A background image of a molecular model with various colored spheres (red, green, blue, white) connected by grey rods, set against a light blue gradient background.

USP Workshop on Therapeutic Peptides and Oligonucleotides

November 4-5, 2019 • Rockville, MD



Speaker Biographies & Abstracts (listed alphabetically)

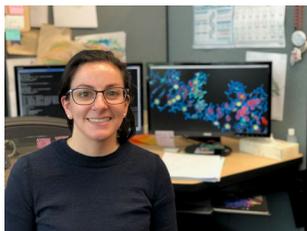
**Fouad Atouf, Ph.D.**

Vice President, Science-Global Biologics
U.S. Pharmacopeia
Rockville, Maryland

Fouad Atouf is Vice President, Science—Global Biologics, for USP. He leads all scientific activities related to the development and maintenance of documentary and reference standards for biologics and antibiotics and oversees the biologics laboratories in USP–U.S. and USP–India. His department supports the work of the associated USP Expert Committees. Dr. Atouf has been at USP for over 10 years and served in a variety of scientific leadership roles including being the regional champion for the Middle East and North Africa Region, where he helped facilitate programs designed to enhance the understanding of the role of regulations and standards in the registration of medicinal products. Dr. Atouf has strong background and experience in the development and regulation of *cellular and tissue-based products*. Prior to joining USP in 2006, his research at the U.S. National Institutes of Health focused on developing methods for the *in vitro* generation of cell-based therapies for diabetes. Dr. Atouf is the author of numerous publications in peer-reviewed journals and a frequent speaker at national and international scientific conferences. Dr. Atouf earned his Master's degree in Biochemistry and his Ph.D. in Cell Biology from the Pierre & Marie Curie University, Paris, France.

Presentation

USP Welcome

**Christina Bergonzo, Ph. D.**

NIST (National Institute of Standards and Technology)

Dr. Christina Bergonzo is an expert in computational simulations of nucleic acid systems. Dr. Bergonzo earned her Ph.D. in Chemistry (2012) from Stony Brook University under the guidance of Professor Carlos Simmerling. Dr. Bergonzo helped pioneer an implementation of the Nudged Elastic Band method, which was then used to study a DNA repair mechanism with atomic level detail for both solute and solvent. She has also been on the forefront of correcting the deficiencies present in current force fields for nucleic acid simulations. She is currently a Research Chemist at the National Institute of Standards and Technology, where she is developing protocols to better integrate experimental data from a variety of experimental sources (including NMR and SAXS experiments) with state-of-the-art MD simulations to better refine RNA structure and dynamics

Session III – Novel Approaches for Characterization*Computational Modeling of the Structure-Function Relationship in RNA*

Nucleic Acids Through a Virtual Microscope: Simulating the Structure-Function Relationship in RNA: RNA based therapeutics beyond simple oligonucleotides will need to fold to be effective, stable, and safe biopharmaceutical products. Characterization of structure is central to this effort. RNA has a propensity to adopt multiple conformations, and in larger RNAs this conformational promiscuity works by stabilizing specific structures from an ensemble. Additionally, chemical modifications to improve delivery and misfolding can cause off-target effects. Structure determination methods, such as small angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR), and multiscale computational methods, provide a robust way of isolating the structure-function relationship of RNAs, including conformational ensembles, formulations, and differences between chemically modified RNAs used in therapeutics and the unmodified molecules. In this work, we provide evidence that reliable structural quality of prototype RNA can be improved in the limit of experimental information content. Ultimately, this work will advance the NIST goals of creating standard methodologies for biomolecules, and technologies to foster innovation in US industry.

**Walter Cabri**

Senior Vice President API I&D
Fresenius Kabi Business Unit API

I am full Professor of Organic Chemistry at the Alma Mater Studiorum University of Bologna as well as SVP of Fresenius Kabi API I&D. In Fresenius Kabi I coordinate Chemical/Analytical Development, Sourcing and Regulatory Affairs for API. The main area of interest are in the field of protein-protein interactions; Green Chemistry, Peptides and complex drugs. I am co-author of 111 papers; 100 patents; one Book and the H-index is 29.

Session V – Novel Therapeutics and Analytical Strategies

Peptide sameness. Challenges and regulatory requirements: Walter Cabri; Antonio Ricci^a & Klemmens Winna

The definition of specification for API and finish dosage form in the area of generic peptide is not only related to the identification of impurities according to the latest standard techniques but also on several chemiometric approaches based on NMR or UPLC/ms. In particular the 2D NMR technique monitor the impact of all components API, impurities, counter ion, salts, solvents etc. at the same time. We will discuss the impact of the evolution state of the art analytical technologies on the RLD quality assessment and the impact of the generic regulatory journey to prove sameness using a case study. A focus will be on terminal sterilization of peptides.



Anne DeGroot, BA, M.D.
EpiVax, Inc.

Dr. Annie De Groot earned a BA from Smith College and her M.D. from Pritzker School of Medicine at the University of Chicago. After vaccinology training under Jay Berzofsky (National Cancer Institute), and training in Infectious Diseases at Tufts New England Medical Center, De Groot joined the faculty of Brown Medical School. In 1998 she and colleague Bill Martin founded EpiVax, Inc. Dr. De Groot is the founder and director of the Institute for Immunology and Informatics at the University of Rhode Island, and volunteers as medical director of Clinical Esperanza/Hope Clinic, and scientific director at GAIA Vaccine Foundation.

Session IV – Impurities and Immunogenicity

New Dogs/Old Tricks: Methods for Assessing the Immunogenicity of Peptide Drugs and Their Impurities: Anne S. De Groot, Brian Roberts, Frances Terry, Aimee Mattei, Lenny Moise, Bill Martin University of Rhode Island Institute for Immunology and Informatics, and EpiVax, Inc. Providence, Rhode Island

Immunogenicity is a term that describes immune responses to protein or peptide biologics. Due to several well-documented adverse events that were attributed to immunogenicity (PRCA, Erythropoietin, for example), immunogenicity risk assessment using in silico and in vitro methods recently achieved “standard practice” status in the biologics preclinical development pipeline. An industry white paper on T cell-dependent immunogenicity was developed by a consortium of researchers and leading biologics manufacturers and published in 2013. The

approaches described in the white paper are now widely adopted by biologics developers. A new draft guidance for generic peptide drugs was recently published, highlighting FDA concern about the potential for peptide drugs and their impurities to drive unwanted immunogenicity and adverse effects (<https://www.fda.gov/downloads/drugs/guidances/ucm338856.pdf>). More specifically, peptide drug-substance (DS) and impurities that are derived from solid phase synthesis may contain T cell epitopes that can be presented on the surface of antigen presenting cells by class II HLA molecules, priming T cells and driving unwanted immune responses. Recent updates to this approach now permit the following three-step approach to immunogenicity risk assessment for peptide drugs and their impurities.

Step 1. Immunoinformatics assessment: The potential of the DS to stimulate a T cell response can be rapidly assessed computationally using T cell epitope mapping algorithms. We use EpiMatrix for this purpose and focus on HLA DR (Class II) HLA binding predictions. In a typical DS analysis, the EpiMatrix algorithm is used to screen the primary amino acid sequence of the DS and its impurities, for the presence of HLA DR ligands, which can be considered putative T cell epitopes. Discriminating between potential inflammatory “T effector” epitopes and regulatory “T reg” epitopes is performed with a second algorithm, known as JanusMatrix. The latter algorithm identifies putative Treg epitopes, defined as HLA/epitope complexes that present a human-like outer contour (TCR face). HLA/epitope complexes that do not present an outer contour (TCR face) that is ‘human-like’ are more likely to drive effector T cell response. Following assessment of T cell epitope phenotype, the next step is to combine the scores for effector and regulatory T cell epitope content, providing an overall assessment of immunogenic potential of the DS and impurities. The resulting Treg adjusted EpiMatrix scores are highly correlated with immune responses in vivo.

Step 2: In vitro - HLA binding: The DS, its impurities, and peptides representing predicted T cell epitopes can be evaluated for binding to human HLA in assays that measure binding affinity in competition-based binding studies, in vitro. HLA binding is used to confirm in the in silico analysis and inform the design of in vitro assays (see step 3). **Step 3: In vitro**

assay – Teff Assay: Measurement of de novo T cell response: Cell culture protocols have been developed to emulate in vivo conditions that support differentiation of naïve T cells to effector T cells by antigen stimulation with biologics or their constituent T cell epitopes. In vitro stimulations using the biologic drug and human peripheral blood cells (PBMC) allow for natural antigen processing of the DS and other product components including impurities. In vitro assays using predicted epitopes derived from the DS product impurities provide information about the ability of these defined sequences to drive a T cell response using human T cells. At the end of the culture period, T cell phenotype and/or function are characterized in assays that measure the magnitude and quality of effector T cells that have potential to drive ADA development. Treg Assay: Co-incubation of the putative Treg epitopes with known T effector epitopes (such as Tetanus Toxoid-derived T effector epitopes) allows the assessment of bystander suppression. Bystander suppression is a feature of Treg epitopes. In summary, in silico and in vitro assessment of novel T effector (inflammatory) and Treg (suppressive) epitopes is necessary to best evaluate the impact of novel impurities on the immunogenicity risk in humans. In addition to the standardized tools described above, we will describe novel in silico methods that have been developed to anticipate well-known synthetic peptide impurities (The What If Machine). Several case studies (Tasuglutide, Salmon Calcitonin) will be provided to illustrate these well-established (and more recently developed) Immunogenicity Risk Assessment methods.

**Michael DeFelippis, PhD**

Chair, USP Peptide and Oligonucleotide Therapeutics Workshop Steering Committee, and Chair, USP BIO1 – Peptides and Insulins Expert Committee

Michael R. De Felippis joined The Lilly Research Laboratories of Eli Lilly and Company in 1990 after obtaining his doctorate in biochemistry from The Ohio State University. He is currently a Distinguished Research Fellow in the Bioproduct Research and Development division. His work focuses on commercializing biopharmaceutical products with particular emphasis on characterizing physicochemical properties, devising control strategies, and preparing CMC-related documentation to support product licensure in global markets. Dr. DeFelippis has published manuscripts, review articles and book chapters on the subjects of protein and peptide structural characterization and formulation design/delivery strategies. Dr. De Felippis is a USP Council of Experts member and Chair of the USP Expert Committee on Therapeutic Peptides and Insulin. He also Chairs the Parenteral Drug Association Biopharmaceutical Advisory Board.

Workshop Overview**Session V – Novel Therapeutics and Analytical Strategies****Workshop Wrap-up**

**Robert Duff, Ph.D.**

Amgen

Dr. Duff joined Amgen in 2013 as a Principal Scientist in Attribute Sciences. He has led the analytical efforts for multiple programs including monoclonal antibodies with his current focus centering on the advancement of siRNA at Amgen. Prior to Amgen, he was the manager of the Biochemistry department at Eurofins Lancaster Laboratories, Inc. His team developed and validated methods/assays through all phases of clinical development. Earlier research centered on ligand-directed drug delivery of modified antisense oligonucleotides. The study of the use of complex carbohydrate in conjunction with C-type lectins for the purposes of targeted drug delivery was the primary focus. A publication on the use of a GalNAc cluster ligand for delivery of antisense against hepatitis B virus demonstrated that dose dependency for efficacy correlated with asialoglycoprotein receptor uptake. He has various publications, posters and patents on subjects ranging from the study of stereoelectronic effects of enzyme active site functional groups, mechanisms of anticancer drugs, ligand-directed drug delivery, and antisense technology. Dr. Duff completed postdoctoral research on mechanistic studies of Drug-DNA interactions at Johns Hopkins University. He received a bachelor's/master's of science degrees in Chemistry from the University of Pittsburgh, and a Ph.D. in Organic Chemistry from The University of Virginia.

Session II – Analytical and Control Strategies*Quality Attributes for siRNA*

Small interfering RNA (siRNA) molecules are an emerging class of therapeutics with tremendous potential in the clinic. Generally siRNA molecules have been described as “big small molecules” since they possess attributes of synthetic as well as biologic molecules. Nevertheless, the principles of Quality-by-Design (QbD) apply so the attributes must be assessed and scored for criticality. The purpose of a product quality attribute (PQA) assessment is to provide severity scores ranking the criticality for the molecule's quality attributes. A severity score reflects the expected or perceived impact on the drug's safety or efficacy if the attribute levels were to be out of control (elevated or reduced in comparison to expected levels). Product understanding and the relationship of process parameters to critical product quality attributes is an expectation in regulatory filings for product characterization, analytical method capability, process evaluation (formerly comparability), and justification of control strategy. This presentation focuses on the assessment of the critical quality attributes for this modality as based on mechanism of action, forced degradation experiments and the literature. Critical attributes are described and scored as related to efficacy and safety within this presentation.



Liqing Gu Ph. D.
SGS West Chester

Dr. Liqing Gu joined SGS Life Science Services in West Chester PA in 2016 and currently serves as the Senior Scientist and Biologics Mass Spectrometry Group Leader. He specialized in qualitative and quantitative characterization of large molecule drugs (including proteins, peptides and oligonucleotides) using high resolution LC-MS instruments with a focus on the product Critical Quality Attributes (CQAs) such as intact masses, sequences, post-translational modifications, impurities, glycosylations and disulfide bridge patterns. He also has expertise of complexed biologics characterization using innovative approaches such as statistical OMICS analysis and cysteine redox quantitation. In addition, he has extensive hands-on experiences of characterizing impurities from generic peptide drug products and comparability studies of generic peptide drugs versus RLD, especially with the deep knowledge of current FDA guidance as well as the technical challenges and possible analytical strategies. Prior to joining SGS, Dr. Gu obtained his Ph.D. from University of Pittsburgh. His Ph.D. research covers the fields of mass spectrometry, redox chemistry and quantitative proteomics with an emphasis on hypermultiplexing proteomics methodology. He is the author of a number of scientific papers published on peer-reviewed journals such as *Mass Spectrometry Reviews*, *Analyst*, *Journal of the American Society for Mass Spectrometry* and *Analytical and Bioanalytical Chemistry*. He is also the invited reviewer for journals including *Analytical Methods*, *Journal of the American Society for Mass Spectrometry* and *Journal of Mass Spectrometry*.

Session IV – Impurities and Immunogenicity

Product-related impurity quantitative and qualitative analyses in the context of Complex Generic peptide products

Generic medicines are now an established part of the pharmaceutical supply chain and offer significant savings to health services, insurers and patients alike. Complex generics are products that may include complex: active ingredients, formulation, route of delivery, or even a mixture of ingredients. The key to creating a new generic drug and gaining regulatory approval is proving that it is safe and comparable to the originator product. This includes demonstrating that their impurity profiles are comparable. Over the past few years, the FDA issued a series of DRAFT guidance documents on Impurities and how to handle their monitoring or understanding in the context of ANDA applications in general, and in the context of synthetic peptides in particular. Navigating these guidelines is not a trivial exercise from an analytical and/or regulatory standpoint. Peptides would appear to be one class of complex API for which product-related impurities are relatively straightforward to characterize using high end instrumentation but this would be an oversimplification of the work which needs to be done. On one hand, the presentation will focus on the interpretations of the guideline currently available to the industry. On another hand, the analytical difficulties presented by these complex APIs will also be covered: from the differences in impurity levels and nature between synthetic and recombinant products, the frequent use of unnatural amino acids or D-amino acids in the sequence of therapeutic peptides, the complexity of the formulation of the commercial products, as well as the presence of many types of modifications related to process or related to degradation. Case studies will be used to exemplify the complexity of this part of the analytical sameness demonstration between complex generic peptide product and their commercial counterparts. Modern LC-MS and MS/MS methodologies will be emphasized on addressing various types of analytical challenges related to impurity characterization and impurity profile comparability.



Elena Gubina, Ph.D
CBER, FDA.

Elena Gubina joined FDA in 1997, first in the Division of Monoclonal Antibodies, CDER. For the last twelve years, she has been working as a full-time regulator in the Office of Tissues and Advanced Therapies, CBER.

Session I – Regulatory Considerations

Challenges of Personalized Neoantigen-specific Therapeutic Vaccines

**Gerhard Haas, Ph.D.**

USP Peptide and Oligonucleotide Therapeutics Workshop Steering Committee, and Member, USP BIO1 – Peptides and Insulins Expert Committee

Gerhard Haas is currently heading the Central Function Quality of Bachem's European sites. Between 2006 and 2018, he lead the local QA/RA department of the Bubendorf site. He has been with Bachem since 1992 in various positions including QA, Production, and R&D. Gerhard received his Ph.D. in Organic Chemistry from the University of Stuttgart, Germany

Chair: Session III – Novel Approaches for Characterization

Tanja Huth, Ph.D.,
Bachem



Tanja Huth is heading the QA/RA project management team supporting API development at Bachem AG in Switzerland. She started at Bachem in 2013 as QA Release Manager soon also being deeply involved in the development of peptide APIs. Before, Tanja Huth worked for 6 years at Vetter-Pharma as project manager and in various roles of the quality unit supporting aseptic manufacturing. She earned her PhD in biology at the Institute of Biochemistry at the University of Heidelberg.

Session II – Analytical and Control Strategies

Phase-Appropriate CMC Development for Synthetic Peptides

Bachem's modular CMC development concept will be presented. The concept is based on experience with a wide range of customers (virtual to big Pharma) and represents Bachem's *best practice* recommendations for the successful development of a robust manufacturing process and control strategy in compliance with current regulatory and quality requirements. In this presentation, the emphasis will be on the timing of analytical development and validation commensurate with clinical development and batch manufacturing. Approaches to other deliverables such as the assessment of genotoxic and elemental impurities will also briefly be discussed.

**Edwin Kellenbach, Ph.D.**

Aspen OSS

October 1986: M. Sc. (cum laude) in organic chemistry (Leiden University) and in-vivo NMR (Max Planck Institute Dortmund).

November 1991: Thesis: "DNA-binding by the Glucocorticoid Receptor", Utrecht University.

November 1991-April 1992: post-doc at the Department of Molecular Physics, Wageningen University. May 1992-April 1994: NMR specialist within the Structure Analysis section, Solvay-Pharma, Weesp.

May 1994-October 2001: Section Leader of the Structure Analysis section, Organon, Oss, the Netherlands. November 2000-October 2002: Section Head Analytical Chemistry, Organon, Riom, France.

November 2002-September 2011: Senior Director Analytical Development, MSD, Oss. September 2011-October 2013: Section Lead GTO Biochemistry (15 persons). November 2013- April 2016: Program Manager Biochemistry.

May 2016-present: Principal Scientist Biochemistry.

Member of European Directorate for the Quality of Medicine (EDQM) expert group 6 (biologicals). Member of the USP expert panel on synthetic peptides.

Coauthor of over 30 articles on proteins and protein-DNA interaction, analytical chemistry and physicochemical characterization in peer reviewed journals.

Research interests

Spectroscopy, peptides and proteins, heparins and heparinoids, physicochemical and extended characterization of biopharmaceuticals, chirality, polymorphism, structural biology, structure-activity relations.

Session V – Novel Therapeutics and Analytical Strategies*Discrimination of Strongly related Peptides and Detection of Peptide Aggregation by NMR***Introduction**

Applications of NMR to the discrimination of strongly similar peptide (-impurities) and peptide aggregation are presented.

Discrimination of Strongly related Peptides

NMR is shown to be able to discriminate 4 different insulin types with strongly resembling sequences by both one-dimensional proton- and two dimensional ¹H-¹³C NMR. The parallel and antiparallel Oxytocin impurities have identical amino acid composition, sequence and mass and therefore cannot be discriminated by these techniques. However, these two impurities are readily distinguishable by both one-dimensional proton- and two dimensional ¹H-¹³C NMR. No laborious and time-consuming sample pretreatment is required for these analyses.

Detection of peptide aggregation

Detection of additional signals in an Oxytocin sample triggered the investigation of 2D diffusion ordered spectroscopy (DOSY) allowing the determination of (differences in) diffusion time due to differences in hydrodynamic radius. Differences in NOE buildup curves also indicate the presence of aggregates.

Conclusion

NMR is a highly specific tool to distinguish strongly related peptides *without* the need of time consuming, laborious sample pretreatment and is able to detect peptide aggregation in a number of orthogonal techniques based on different principles.

**Marc Lemaire, Ph.D.**

USP Peptide and Oligonucleotide Therapeutics Workshop Steering Committee
ML Consult LLC

Marc Lemaître holds a degree in Organic Chemistry and a PhD in Biochemistry from the University of Liège, Belgium. Since 1985 Marc's professional interests has been the study of Nucleic Acids and therapeutic as well as diagnostic applications. After 5 years of post-doc research including 2 years at the Pasteur institute in the team of Nobel Prize Luc Montagnier, Marc held positions of increasing seniority in R&D, operations, business development, and general management within CMO's, Pharma and Biotech companies. Based in the USA since 2006 he worked for Glen Research [Sterling, VA], then as CEO of Girindus America [Cincinnati, OH], a CMO with a focus on the cGMP manufacture of oligonucleotides for therapeutic applications. Since 2013 Marc is working as independent consultant specialized in CMC and working mostly with start-up and Pharma companies developing therapeutic oligonucleotides and some peptides. He is member of the Board of Director of the Oligonucleotide Therapeutics Society since 2015 – treasurer 2015-2018.

Session I – Regulatory Considerations

CMC, Regulatory, and Quality Strategies for Production of Therapeutic Oligonucleotides – EU vs. US

There is an increasing number of oligonucleotides reaching late stage clinical development. For that reason, there is considerable interest in integrated approaches to manufacturing and quality systems towards global product registrations. Synthetic oligonucleotides, due to similarities in manufacturing and analysis, offer a unique opportunity to accelerate the development of both drug substance and drug product – compared to small molecules for instance. And a good understanding of the regulatory guidelines is important to achieve this goal. Also, recent advances in oligonucleotide-based therapeutics include the development of complex formulations for targeted delivery to various sites. Utilization of these novel systems for the delivery of complex oligonucleotides presents additional challenges for characterization and control. Leveraging on real-world cases, this presentation will provide strategies for the management of CMC operations towards successful global development/registration of oligonucleotide therapeutics and for addressing the regulatory expectations for assuring quality and performance of those delivery systems.

Chair: Session II – Analytical and Control Strategies

**Ranajoy Majumdar, Ph. D.***Eli Lilly and Company*

Ranajoy Majumdar, PhD joined the Lilly Research Laboratories of Eli Lilly and Company in 2014 after earning his doctorate in Pharmaceutical Chemistry from the University of Kansas. He is currently a Senior Research Scientist in the Bioproduct Research and Development organization. He is working on development of peptide, oligonucleotide, and protein biotherapeutics with focus on characterizing higher order structure and interactions needed to understand physicochemical properties and analytical control strategies to develop biotherapeutics and prepare data packages to support worldwide regulatory submissions. He has published several manuscripts, review articles, and a book chapter in the subject area of interrelationship between protein structural characterization and formulation design. He is also a co-inventor on patents for insulin compositions.

Session III – Novel Approaches for Characterization*Considerations for Biophysical Characterization in Developing Peptide Biologics*

A wide variety of peptide biologics are currently in preclinical and clinical testing with majority of indications in metabolic disorders, oncology, and dermatology. The focus of biophysical characterization of peptides at early stages of drug product development is to elucidate any connections between changes in higher order structure and corresponding impacts on stability and/or pharmacological properties. This talk will highlight approaches to select fit-for-purpose biophysical techniques to answer specific questions during early periods of peptide DP development. Considerations and approaches for comprehensive understanding of peptide biophysical properties to enable drug substance characterization, formulation development of drug product, and comparability of peptide therapeutics will be discussed along with few case studies.



Gareth Murphy BSc
Ipsen Manufacturing Ireland Ltd

Employment

Company: Ipsen Manufacturing Limited Ireland
Blanchardstown Dublin 15 Rep. of Ireland

- Current Role: Analytical Development Manager – Managing 8 analytical chemists and the development of peptides and radiopharmaceutical precursors. I've been with Ipsen 5 years
- Previous Employment: 12 years experience working in Mylan Generic pharmaceuticals as a Technical Development Senior Analyst working on method development, validations and method transfers

Education

BSc Honors Degree in Pharmaceutical Technology from
Dublin Institute of Technology (DIT)

Session III – Novel Approaches for Characterization
Chiral Purity Testing of Amino Acids by Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is a form of [chromatography](#) that uses a [supercritical fluid](#) such as [carbon dioxide](#) as the mobile phase. It is used for the analysis and purification of low to moderate [molecular weight](#), thermally labile molecules and can also be used for the separation of [chiral](#) compounds. Principles are similar to those of [high performance liquid chromatography](#) (HPLC), however SFC typically utilizes carbon dioxide as the mobile phase; therefore the entire chromatographic flow path must be pressurized. Because the supercritical phase represents a state in which liquid and gas properties converge, supercritical fluid chromatography is sometimes called convergence chromatography. An advantage of using Supercritical fluid chromatography is that you get to extend the polarity range of reverse phase chromatography to avail of the benefits of normal phase chromatography without using a lot of solvent so it would be considered a “green” analytical technique. For this workshop I propose to present how we have used Supercritical fluid chromatography to determine the chiral purity of some naturally occurring and custom-made amino acids with up to two chiral centres present. As per ICHQ6A, we are controlling the enantiomeric purity of the final drug substance by testing the starting materials (amino acids) individually for enantiomeric purity. So far it has been a challenge to develop a suitable method for the drug substance (peptide + metal chelator). The Supercritical fluid chromatography system (Waters UPC²) we have in Dublin has proved to be very useful when trying to develop enantiomeric purity methods for the amino acids, which has allowed us to be confident that there is no enantiomeric impurity present in the final drug substance. Examples of methods, chromatography and analytical method development will be used throughout the presentation to show how Supercritical fluid chromatography can be used to great affect to control the enantiomeric purity of raw materials and therefore the final drug substance.

**Lubomir (Lubo) Nechev, Ph. D.***Alnylam Pharmaceuticals*

Dr. Nechev leads the Process and Analytical Sciences Department at Alnylam Pharmaceuticals. He joined Alnylam in 2004 and has since been responsible for the CMC aspects of the drug substance and drug product development, characterization and manufacturing as well as the overall control strategy. Prior to joining Alnylam, Dr. Nechev worked at Ribozyme Pharmaceuticals (RPI) and the Nucleic Acid Synthesis unit of Transgenomic Inc. He received his Ph.D. degree in Organic Chemistry from St. Kl. Ohridski University, Sofia, Bulgaria and completed his post doctoral training at the Department of Chemistry and Center in Molecular Toxicology at Vanderbilt University, Nashville, Tennessee.

Session II – Analytical and Control Strategies*Manufacturing Process and Control Strategy for GalNAc Conjugated siRNAs*

Several GalNAc conjugated siRNAs are currently at different stages of clinical development for various programs at Alnylam. The presentation will describe the Alnylam manufacturing process for this type of molecules as well as the development of the CMC control strategy from early stage to NDA. The possibility of using a platform approach to enhance this strategy will also be discussed.



Lawrence Perez, Ph.D.
CDER, FDA

Lawrence B. Perez obtained his PhD from Massachusetts Institute of Technology under the direction of Professor George Whitesides. After a post-doctoral study with Professor Dale Poulter at the University of Utah, Dr. Perez started his professional career as medicinal chemist with Novartis, first in cardiovascular diseases and then in oncology. Recently, Dr. Perez has moved to the Food and Drug Administration where he reviews the CMC section of new drug applications, specializing in the chemistry of small molecules, peptides, oligonucleotides and botanicals.

Session I – Regulatory Considerations

CMC and Regulatory Challenges on Oligonucleotide Drugs

In the last few years, the FDA has received a number of new drug applications involving oligonucleotides. This class of therapeutic agents has presented some unique scientific and regulatory challenges. This presentation will review a few of these challenges involving the regulatory aspects for chemistry, manufacturing, and controls in new drug applications submitted to CDER within the FDA.



Stefania Pfeiffer-Marek, Ph.D.,
Sanofi-Aventis Deutschland GmbH

Dr. Stefania Pfeiffer-Marek is physicist by training and obtained her Ph.D. in Biophysical Chemistry at the Johann-Wolfgang-Goethe University in Frankfurt (Germany) in 1997. Afterwards she joined the Rockefeller University New York as postdoctoral fellow before she started at Sanofi in 2001 being responsible for rational drug design and multi-dimensional optimization in numerous discovery projects. In 2012, she moved to Sanofi's pharmaceutical development platform and focused on early formulation development and developability assessment of therapeutic peptides. She has grown strong background in using developability criteria during drug discovery, biophysical characterization of peptides as well as in early clinical formulation development.

Session V – Novel Therapeutics and Analytical Strategies

Tailor-made therapeutic peptides for accelerated clinical development

Physicochemical properties of therapeutic peptides need to be compatible with the manufacturing process and formulation requirements to ensure developability towards the commercial drug product. This aspect is often disregarded and only evaluated late in discovery, imposing a high risk for delays in development, increased costs, and finally for the project in general. Peptide drugs that are formulated in a ready-to-use solution for subcutaneous injection are frequently presented to the patient using multiple-dose pen devices. Such drug-device-combinations require a low-viscosity solution, long-term chemical and physical stability of the drug product solution including an in-use period, and the compatibility of the peptide drug with antimicrobial preservatives and with siliconized packaging material. We have established and successfully applied our concept towards developability of such peptide drugs aiming at stable prototype compositions for Phase 1 clinical studies and beyond. The process links rational peptide design based on experimental and in silico profiling during discovery with pre-clinical formulation development.

**Claus Rental, Ph. D.**

Ionis Pharmaceuticals, Inc.

Dr. Rental is currently Executive Director, Analytical Development and Quality Control at Ionis Pharmaceuticals, Inc., Carlsbad, California. Prior to joining Ionis in 2001 he worked in Quality Control and Special Analytics at CarboGen Laboratories AG, Aarau, Switzerland. He received his Ph.D. (summa cum laude) from the University of Tuebingen, Germany. Dr. Rental has 20 years of experience in Quality Control and analytical method development and validation. He is an expert in mass spectrometric techniques, and has extensive expertise in the development of oligonucleotide therapeutics in regards to specifications, testing of starting materials, reagents, drug substance intermediates, drug substances, drug products and toxicological samples, as well as installation and validation of IT Quality Systems. He has been responsible for the CMC drug substance section of IND filings for more than 75 oligonucleotides, and participated in the NDA filings for KYNAMRO® (mipomersen), SPINRAZA™ (nusinersen), WAYLIVRA® (volanesorsen), and TEGSEDI™ (inotersen).

Session III – Novel Approaches for Characterization*Analytical Methods for Oligonucleotide Therapeutics*

**Sabrina Rüggeberg Ph. D.***Sanofi*

- 2001 Graduated in biotechnological engineering at the Mannheim University of Applied Sciences, Germany.
- 2001 to 2008 Scientific staff at EMBL (European Molecular Biology Laboratory) Heidelberg, Germany, in the Proteomics Core Facility
- 2008 to 2011 Scientist in the field of bioanalytics for therapeutic antibodies at Sanofi
- 2011 to 2018 Head of a laboratory called "Bioassays Biosynthetics" specialized on cell based assays for potency determination of insulins and peptides.
- Since 2019 Head of a laboratory called "Innovation and Technology" focused on methods for compound characterization..

Session V – Novel Therapeutics and Analytical Strategies*Cell Based Potency Assays for Peptides: Challenges and Strategies*

Potency tests are essential for biopharmaceutical compounds in order to understand and monitor their mode of action. Also for peptides e.g. incretin mimetica, those potency assays have become more important during clinical development. Potency data are mandatory within the CMC package in dossiers (NDA). GLP1 receptor agonist activity is used for Sanofi's therapeutic pipeline of synthetic peptides, e.g. for the already marketed products (Lyxumia, iGlarLixi) and for development projects being in clinical trials. The development and validation of a platform potency assay targeting GLP1R is reported with special emphasis on recent technologies, like assay ready cells or reporter gene read out technologies.

**Jessica Stolee Ph. D.***Biogen*

Dr. Stolee leads the pharmaceutical and antisense oligonucleotide analytical development group at Biogen. Her group supports pharmaceutical and oligonucleotide process development and manufacturing and is responsible for evaluating and implementing new technologies for their analysis. Dr. Stolee was one of the primary authors of the CMC portion of Biogen's first oligonucleotide NDA (Spinraza) and continues to support the global filings. She has also contributed to numerous pharmaceutical and oligonucleotide IND filings at Biogen. She received her Ph.D. in Chemistry from George Washington University where her work focused on the development of novel ionization methods for mass spectrometry.

Session IV – Impurities and Immunogenicity*Purge-based Risk Assessment for Solvent and Small Molecule Impurities Generated During Oligonucleotide Manufacture*

Through the European Pharmaceutical Oligonucleotide Consortium, a team of companies is exploring opportunities to justify the exclusion of small molecule impurities and solvents from release testing through the use of risk-based purge arguments. To support the theoretical purge arguments, spike and purge studies have been performed in different processes and by multiple companies. Results show that process related impurities generated during the synthesis are well purged by the downstream unit operations and justify the exclusion of testing

**Ved Srivastava Ph. D.**

USP Peptide and Oligonucleotide Therapeutics Workshop Steering Committee, and Member, USP BIO1 – Peptides and Insulins Expert Committee

Dr. Ved Srivastava is Vice President of Chemistry at Intarcia Therapeutics at North Carolina, USA; and is the President of the American Peptide Society. Prior to that, he was the Head of Peptide Chemistry at GlaxoSmithKline (USA), and in the senior leadership role at Amylin Pharmaceuticals (Astra Zeneca, USA). He co-founded, Phoundry Pharmaceuticals, a biotech company focused on the discovery of peptide hormone therapeutics. Phoundry was acquired by Intarcia Therapeutics in 2015. Ved has over 25 years of experience with expertise in drug discovery and development in the area of metabolic diseases, CNS, and inflammation with major emphasis in peptide medicinal chemistry, chemistry manufacturing and control (CMC) and peptide drug delivery. Ved has participated in the development and commercialization of SymlinTM, ByettaTM and BydureonTM first-in-class medicines for the treatment of diabetes. Ved is the Editor of four peptide books (1) *Peptide Therapeutics: Strategy and Tactics for Chemistry, Manufacturing, and Controls*, (2) *Peptide-based Drug Discovery: Challenges and New Therapeutics*, (3) *Comprehensive Medicinal Chemistry III, Volume 6, Biologics Medicine* and (4) *Peptide 2015*. Ved is also a member of the BIO1- Peptides & Insulins Expert Committee. He earned a Ph.D. in organic chemistry from the University of Lucknow, India; and had subsequent postdoctoral appointments at the University of Georgia and the University of Colorado Medical School.

Session I – Regulatory Considerations

Session Chair:



Renata Varga Ph. D.
Teva Pharmaceuticals

Renata Varga graduated as a Chemist working with solid phase peptide synthesis and enzymatic reactions. She obtained a PhD in Hungary in analytical chemistry by analyzing surface water samples for pharmaceutical residues with LC-MS/MS after solid phase extraction. She started to work in Teva Pharmaceuticals as a Research Analyst in the Sterile R&D, Godollo, Hungary. She has expertise in the development and NDA/ANDA regulatory filing of sterile infusions, injections, eye-drops, lyophilized powders and therapeutic peptides. She moved to CMC Biologics in West Chester, PA, where she was working as a Characterization Scientist, providing Critical Quality Attribute Assessments for all peptides and monoclonal antibodies under development in Teva Pharmaceuticals, performed characterization studies for product variants, mainly size- and charge variants, and for higher-order structure and contributed to IND/BLA authoring. Currently she is the Biosimilars Champion overseeing and providing strategic support for all biosimilar product development in Teva Pharmaceuticals on top of serving as the analytical lead for one of the biosimilar projects.

Chair: Session IV – Impurities and Immunogenicity



Thomas Wucherpfennig, Ph. D.
Bachem

Thomas Wucherpfennig works as a Project Chemist in the quality control department at Bachem in Bubendorf, Switzerland. His work in the mass spectrometry service covers various aspects of the MS-based analysis of peptides and peptide conjugates, including the structure elucidation of peptidic impurities and the analysis of aggregates or other high molecular weight species. Besides, he is responsible for method validation, the implementation of advanced MS techniques into routine tests and the evaluation of new MS-related tools to address challenges at the frontier of peptide analytics. Thomas obtained his PhD in Chemistry from ETH Zurich in 2016, where his work in the group of Prof. Jeffrey Bode focused on chemical protein synthesis. Afterwards, he went for a postdoc position with Prof. Ben Cravatt at The Scripps Research Institute, La Jolla, CA, USA, where he combined mass spectrometry and chemical proteomics to investigate and manipulate cellular protein stability. He joined Bachem in 2018.

Session II – Analytical and Control Strategies

Cell Based Potency Assays for Peptides: Challenges and Strategies

Peptide conjugates are a promising and growing class of peptide therapeutics. They require a specific set of analytical test methods to fully characterize the manufacturing process, its intermediates and the final drug substance. Typically, the physical and chemical properties of the peptide are substantially influenced or dominated by the properties of the conjugated moiety. When a protein is conjugated to a peptide, biochemical methods are best suited as analytical test methods, while PEGylated peptides require analytical test methods that adequately characterize the polymeric moiety. In addition, the determination of the degree of conjugation and the identification and structural elucidation of peptide related impurities in the drug substance is important. An overview on challenges during development of assay and purity methods using different chromatographic and MS techniques will be presented.



Deyi Zhang Ph. D.
CDER, FDA

Deyi Zhang is a senior chemist in the Office of Research and Standards, Office of Generic Drugs (OGD) at FDA specializing in complex drug substances, including providing scientific input for regulatory policy and product-specific guidance development on such products and managing related research activities. He has over 20 years of experience from academia, industry and regulatory agency. Prior to joining FDA in 2015, he was an Executive Director in Crown Bioscience, a biotech company focusing on oncology drug discovery and translational medicine. Before joining Crown, he was a Principal Research Scientist at Eli Lilly and Company. Prior to his career at Eli Lilly, he was a NIH Postdoctoral Fellow at University of Pennsylvania. He received his Ph.D. in Organic Chemistry from University of Notre Dame. He has over 10 US patents and 40 publications and presentations.

Session I – Regulatory Considerations

Cell Based Potency Assays for Peptides: Challenges and Strategies

In this presentation, we will discuss some of the considerations in developing generic peptide and oligonucleotide drug products. Peptides and oligonucleotides are complex active ingredient drug products. Demonstrating complex active ingredient sameness is critical in the development of generic peptide and oligonucleotide drug products. For peptides, we will discuss some general characterization strategies with the focus on comparative impurity profile analysis; for oligonucleotides, we will discuss their unique features and challenges, and provide some preliminary thinking on how to address those challenges. The goals of this presentation are to better communicate with generic sponsors and generic industry in general the current regulatory thinking on the development of generic peptide and oligonucleotide drug products.