analogues that enable the conversion of nontoxic fibrils into toxic oligomers in a spatiotemporally controlled fashion simply by illumination.

P13 Photoinactivation of Gram positive and Gram negative bacteria with the antimicrobial peptide (KLAKLAK)2 conjugated to the hydrophilic photosensitizer eosin Y

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We test the hypothesis that the antimicrobial peptide (KLAKLAK)2, enhances the photodynamic activity of the photosensitizer eosin Y upon conjugation. The conjugate eosin-(KLAKLAK)2 was obtained by solid-phase peptide synthesis. Photoinactivation assays were performed against the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and multi-drug resistant *Acinetobacter baumannii* AYE, as well as the Gram positive bacteria *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Partitioning assays were performed with E. coli and S. aureus. Photohemolysis and photo-killing assays were also performed to assess the photodynamic activity of the conjugate towards mammalian cells. Eosin-$(KLAKLAK)_2$ photo-inactivates 99.999% of 10^8 CFU/mL of most bacteria tested at a concentration of 1 μM or below. In contrast, neither eosin Y nor $(KLAKLAK)_2$ cause any significant photoinactivation under similar conditions. The increase in photodynamic activity of the photosensitizer conferred by the antimicrobial peptide is in part due to the fact that $(KLAKLAK)_2$ promotes the association of eosin Y to bacteria. Eosin-$(KLAKLAK)_2$ does not significantly associate with red blood cells or the cultured mammalian cell lines HaCaT, COS-7 and COLO 316. Consequently, little photo-damage or photo-killing is observed with these cells under conditions for which bacterial photoinactivation is achieved. The peptide $(KLAKLAK)_2$ therefore significantly enhances the photodynamic activity of eosin Y towards both Gram-positive and Gram-negative bacteria while interacting minimally with human cells. Overall, our results suggest that antimicrobial peptides such as $(KLAKLAK)_2$ might serve as attractive agents that can target photosensitizers to bacteria specifically.

P14 Prepaying the Entropic Cost is Detrimental for Inhibitor Development against Cbl(TKB)

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Imposing conformational constraints onto lead peptides is a common strategy fore developing inhibitors of protein-protein interactions (i-PPI). There are a number of examples where such constraints improve the binding affinity. Here, we describe our studies toward the design and development of a peptidomimetic inhibitor of the tyrosine kinase binding domain of Cbl, Cbl(TKB). Solving the crystal structure of Cbl in complex with the most active peptide, pYTPEP, confirmed that the peptide adopted a similar binding mode as the long peptide. To develop such a defined system, an unnatural amino acid termed a proline templated glutamate (ptE) was synthesized. The ptE was used to generate a series of peptides that could be used to directly investigate the result of backbone rigidity with the sequence pYTPXP. NMR NOE experiments on the conformation of the peptides confirmed that the peptide panel adopted the bioactive conformation in solution. The panel of peptides was then quantitatively compared in a competitive fluorescence polarization (FP) assay. We expected nearly an order of magnitude increase in binding affinity with the designed peptide with ptE residue. The results, however,

reveal a loss of ~5-fold activity. To the best of our knowledge, these are the first of such results where preorganizing a lead peptide to the bound conformation results in a loss of activity. This lends support to the hypothesis that Cbl-phosphopeptide binding follows an induced fit model of ligand binding. As a result, rational design of Cbl inhibitors should require more flexible ligands instead of rigid ligands.

P15 Development of Modified Human Serum Albumins for Peptide and Protein Half-life Extension: Towards Monthly Dosing

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Purpose

Human Serum albumin (HSA) is a plasma protein with a long plasma half-life. This extended half life has been utilised therapeutically as a mechanism for increasing the half-life of attached peptides and proteins. The long half life of the albumin is due to a receptor mediated process called, FcRn recycling. This process is similar to that characterised for IgG, where a direct relationship between affinity for the FcRn receptor and plasma half-life has been determined. Here we describe modification of albumin with the aim of altering its affinity to the human FcRn (hFcRn) receptor and ultimately it's pharmacokinetics. The therapeutic potential of these modifications will be discussed.

Methods

A range of variant albumin molecules with a single point mutation and number of albumin fusion proteins were produced in a Saccharomyces cerevisiae expression system.

The binding affinity of the albumin variants and albumin fusions to the hFcRn receptor was evaluated by surface plasmon resonance (SPR). The pharmacokinetics of a range of variant molecules was evaluated in a rat model.

Results

SPR analysis of albumin variants with single point mutations demonstrated both increased and decreased affinity to the human FcRn receptor. A summary of the binding affinities from specific mutations is illustrated in Figure 1. Furthermore, pharmacokinetic data from a rat model demonstrated that an albumin variant with a high affinity to the hFcRn receptor had a half-life more than double that of a wild type albumin.

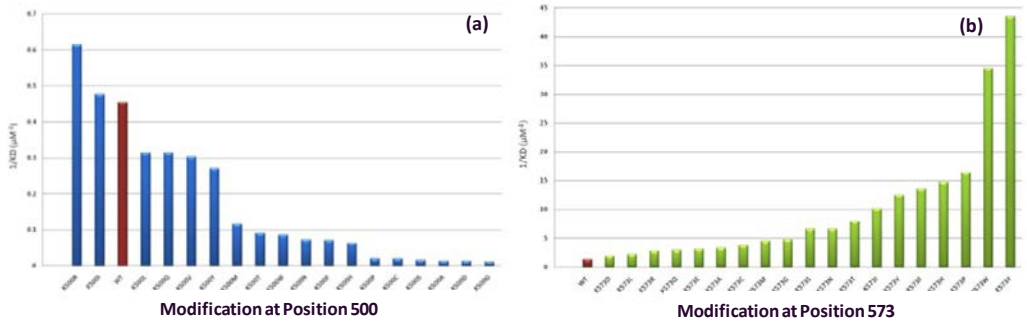

Figure 1. A summary of the binding affinities of variant albumin molecules to the human FcRn receptor obtained by SPR. (a) Modifications of the albumin molecule at position 500. (b) Modifications of the albumin molecule at position 573. The red line in each graph denotes the wild type albumin. The amino acid substitution is denoted by the single letter amino acid code.

In further studies evaluation of variant albumin fusions by SPR highlighted that attachment of a peptide or protein did not significantly alter binding to the hFcRn receptor.

Conclusions

Single point mutations in the albumin molecule can significantly alter binding to the hFcRn receptor. Furthermore, pharmacokinetic studies have confirmed that a specific variant with a high affinity for hFcRn had a half-life more than double that of wild type albumin in a rat model. We have also demonstrated that the effect of these modifications on hFcRn binding can be retained following the attachment of a protein or peptide to albumin. This technology has the potential to open the door to longer dosing intervals for therapeutic molecules.

P16 CAR Peptide, A Disease Selective Therapeutic Adjuvant For Targeted Drug Therapy

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A major limitation in the treatment of disease is the lack of therapeutic selectivity. Recent studies have shown that pathologically altered blood vessels express specific cell surface markers. These markers are detectable by specific homing peptides, which can be used for cell-targeted drug delivery. Tissue-penetrating homing peptides can activate a bulk transport pathway that translocates co-administered substances from the blood vessels into the target tissue. This "bystander effect" mechanism provides the advantage of delivery of a drug to target tissues without covalent coupling of the drug to the peptide. An adjuvant that enables therapeutic agents to specifically target areas of disease via a bystander effect without conjugation would be a significant advance that would allow for greater treatment efficacy with reduced side effects.

We have discovered a 9 amino acid cyclic peptide, CARSKNKDC (CAR), that selectively targets and internalizes into a range of diseased tissues. CAR targets hypertensive vessels, angiogenesis, fibrotic lesions, areas of inflammation, acute lung injury, asthma, wounds, and tumors. Interestingly, CAR peptide also enables a multiplicity of co-administered drugs (fasudil, Y-27632, imatinib, and sildenafil) to preferentially affect disease tissues to increase localized concentrations of therapeutics without requiring the drug to be conjugated to the CAR peptide adjuvant. There is also evidence to indicate that CAR possesses oral bioavailability. In addition to oral availability, CAR peptide has a long half-life (27 hours), and no toxic effects seen to date in pre-clinical models.

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P17 Novel Room Temperature Formulation of PEGylated Peptides and Proteins

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Conjugation of peptides and proteins to polyethylene glycol (PEG) has proven an effective strategy to improve drug solubility, increase retention of drugs in the circulation, protect against enzymatic digestion, slow filtration by the kidneys, and reduce immunogenicity. Recent improvements in PEG purity and monodispersity, as well as in monofunctional and efficient ligation steps has moved PEGylation to the early development stages as part of a strategy to improve biotherapeutic properties.

Despite these improvements, many PEGylated drugs require refrigerated storage at 2 to 8°C, adding significant cold chain management complexity and associated costs. We have developed a versatile formulation/delivery system that enables up to 2-year physicochemical and biological stability at room temperature. This delivery system consists of a proprietary combination of FDA approved excipients (GRAS) that have been safely employed in multiple products and is compatible with most commonly used preservatives. Experimental evidence suggests that the excipients work synergistically with the PEG to protect the peptide/protein from typical degradation pathways, such as hydrolysis, oxidation, and deamidation. This delivery system enables lower storage/transportation costs and facilitates patient compliance.

P18 Delivery of Macromolecules Using Fluorescent Cell-penetrating Peptides and Light: A Focus on Photo-endosomolysis

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Macromolecules that target intracellular processes are potential therapeutic agents. Attaching macromolecules to cell-penetrating peptides (CPP) like TAT facilitates their cellular internalization by endocytosis. Cytosolic delivery of macromolecules however, is inefficient due to entrapment of cargo within endocytic vesicles. Endocytosis is a bottleneck also because macromolecules subsequently get degraded in lysosomes if not delivered into cytosol.

TMR-TAT, a fluorophore-CPP conjugate, efficiently disrupts endosomal membranes on light irradiation using a mechanism that involves production of singlet oxygen. Mechanistic studies suggest that TAT acts on partially photo-oxidized membranes to cause lysis of membranes. As a result, TMR-TAT can cause delivery of co-incubated cargo molecules to the cell cytosol. However, endosomal release of TMR-TAT is accompanied by a dramatic membrane blebbing phenomenon that subsequently results in cell death. Our aim is to understand the mechanism behind TMR-TAT mediated cell death in order to circumvent cell death problem during FI-CPP mediated delivery.

Our data demonstrates that the endosomal release of TMR-TAT is accompanied by a sudden influx of calcium ions into the cytosol that originate from endocytic vesicles. This is followed by accumulation of calcium in the mitochondria. Inhibition of cell death in the presence of cyclosporin and ruthenium red, inhibitors of calcium import in mitochondria and the mitochondrial permeability transition pore (mPTP) suggests that opening of the mPTP leads to cell death. Therefore, lowering the rate of endosomolysis or reducing the dosage of light irradiation can help the cell cope up with the calcium entering the cytosol. Thus, understanding the relationship between endosomolysis and cell death can aid n development of better delivery protocols without causing cell killing.

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P19 First Uncyclotide from the Monocot Plant Panicum laxum of the Poaceae family and its Activity Against Drug Resistant Bacteria

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Cyclotides are disulfide-rich macrocyclic peptides from plants that display a wide range of biological activities. Their linear variants are uncyclotides which share high sequence homology but are unzipped at both ends as linear peptides. All known examples of cyclotides and uncyclotides thus far are isolated from dicot plants of the Rubiaceae, Violaceae, Cucurbitaceae, and recently the Fabaceae and Solanaceae. Here, we report the first example of uncyclotides, designated as panitide, in the monocot plant *Panicum laxum* of the Poaceae or commonly known as grass family. Activity testing showed that it was active against Gram-negative bacteria at low micromolar range. Interestingly, it also displayed potent activity against drug-resistant E. coli strains carrying NDM-1 gene. This is an exciting finding as NDM-1 producing bacteria are known to resist against most commercially available antibiotics. Overall, our study provides new lead compounds for drug development, and sheds new understanding about the distribution and evolution of uncyclotides in plants.

P20 Membrane-active Cyclotides from the Medicinal Plant *Clitoria ternatea*

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Cyclotides, which are plant-derived miniproteins with unique structural features, have attracted great interests from researchers because of their high potential as candidates for novel peptide therapeutics. Cyclotides possess three disulfide bonds and a cyclic backbone resulting from the ligation of the N- and C-termini. These properties make them extremely resistant against thermal, chemical and enzymatic degradation. In this study, we investigate the antibacterial and hemolytic

activities of cliotides, which are cyclotides from the butterfly pea *Clitoria ternatea*, a famous Ayurverdic herbal medicine with various therapeutic benefits. Cliotides displayed potent antibacterial activity specifically towards Gram-negative bacteria such as *E. coli*, *K. pneumonia*, *K. species*, *P. aeruginosa*, *A. baumanni* and *E. cloaceae* with minimal inhibitory concentrations (MICs) of as low as 0.5 μM, while being inactive against Gram-positive bacteria. Atomic force microscopy scanning showed that this specificity of cliotides is due to their ability to perturb the outer and/or cytoplasmic membranes of Gram-negative bacteria. Next, hemolysis assay revealed that cliotides are also able to lyse human red blood cells at concentrations of approximately 10 folds their respective MICs. These findings suggest that cliotides are membrane-active peptides that may serve as promising candidates for novel therapeutics development, as well as drug potentiators for use in combination with conventional antibiotic.

P21 Discovery and Characterization of Small Proteinaceous α-Amylase Inhibitors from *Allamanda oenotheraefolia*

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Proteinaceous α-amylase inhibitors play important roles in the regulation of starch metabolism and the plant defense against pests and pathogens. They are useful tools in crop protection and the management of obesity and diabetes. Here, we report the discovery and characterization of a novel family of small proteinaceous α-amylase inhibitors, allatide O1-O7 (aO1-aO7), from *Allamanda oenotheraefolia* of the Apocynaceae family using both proteomic and genomic methods. All seven allatides are comprised of 30 amino acids with six Cys residues arranged in a cystine knot (CK). This knotted structure confers allatides extraordinary tolerance to heat and enzymatic degradation by trypsin and chymotrypsin. Sequence analysis revealed their high homology to the Amaranth α-amylase inhibitor (AAI), the first and only member of the superfamily of CK α-amylase inhibitors reported to date. Genomic analysis showed that the allatide precursors contain three domains: an ER- signal sequence, a prodomain, and a mature peptide domain. Allatides displayed inhibitory activity specifically against the α-amylase from yellow mealworm with IC50 in micromolar range. With their stability against heat and digestive enzymes, allatides could be promising leads to develop α-amylase inhibitors for obesity and diabetes management

P22 Artificial Therapeutic Peptides Designed Through Disulfide Bond-Replacement Strategy

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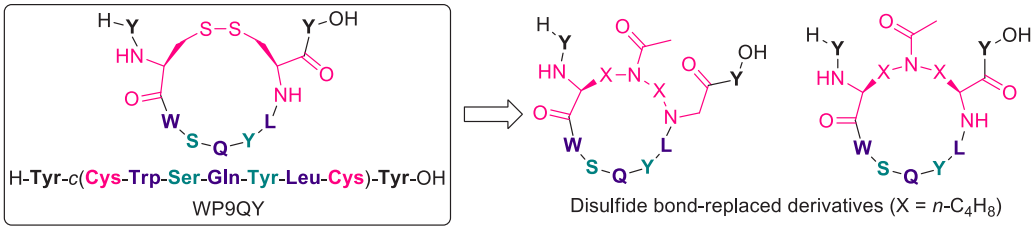
We have successfully demonstrated that the disulfide-bond replacement strategy is efficient for engineering therapeutic peptides using WP9QY that mimics TNF receptor ligand contact site as a model. Based on the soluble tag-assisted liquid-phase peptide synthesis using hydrophobic benzyl alcohol as supports, we prepared several WP9QY derivatives incorporating artificial amine cross-linkage instead of disulfide bond. Their biological activities were evaluated using murine bone marrow cells, suggesting that the bone resorption-blocking properties of the synthetic WP9QY derivatives were maintained comparable in magnitude to its native form, while the enzymatic stabilities were significantly improved. The WP9QY derivative was also found to function in murine low dietary calcium model, even the native form was not effective under same condition.



P23 Q Sphera: Letting-go of the Emulsion

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The Q Sphera microencapsulation platform has been developed to enable therapeutic peptides to be formulated into long-acting/sustained-release PLGA formulations with unprecedented precision and control. The technology seeks to arm the peptide formulator with another, significantly more subtle tool, with which to address the ever-increasing need[1] for long-acting delivery solutions for new peptide therapeutics. The Q Sphera process is a ***flow*** process, differing markedly from traditional batch reactors, and enabling exceptional control over the particle formation and encapsulation steps.

Building upon an industry-wide critical evaluation of existing emulsion-type processing methods for PLGA encapsulation, Q Chip has striven to develop an improved system, which offers a menu of options for the peptide formulator, including *monodispersity of product*, *uniformity of morphology*, *porosity control*, *density control*, and most importantly ***release-profile modification & initial burst control***.

In our previous work with microfluidic chip-based systems for PLGA encapsulation, Q Chip set out to eliminate FDA Class II fluid components (e.g. dichloromethane) from the PLGA encapsulation process, due to their difficult handling, impact upon API integrity and general safety concerns[2]. Both the Q Sphera development platform and pilot-scale aseptic microsphere manufacture facility use **only biocompatible solvents** such as DMSO, triacetin, glycofurol, alcohols (and also PEGs).

By combining the principles of microfluidic flow devices with smart engineering and high-throughput components[3], Q Chip has developed a proprietary peptide-formulation platform, designed to provide a more flexible, useful alternative in PLGA encapsulation, to accelerate and simplify the development of new peptide long-acting formulations[4].

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P24 A Human-Based Receptor Antagonist of Sustained Action Reveals Body Weight Control by Endogenous GLP-1

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Ex-4 (9-39)a is a well characterized GLP-1 receptor antagonist that suffers from two notable limitations, its nonhuman amino acid sequence and its relatively short in vivo duration of action. Comparable N-terminal shortening of human GLP-1 lessens agonism but does not provide a high potency antagonist. Through a series of GLP-1/Ex-4 hybrid peptides, the minimal structural changes required to generate a pure GLP-1-based antagonist were identified as Glu16, Val19, and Arg20, yielding an antagonist of approximately 3-fold greater in vitro potency compared with Ex-4 (9-39)a. The structural basis of antagonism appears to result from stabilization of the α-helix combined with enhanced electrostatic and hydrophobic interactions with the extracellular domain of the receptor. Site-specific acylation of the human-based antagonist yielded a peptide of increased potency as a GLP-1 receptor antagonist and 10-fold greater selectivity relative to the GIP receptor. The acylated antagonist demonstrated sufficient duration of action to maintain inhibitory activity when administered as a daily subcutaneous injection. The sustained pharmacokinetics and enhanced human sequence combine to form an antagonist optimized for clinical study. Daily administration of this antagonist by subcutaneous injection to diet-induced obese mice for 1 week caused a significant increase in food intake, body weight, and glucose intolerance demonstrating endogenous GLP-1 as a relevant hormone in mammalian energy balance in the obese state.

P25 Ultracentrifugation as an Approach for Measurement of Plasma Protein Binding of Peptidic Drug Candidates

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Purpose:
Commonly used methods for the assessment of plasma protein binding (PPB), such as ultrafiltration (UF) and equilibrium dialysis (ED), utilize membranes to separate free drug from protein bound drug. However, these approaches pose two major disadvantages for larger molecules such as peptides: (1) non-specific binding of molecules to the membranes/containers and (2) pore size influencing/restricting free transit of unbound peptides across the membrane. Since the above issues interfere with an accurate measurement of PPB, we developed an alternate "membrane free" PPB measurement approach, ultracentrifugation (UC), for studying PPB of peptidic drug candidates.

Methods:
UC was performed using a micro-ultracentrifuge at various speeds and durations. Rat plasma and four synthetic peptides were used to optimize the system; arginine vasopressin (MW 1084), Dynorphyn A 1-17 (MW 2149), ghrelin (MW 3315) and NPY (MW 4255). "Protein free" middle layers obtained post centrifugation were studied to determine any remaining protein contamination, unbound peptide concentration loss, and ultimately PPB. Samples taken from the UC middle layer were analyzed for total protein using protein assay kits or for unbound drug concentrations using UHPLC or LC/MS/MS.

Results:
The endogenous plasma protein residue in the UC middle layer after ultracentrifugation for 30, 60 and 120 min at 535,000g and 770,000g were found to be < 0.1%-4.5%. After UC at the above mentioned speeds for 30-60 min, the loss of the 4 test peptide analytes in the "protein free" middle layer was 3- 47%. The optimal UC condition for the peptides tested was determined to be 770,000g for 30 min at 370C.

Conclusions:
The above UC method has been proven to be more reliable for larger molecules such as peptides than the commonly used (membrane based) UF or ED methods. Under the optimal condition described herein, the total protein contamination in the UC middle layer was < 0.1%. This might result in an unbound fraction over estimation with a relative error of < 2% for compounds with PPB up to 95%. The peptide loss under this condition was between 3 and 12% for molecules weighing from 1 kDa to 4 kDa.

P26 Impact of the Oligomerization State of the MPER on HIV-1 Neutralizing Antibody Affinity

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The membrane proximal external region (MPER) of the HIV surface glycoprotein gp41 is the binding site for several potent broadly neutralizing antibodies. These antibodies recognize highly conserved linear peptide epitopes adjacent to the viral membrane. By studying epitope-antibody interactions near membrane surfaces, we seek to design better structural mimics of the MPER. Peptide mimics derived from HIV gp41 have been chemically synthesized with a variety of structural changes including the presence and nature of an anchoring transmembrane (TM) domain. These peptide mimics have been incorporated into membrane bilayer mimics called Nanodiscs.

Significant differences in binding have been observed between soluble and membrane-anchored peptides. The artificial monomeric TM domain introduces non-native behavior of the MPER, suggesting the membrane and transmembrane domain influence the structure of the MPER. A trimeric TM domain shows better and more native binding than the monomeric TM domain or soluble peptide. The native HIV TM domain shows very similar results to the trimeric TM domain, suggesting that 1) the transmembrane domain imparts significant structural information to the MPER; and 2) neutralizing antibodies differentiate between oligomerization states of the MPER. Membrane presentation of peptide antigens with an appropriate transmembrane domain may be an important feature of vaccine design.

P27 Peptide Purification using a New 150A Reversed-Phase Silica

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Grace Materials Technologies - Discovery Sciences

The demand for high purity peptides is increasing. Small synthetic peptides to large cellular produced peptides are being investigated for possible therapeutic benefits. Both can be difficult to purify to high levels, >98%, because of the very similar products, many times differing by only one amino acid. Optimized purification techniques are required to meet these high purity demands in an economical manner. Reversed-phase chromatography, because of its high resolving power, has been the technique of choice for achieving the high level of purity necessary in the pharmaceutical industry. For industrial purification, important consideration and selection of particle size, pore size, and stationary phase in relation to the peptide can optimize purification. We illustrate how the new Vydac® 150Å reversed-phase media is highly effective at purifying peptides with greater loading capacity and improved productivity compared to competitive media. The media has unique selectivity that can reveal peaks masked by other C18 phases and improves resolution of closely related peptides and impurities for higher purity target peptides. The bulk media incorporates bonded phase chemistries identical to those used in analytical and prep columns, thereby assuring economical method development and reliable scale-up for preparative and process purification. Media packed in dynamic axial compression MODcol® Spring® columns demonstrate high efficiency and extended lifetime.

P28 Predictable Half-life Extension Demonstrated with the Peptide TRI-1144

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ProLynx recently reported a new format for releasable macromolecule-drug conjugates that does not require enzyme hydrolysis, is highly tunable, and gives predictable cleavage rates both in vitro and in vivo (Santi DV, et al. (2012) PNAS 109:6211-6216). The technology uses novel linkers that undergo β-elimination reactions at pre-programmed rates to release native drugs from macromolecular conjugates, e.g. those using polyethylene glycol (PEG). These linkers were shown to provide predictable, tunable release rates over a range spanning hours to months at physiological pH and were used to increase the in vivo half-life of exenatide by > 50-fold in rat (from 30 min to 28 hours). In collaboration with Janssen Research and Development, application of the technology to the 38 amino acid fusion inhibitor reference peptide TRI-1144 (previously intended for once-a-day treatment of HIV) demonstrated the predictability of the linker technology. From the release kinetics of the conjugate and the pharmacokinetics of the native peptide, we were able to identify a linker and dosage that provided the desired minimal free drug concentration with once-a-week dosing. By conjugation of the peptide to 40 kDa PEG via the appropriate linker, the free peptide clearance half-life was increased 8-fold in rats, from 4 hrs to 34 hrs, keeping the free peptide concentration in plasma above 4 nM over 7 days with an initial injection of 3.6 mg/kg of peptide. The experimental data were in excellent agreement with that predicted from modeling.

P29 Functional Differences between Synthetic and Recombinant Amyloid Peptides

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There is an increasing demand for the manufacture of longer chain, modified and more complex peptides that are difficult to manufacture by either traditional chemical synthesis or recombinant methods. AmideBio has addressed this trend with the production of recombinant Aβ42 manufactured by AmideBio's BioPureTM process called CAP-CLIPTM. Recombinant Aβ42 has reproducibly been shown to have higher in vitro toxicity and aggregate faster than synthetically prepared Aβ42 due to a 99% level of purity by RP-HPLC and SDS-PAGE with mass confirmation by ESI-MS and MALDI-MS and an absence of (n-1) deletion products and racemized amino acids. AmideBio's proprietary advantage resides in its innovative hybrid technology platform that combines unique recombinant fusion constructs for robust expression of the peptides of interest, and selective chemical cleavage to produce the native peptide while eliminating the potential for chemical modifications that are common to the synthetic process. The AmideBio technology has been developed to allow efficient capture of the fusion peptide on standard, scalable chromatography resins, followed by on-column processing and selective cleavage to release the target peptide. The CAP-CLIPTM process has the potential of significantly streamlining peptide purification processes by reducing the number of steps, increasing the yield, reducing the manufacturing cost, all while at the same time improving the quality of the final product.

P30 "New" Uses for "Old" Arginine Specific Conjugation Reagents

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The remarkably specific, moderately reactive reagent phenylglyoxal covalently modifies guanidino groups forming a meta-stable, heterocyclic dihydroxydehydroimidazole linkage. Arginine represents just 4.2% of all amino acids in vertebrate proteins, but is highly abundant on the surface of proteins. In contrast to the widely popular lysine and cysteine directed conjugates, arginine specific reagents have traditionally been underutilized, due, in part, to the lack of readily available linkers. We have developed a straightforward approach for the introduction of a commercially available phenylglyoxal group into peptides, generating a payload attached arginine targeted conjugation agent. Analysis of the reactivity, selectivity and stability of this conjugation reaction have been performed through chemoselective attachment to RNAse A 1-16 and through covalently linked trimers of HIV Env.

P31 On the Mechanism of Degradation of Oxytocin and its Analogues in Aqueous Solutions

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Oxytocin (OT) is extensively used for induction of labor. Surprisingly, little is known about its mechanism of degradation in solution or the degradation products. A recent study identified monomeric polysulfides and dimeric degradation products, postulated to derive from β-elimination followed by deamidation and dimerization.[1]

We recently reported that degradation of oxytocin and its analogues in aqueous solution at pH 7.4 produced monomeric polysulfides with up to 6 sulfur atoms as well as dimeric products.[2] Unexpectedly, incubation of OT or of various analogues modified in position 1 resulted in identical dimeric degradants. We concluded that β-elimination via breakage of the C-S bond of Cys^1 must be a key step of the process, and that the resulting ΔAla^1 residue would have to undergo further modifications to yield the same dimeric products independently of the substitution of the N-terminal nitrogen.

Here we further clarify the degradation mechanism and propose a structure for the dimers. We postulate that hydrolysis of the ΔAla^1 residue yields an N-pyruvoyl linear peptide, which loses one sulfur atom and subsequently forms dimers, which we found are linked by one disulfide bridge and one non-reducible bond. The putative linear N pyruvoyl oxytocin intermediate was synthesized and found to degrade to the same dimers as the ones in the incubations of OT. A [U-^{13}C]Cys^1 OT analogue gave degradation products with ^{13}C-NMR spectra consistent with a non stereospecific aldol-type condensation.

Detailed experimental procedures, structures of the degradants and the postulated mechanism of OT degradation in near neutral solutions will be presented.

1. Hawe, A.; Poole, R.; Romeijn, S.; van der Heijden, R.; Jiskoot, W. Pharm. Res. 2009, 26, 1679-1688.
2. Wisniewski, K.; Finnman, J.; Flipo, M.; Galyean, R.; Schteingart, C. D. In Peptides. Building Bridges: Proceedings of the 22nd American Peptide Symposium. M. Lebl (Ed), Prompt Scientific Publishing, San Diego 2011, pp 60-61.