Introduction to Glycomic and Glycoproteomic Analysis

August 16-18, 2021

Course Overview:

In this 3-day virtual course, participants will learn basic techniques for glycomic and glycopeptide analysis via a combination of virtual demos, hands-on data interpretation, and lectures. Topics to be included are: release and structural characterization of N- and O-linked glycans, glycopeptide and glycoproteomics analysis, sequencing by MS/MS, biology of glycosylation, glycolipid analysis, and data interpretation. The final day will include optional additional modules on using mass spectrometry, and NMR spectroscopy for the analysis of glycoconjugates.

Takeaways:

- Understand basic principles of protein and lipid glycosylation, structure, and function within biological systems.
- Learn analytical strategies to release, isolate, and permethylate N- and O-linked glycans, or to prepare solutions of glycopeptides from proteins, tissue, or cells
- Familiarize with methods to analyze glycans and glycopeptides using MALDI, LC-MS, tandem MS, and multiple fragmentation techniques
- Additional analytical techniques including chromatography and NMR, and molecular modeling techniques
- Experience hands-on data interpretation with provided example datasets, available databases and downloadable software.

Preparation:

- This class is to be taken completely online
- We will provide a link to the platform on the day of the class
- This class requires a computer and strong internet connection
- Participants should be familiar with basic fundamentals of biochemical analysis.

Instructors:

Dr. Parastoo Azadi - Dr. Parastoo Azadi received her B.Sc. in Chemistry in 1987 from University of North London, UK and her Ph.D. degree in biochemistry in 1991 from Imperial College of Science and Technology, University of London, studying structural characterization of carbohydrates and glycoproteins by mass spectrometry under the supervision of Profs. A. Dell and H.R. Morris. Since 2001, Dr. Parastoo Azadi has been the Technical Director of Analytical Service and Training at the Complex Carbohydrate Research Center. The samples submitted for these types of analyses come from academic, government, non-profit organizations and private companies, throughout the United States and internationally.

Dr. Kelley Moremen - Dr. Moremen received his B.S. in Biology and Chemistry (1978) from Dickinson College and his Ph.D. in Molecular Biology (1984) from Vanderbilt University and a pursued postdoctoral training at the Massachusetts Institute of Technology. In 1991, Dr. Moremen joined the faculty of the Complex Carbohydrate Research Center at the University of Georgia where he is now Professor in the Department of Biochemistry and Molecular Biology. Dr. Moremen has chaired the Glycobiology Gordon Research Conference, served as President, member of the Board of Directors, and Secretary of the Society for Glycobiology. He presently directs efforts on an NIH funded multi-investigator 'Resource for Integrated Glycotechnology', is a senior investigator on the NIH-funded 'National Center for Biomedical Glycomics', and is a lead Principal Investigator or Senior Investigator on eight additional grants from the NIH and Department of Energy. He has served on editorial boards of Journal of Biological Chemistry, Glycobiology, and Glycoconjugate Journal, numerous NIH grant review panels, and Scientific Advisory Boards of four biotech companies. In 2014 Dr. Moremen was appointed the Distinguished Research Professorship in Biochemistry and Molecular Biology at the University of Georgia and has a total of 10 patents and over 150 peer-reviewed publications. In 2018, he launched the biotech startup, Glyco Expression Technologies, Inc., that is located in the UGA Innovation Gateway.

Dr. Robert Haltiwanger - Dr. Haltiwanger received his B.S. in Biology (1980) and Ph.D. in Biochemistry (1986) from Duke University. He went on to do postdoctoral work at Johns Hopkins University School of Medicine, and took his first independent position as an Assistant Professor in the Department of Biochemistry and Cell Biology at Stony Brook University (1991). He rose through the ranks to full Professor and served as Chair of that Department for 8 years. He moved to the CCRC in 2015 as the GRA Eminent Scholar in Biomedical Glycosciences. He has served

as President of the Society for Glycobiology, Chair of the Glycobiology Gordon Conference, and currently serves as Editor-in-Chief of the journal Glycobiology.

Dr. Michael Tiemeyer - Dr. Tiemeyer received his B.A. in biology in 1982 and his Ph.D. in neuroscience in 1989 from The Johns Hopkins University. He was a Helen Hay Whitney postdoctoral fellow in developmental neurobiology at the University of California at Berkeley. Prior to joining the CCRC faculty, Dr. Tiemeyer was a faculty member in cell biology at Yale University School of Medicine and Director of Biochemical and Clinical Analytics and New Methods Development at Glyko/Biomarin, Inc.

Dr. Christine Szymanski - Dr. Szymanski has been exploring bacterial glycomics for more than two decades, working on food pathogens since the early 1990s, with a particular emphasis on Campylobacter jejuni. She combines her expertise in food safety and animal health with novel therapeutic diagnostic platforms developed during her postdoctoral fellowship at the Naval Medical Research Center vaccine program (1996-2000), the key findings while employed at the National Research Council of Canada (2000-2008), and the translational advances during her tenure as an Alberta Innovates Technology Futures Scholar at the University of Alberta (2008-2016). She was the first to demonstrate that bacteria are capable of N-glycosylating proteins and is now exploiting these systems to create glycoconjugate vaccines and oral therapeutics through recombinant expression in Escherichia coli. Dr. Szymanski was also the first to demonstrate that viruses specific for bacteria express proteins that can be used as novel therapeutics in addition to their recognized diagnostic value. These viruses (bacteriophages) are the most abundant biological entity on earth (1031) and are therefore a limitless resource for exploitation, especially in the area of glycomics.

Dr. Lauren Pepi - Dr. Lauren Pepi received her B.A. (ACS certified) in Chemistry and Biology in 2015 from Assumption University (Worcester, MA) and her Ph.D. in Analytical Chemistry in 2020 from the University of Georgia under the direction of Dr. Jon Amster. Her Ph.D. work focused on fundamental studies of tandem mass spectrometry of glycosaminoglycans. Lauren joined the Analytical Service and Training team at the end of 2020, where her research focuses on glycomic and glycoproteomic characterization of N- and O-glycans using mass spectrometry techniques. Lauren has experience using a wide range of mass spectrometers, including FT-ICR MS, Orbitrap MS, MALDI MS and Ion trap MS.

Dr. Asif Shajahan – Dr. Shajahan received his master's degree in Pharmaceutical Chemistry at Hamdard University, India, and his Ph.D. at the National Institute of Immunology, India in 2014. His doctorate study was in the interface of chemistry and biology, where he synthesized various glycoconjugates for the glycoengineering of brain glycans (across BBB) in mice models. He studied glycoengineering in both in vitro and in vivo models using molecular biological and glycoproteomics techniques. He is now an assistant research scientist at the Analytical Service and Training Laboratory, Complex Carbohydrate Research Center (University of Georgia), as a part of glycoprotein analysis team. His research focuses on method development for glycoprotein characterizations by both glycomics and glycoproteomics.

Dr. Christian Heiss – Dr. Christian Heiss received his B.Sc. in Chemistry in 1991 from the University of Erlangen, Germany, and his Ph.D. in Organic Chemistry in 1999 from the University of Georgia. He serves as the Assistant Technical Director of Analytical Service and Training at the Complex Carbohydrate Center. He has written multiple papers on the analysis of carbohydrates, and established the expansion of the CCRC's analysis to glycosaminoglycans in 2006.

Introduction to the Basics of Glycomic and Glycoproteomic Analysis

August 16-18, 2021

Monday, August 16, 2021

8:45 a.m. - 9:00 a.m.

Introduction and Welcome

Dr. Parastoo Azadi

9:00 a.m. - 10:30 a.m.

Lecture -*"Overview of Glycoprotein Structures, Biosynthesis and Function"*

Dr. Kelley Moremen

10:30 a.m. – 11:15 a.m.

Lecture –*"Introduction to Glycomics and Glycopeptide Analysis"*

Dr. Parastoo Azadi

11:15 a.m. – 12:00 p.m.

Lab Exercise discussion – Glycomics

- N-glycan and O-Glycan Release
- Permethylation

12:00 p.m. – 1:00 p.m. – **Lunch Break**

1:00 p.m. – 3:30 p.m.

Laboratory Discussion: Glycomics Analysis by MALDI-TOF and ESI-MS

- MALDI-TOF Profiling
- Direct Infusion – Mass Spectrometry
- MS/MS and MSn Methods

Laboratory Discussion – Glycopeptide Analysis and Glycoproteomics

- Protease and Enzymatic Treatments
- Chromatography and Instrument Settings
- Data Considerations

3:30 p.m. – 4:00 p.m.

Break

4:00 p.m. – 5:00 p.m.

Lecture – *"Glycans Linked to Lipids and Lipid Precursors"*

Dr. Michael Tiemeyer

Tuesday, August 17, 2021

8:45 a.m. – 9:00 a.m. -Zoom Room Opens

9:00 p.m. – 10:30 a.m.

Lecture – *"Regulation of Notch with Glycosylation"*

Dr. Robert Haltiwanger

10:30 a.m. – 11:00 a.m.

Break

11:00 a.m. – 12:00 p.m.

Laboratory Discussion: Glycopeptide and Glycoproteomics Analysis by LC-MS

- MALDI-TOF of Glycopeptides
- Chromatography Considerations
- MS/MS and MSn Methods

Laboratory – Glycopeptide Analysis by Mass-Spectrometry – Data Interpretation

- Databases - Uniprot, MS-Prospector, NetNGlyc, NetOGlyc
- Manual Interpretation
- Software Assisted Analysis

12:00 p.m. – 1:00 p.m. – **LUNCH**

1:00 p.m. – 2:30 p.m.

Introduction to Software Packages for Glycomic and Glycoproteomics Experiments

1:15-1:45 p.m.

Software Demo: Introduction to Byonic

Marshall Berns, PhD
Protein Metrics

1:45 – 2:30 p.m.

Software Demo: Introduction to SimGlycan

Rupanjan Goswami
Premier Biosoft

2:30 p.m. – 3:30 p.m.

Laboratory Demo – "Introduction to Glycan Imaging with Bruker"

Peggi Angel
Medical University of South Carolina

3:30 p.m. – 5:00 p.m.

Lecture – *Bacterial Glycoproteins*

Dr. Christine Szymanski

Wednesday, August 18, 2021

8:45 a.m. – 9:00 a.m. Zoom Room Opens

<Mass Spectrometry Module>

9:00 a.m. – 10:30 a.m.

Lecture – *Glycomics & Glycoproteomics*

Dr. Ron Orlando

10:30 a.m. – 11: 30 a.m.

Laboratory Discussion: Manual and Software Assisted Interpretation of Permethylated Spectra

- Manual Interpretation
- Glycoworkbench Software

Dr. Asif Shajahan

11:30 a.m. – 12:00 p.m.

Lecture – *High Throughput Glycomics with MicroPermethylation*

Dr. Parastoo Azadi/Dr. Asif Shajahan

12:00 p.m. – 1:00 p.m. – **LUNCH**

<NMR Module>

1:00 p.m. – 3:00 p.m.

Lecture – *"Introduction to NMR of glycoproteins and carbohydrates"*

Dr. Christian Heiss

<Molecular Modeling>

3:00 p.m. – 5:00 p.m.

Lecture – *"Molecular modeling of Protein- Carbohydrate Interactions"*

Dr. Lachele Foley