SEPARATION AND CHARACTERIZATION OF GLYCOPROTEIN AND GLYCOLIPID OLIGOSACCHARIDES - GLYCOPROTEIN COURSE

(AUGUST 12-16, 2024)

Course Overview:

This 5-day hands-on course is focused on hands-on experiments for glycomics and glycoproteomics by mass spectrometry. Participants will release N– and O-glycans, perform permethylation, profiling, and sequencing by NSI-MS/MS and MALDI-MS techniques and also includes a special module on glycoproteomics. Other hands-on experiments include glycolipid analysis and lectin blotting experiments. Lectures by Drs. Archer-Hartmann, Azadi, Wells/Orlando and include topics on monosaccharide and oligosaccharide analysis by HPAEC mapping the glycosylation sites in glycoproteins, glycopeptide analysis, determining the composition, quantitation of glycans, sequencing and branching points of N– and O-linked oligosaccharides, and MS procedures used in these analyses.

Takeaways:

- Understand basic principles of protein and lipid glycosylation, structure, and function within biological systems.
- Learn hands-on analytical techniques to release, isolate, and permethylate N- and O-linked glycans
- Familiarize with methods to analyze glycans and glycopeptides using MALDI, LC-MS, tandem MS, and multiple fragmentation techniques.
- Experience hands-on data interpretation with provided example datasets, available databases and downloadable software.
- Featured lectures include:
    - *"Overview of Glycoprotein Structures, Biosynthesis and Function"*- Dr. Kelley Moremen
    - *"Glycans Linked to Lipids and Lipid Precursors*" – Dr. Michael Tiemeyer
    - "*Bacterial Glycoproteins*"- Dr. Christine Szymanski
    - "*Regulation of Notch with Glycosylation"*- Dr. Robert Haltiwanger
- Your choice of NMR, Mass Spectrometry, or Molecular Modeling breakout session

Preparation:

- All courses are hands-on and in-person
- While we will provide PPE, you are welcome and encouraged to bring a lab coat and pipettors.
- 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.

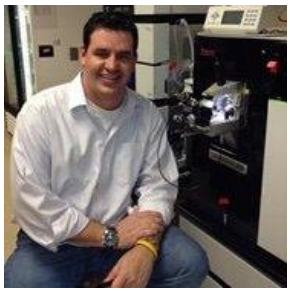
Dr. Robert Haltiwanger - Dr. Haltiwanger received his B.S. in Biology (1980) and Ph.D. in Biochemistry (1986) from Duke University. He went on to do postdoctoral work at Johns Hopkins University School of Medicine, and took his first independent position as an Assistant Professor in the Department of 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. Stephanie Archer-Hartmann – Dr. Stephanie Archer-Hartmann received her B.Sc. in Chemistry in 2006 and her Ph.D in Analytical Chemistry in 2012 from West Virginia University. She has spent more than 10 years working towards improvements for the analysis of carbohydrates, including the isolation, preparation, and analysis of glycosaminoglycans

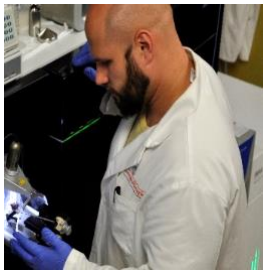
Dr. Bhoj Kumar - Dr. Bhoj Kumar received a BSc. in Biology and MSc. in Biotechnology in 2006 from the Pt. Ravishankar Shukla University and his Ph.D. in Biotechnology in 2014 from the Giwaji University India. After his doctorate, he joined Regional Centre for Biotechnology as Postdoctoral Research Associate and worked on the Mass spectrometry-based Biomarker discovery for Preterm Birth conditions. Dr. Kumar joined the CCRC in Aug 2021.



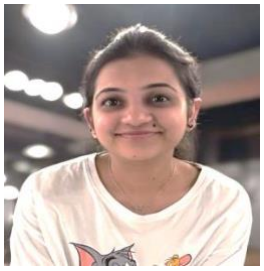
Dr. Lance Wells – Dr. Wells received his B.S. in Chemistry, with a minor in Psychology, in 1991 from the Georgia Institute of Technology, and after spending two years working at the Microchemical Facility, his Ph.D. in Biochemistry and Molecular Biology in 1998 from the Emory University School of Medicine. A postdoctoral research fellowship at the Johns Hopkins School of Medicine in Biological Chemistry followed, which was supported by a National Research Service Award from the National Cancer Institute of the NIH. He is a professor in the Complex Carbohydrate Research Center, conducts research that provides a foundation for understanding O-glycans, which play a critical role in determining protein structure, function and stability.

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.

Dr. Lachele Foley – Dr. Foley received a B.S. in Physical Science from Auburn University in 1988, a M.Ed. in Science Education from the University of Georgia in 1992, and a Ph.D. in Chemistry in 2002. She is currently an Associate Research Scientist at the University of Georgia where she focuses on techniques for modeling of carbohydrates with Dr. Rob Woods.



Dr. Sergei Shalygin - Sergei Shalygin received his B.Sc. in environmental science from Murmansk State Technical University, Russia and his Ph.D. in Plant & Environmental Science from New Mexico State University, USA. His main scientific enquiry during Ph.D. were mostly on environmental toxicology (characterization of cyanobacterial toxins) and plant bioactive metabolites. Now he is working on advanced mass spectrometry, mass spectrometry imaging and bioinformatics.



Dr. Sonali Sunsunwal – Dr. Sonali Sunsunwal received her B.Sc. and M.Sc. in Zoology in 2017 from University of Delhi, India, and her Ph.D. in Microbiology in 2023 from CSIR-IMTech, India. Her Ph.D. work mainly focused on biochemical studies to explore the substrate promiscuity of flagellin glycosyltransferases. Currently, she's a postdoctoral fellow working on advanced mass spectrometry with Dr. Parastoo Azadi.

SEPARATION AND CHARACTERIZATION OF GLYCOPROTEIN AND GLYCOLIPID OLIGOSACCHARIDES

August 14-18, 2023

Monday, August 12, 2024

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

Introduction and Welcome — Dr. Christian Heiss

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

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

Dr. Kelley Moremen

10:00 a.m. - 10:15 a.m. **– Break**

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

Lecture continued – *"Overview of Glycoprotein Structures, Biosynthesis and Function"*

Dr. Kelley Moremen

11:15 a.m. – 12:00 p.m.

Lab Exercise discussion

SECTION I – Monosaccharide composition analysis by HPAEC-PAD

Laboratory

SECTION I – Monosaccharide composition analysis

Begin acid hydrolysis

Dr. Varughese (Alex) Mulamoottil

12:00 p.m. – 1:00 p.m. – **Lunch**

1:00 p.m. – 2:00 p.m.

Lecture – "*Bacterial Glycoproteins"*

Dr. Christine Szymanski

2.00 p.m. – 2.45 p.m.

Lab Exercise discussion

SECTION IV - Permethylation of glycans

Dr. Bhoj Kumar/Dr. Sonali Sunsunwal

2:45 p.m. – 3:00 p.m. **-Break**

3:00 p.m. – 3:30 p.m.

Laboratory

SECTION I – Monosaccharide composition analysis

Recover samples from hydrolysis, freeze digests and lyophilize

Dr. Varughese (Alex) Mulamoottil

SECTION II – Release of N-linked glycans from a glycoprotein (Fetuin)

Denature glycoprotein and start trypsin digestion

Dr. Bhoj Kumar/Dr. Sonali Sunsunwal

3:30 p.m. – 4:15 p.m.

Lab exercise discussion

SECTION VI – Separation of glycolipids by TLC

Laboratory

SECTION VI – TLC analysis

Desialylation of porcine brain gangliosides

Dr. Stephanie Archer-Hartmann

4:15 p.m. – 5:00 p.m.

Lab Exercise discussion

SECTION III – Release of O-glycans from a glycoprotein (Mucin)

Laboratory

SECTION III – Release of O-glycans

β-elimination of O-linked glycans

Dr. Bhoj Kumar

Tuesday, August 13, 2024

9:00 a.m. – 9:15 a.m. – Questions and Discussion

9:15 a.m. – 10:15 a.m.

Lecture – *"Introduction to HPAEC"*

Dr. Parastoo Azadi

10:15 a.m. – 10:30 a.m. – **Break**

10:30 a.m. – 12:00 p.m.

Laboratory

SECTION II – Release of N-glycans

Terminate Trypsin digestion of Fetuin

Dr. Bhoj Kumar/Dr. Sonali Sunsunwal

SECTION III – Release of O-glycans

Neutralization and de-salting of beta-eliminated O-linked glycans

Dr. Bhoj Kumar

SECTION VI – TLC analysis

Sample cleaning by C18 reversed phase cartridge

Dry sample under N_2

Dr. Stephanie Archer-Hartmann

12:00 p.m. – 1:00 p.m. – **LUNCH**

1:00 p.m. – 2:15 p.m.

Lecture – "*Regulation of Notch with Glycosylation"*

Dr. Robert Haltiwanger

2:15 p.m. – 3:00 p.m. **– CCRC Tour** **Dr. Christian Heiss**

3:00 p.m. – 3:10 p.m. – **Break**

3:10 p.m. – 3:40 p.m.

Laboratory

SECTION I – Monosaccharide composition analysis

Preparation of monosaccharide digests for HPAEC analysis

Dr. Varughese (Alex) Mulamoottil

SECTION II – Release of N-glycans

Release N-glycans with PNGase F

Dr. Bhoj Kumar/Dr. Sonali Sunsunwal

3:40 p.m. – 5:00 p.m.

Lab exercise discussion

SECTION V – Labeling of free N-glycans by Reductive Amination

Laboratory

SECTION V – Procainamide labeling

Dr. Stephanie Archer-Hartmann

Wednesday, August 14, 2024

8:45 a.m. – 10:00 a.m.

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

Dr. Michael Tiemeyer

10:00 a.m. – 10:10 a.m. – **Break**

10:10 a.m. – 11:10 a.m.

Lecture –*"Labeling and Separation of Carbohydrates"*

Dr. Stephanie Archer-Hartmann

11:10 a.m. – 12:00 p.m.

Laboratory

SECTION II – Release of N-glycans

Separation of N-glycans from O-glycopeptides/peptides by C18 sep pak

Dr. Bhoj Kumar/Dr. Sonali Sunsunwal

12:00 p.m. – 1:00 p.m. – **LUNCH**

1:00 p.m. – 2:00 p.m.

Laboratory

SECTION III – Release of O-glycans

Removal of Borates from beta-eliminated O-linked glycans

Dr. Bhoj Kumar

2:00 p.m. – 2:30 p.m.

SECTION I – HPAEC Data Discussion

Dr. Varughese (Alex) Mulamoottil

2:30 p.m. – 2:40 p.m. – **Break**

2:40 p.m. – 4:00 p.m.

Laboratory

SECTION VI – TLC analysis

Spot samples

Develop plate

Detection of samples

Dr. Stephanie Archer-Hartmann

4:00 p.m. - 5:00 p.m.

Laboratory

SECTION V – Procainamide Labeling

Removal of free label with SPE cartridges

Lyophilize to dryness.

Dr. Stephanie Archer-Hartmann

Thursday, August 15, 2024

Laboratory

8.45 a.m. – 10:10 a.m.

SECTION IV – Permethylation of released oligosaccharides

(N-glycans from Fetuin or O-glycans from Mucin)

10:10 a.m. – 10:20 a.m. **– Break**

10:20 a.m. – 11:00 a.m.

Laboratory

SECTION IV – Permethylation (continued)/ Introduction to micropermethylation

Dr. Bhoj Kumar

11:00 a.m. – 12:00 p.m.

Glycan Imaging Mass Spectrometry: theoretical overview and quick demo

Sergei Shalygin

12:00 p.m. – 1:00 p.m. – **Group Photo and LUNCH**

1:00 p.m. – 2:15 p.m.

Laboratory

SECTION V – Procainamide Labeling

Overview of HPLC Chromatography

Data Analysis

Dr. Stephanie Archer-Hartmann

2:15 p.m. – 2:55 p.m.

Demonstration

MALDI TOF/TOF MS demonstration

Dr. Bhoj Kumar

2:55 p.m. – 3:05 p.m. – **Break**

3:05 p.m. – 3:45 p.m.

HPAEC and HPLC/CE demonstration

Dr. Varughese (Alex) Mulamoottil /Dr. Stephanie Archer-Hartmann

Discussion of data

3:45 p.m. – 4:00 p.m.

SECTION VI – TLC results

Analysis of TLC data

Dr. Stephanie Archer-Hartmann

4:00 p.m. – 5:00 p.m.

SECTION II, III, IV – MALDI TOF/TOF-MS result

Analysis of N-and O-linked glycans MALDI data & Data analysis

Dr. Bhoj Kumar/Dr. Sonali Sunsunwal

Friday, August 16, 2024

<Mass Spectrometry Module>

9:00 a.m. – 12:00 p.m.

Lecture – *Glycomics & Glycoproteomics* Dr. Lance Wells

10:15 a.m. – 10:30 a.m. – **Break**

12:00 p.m. – 1:00 p.m. – **LUNCH**

1:00 p.m. – 3:00 p.m.:

Mass Spectrometry Facilities Tour – Mass Spec BINGO

MSMS Analysis of Glycans and Glycopeptides

Dr. Stephanie Archer-Hartmann

Or <NMR Module>

9:00 a.m. – 12:00 p.m.

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

Dr. Christian Heiss

12:00 p.m. – 1:00 p.m. – **LUNCH**

Afternoon:

Demonstration and data interpretation

Or <Molecular Modeling Module>

9:00 a.m. – 12:00 p.m.

Lecture – *"Introduction to Molecular Modeling* Dr. Lachele Foley

12:00 p.m. – 1:00 p.m. – **LUNCH**

Afternoon:

Demonstration and data interpretation

Course summary, course evaluation final Q&A