Techniques for Characterization of Carbohydrate Structure of Plant and Microbial Polysaccharides

August 5-9, 2024

Course Overview:

This 5-day hands-on course will provide series of lectures and demonstrations, which cover the theory and techniques of isolation, purification and structure characterization of poly-, oligo-saccharides and glyco-conjugates. Participants will learn the theory and techniques of glycosyl-residue and glycosyl-linkage composition analyses using gas-liquid chromatography mass spectrometry (GC-MS). These methods for derivatization of sugar samples to make alditol acetates and trimethylsilyl derivatives and the production of partially methylated alditol acetates, which the trainees analyze by GC-MS. Lectures and demonstrations will cover such techniques for structural analysis of polysaccharides by mass spectrometry and NMR, and methods for the separation and purification of polysaccharides by chromatographic techniques and monosaccharides and oligosaccharides using HPAEC.

Takeaways:

- Gain an introduction to isolation and structure characterization of poly-, oligo-saccharides and glyco-conjugates.
- Learn various hands-on techniques for the analyses of carbohydrates including:
    - Alditol acetate (AA) composition by GC-MS
    - Methyl glycosides (TMS) composition by GC-MS
    - Partially methylated alditol acetate (PMAA) linkage analysis by GC-MS
- Attend lectures and demonstrations on topics such as: structural characterization by mass spectrometry and NMR, methods for separation and purification of polysaccharides, and monosaccharide analysis by labeling and HPLC or HPAEC.
- Featured lectures include:
    - “Isolation and Characterization of Bacterial Surface Polysaccharides” - Dr. Artur Muszyński
    - “Complex Carbohydrates in Fungal Pathogens Investigated Using Solid-state NMR” – Dr. Liyange Fernando
    - “Different Forms of D-Glucose” - Dr. Geert-Jan Boons
    - “Introduction to Xyloglucan Structure and Biosynthesis” - Dr. Breeanna Urbanowicz
- 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 chemistry and biochemistry.

Instructors:

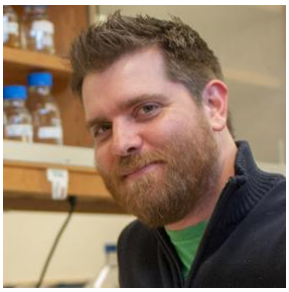
Dr. Parastoo Azadi - Dr. Parastoo Azadi received her B.Sc. in Chemistry in 1987 from University of North London, UK and her Ph.D. degree in Biochemistry in 1991 from Imperial College of Science and Technology, University of London, studying structural characterization of carbohydrates and glycoproteins 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. 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 Research 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. Artur Muszyński - Dr. Artur Muszyński received his Ph.D. degree in Biology in 2004 from the University of Silesia, Poland. He has more than 20 years of study focusing on the microbial glycobiology of bacterial pathogens including isolation and structural analysis.

Dr. Geert-Jan Boons - Dr. Geert-Jan Boons received his M.Sc. in Chemistry in 1987 and his Ph.D. in Synthetic Carbohydrate Chemistry in 1991 from the State University of Leiden in The Netherlands. Prior to joining the faculty at the CCRC in 1998, he spent seven years in the United Kingdom, first as a Postdoctoral Fellow at Imperial College, London, and the University of Cambridge, and then as a lecturer and Professor at the University of Birmingham. In addition to multiple awards for his career and research, he has published more than 250 articles. His research program emphasizes the chemical synthesis and biological functions of complex carbohydrates and glycoconjugates.

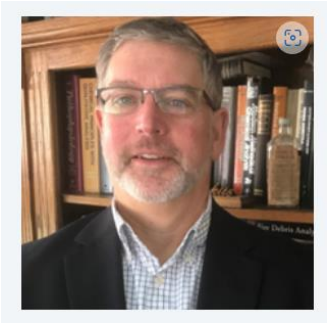
Dr. Breeanna Urbanowicz - Dr. Breeanna Urbanowicz received her B.S. in Biology in 2001 from Purdue University and her Ph.D. in 2008 from Cornell University. Prior to her junior faculty position at the Complex Carbohydrate Research Center, Dr. Urbanowicz was a Postdoctoral Fellow (2008-2013) in the Department of Biochemistry and Molecular Biology at the University of Georgia. She now serves as an Assistant Professor at the CCRC whose work focuses on understanding the integral steps in the molecular pathways used by plants to synthesize complex polysaccharides.



Dr. Ian Wallace – Dr. Ian Wallace received his B.S. and Ph.D. in Biochemistry, both from the University of Tennessee, Knoxville. He graduated in 2008. Wallace has also been a Postdoctoral Research Associate at the Energy Biosciences Institute, UC Berkeley. He was a faculty at the University of Nevada, Reno for nine years, becoming Associate Professor and Graduate Program Director for their Biochemistry and Molecular Biology Department. He arrived at the CCRC in August, 2023.

Dr. Ian Black - Dr. Ian Black received a B.S. in Biochemistry in 2008 and his Ph.D. in Biochemistry in 2021 from the University of Georgia. He is currently a Postdoctoral Researcher with Analytical Services and Training at the Complex Carbohydrate Research Center under the direction of Dr. Parastoo Azadi. His work is primarily devoted to method development, and deriving new protocols for polysaccharide analysis.

Dr. Lachele Foley – Dr. Lachele 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. Robert Woods.



Dr. Kirk Lokits - Dr. Kirk Lokits received his B.S. in Forensic Science and Chemistry from the Eastern Kentucky University. He began his forensic tenure at the Miami Vally Regional Crim Lab in Dayton, Ohio and FDLE in the Orlando and Pensacola laboratories. He left the forensic realm and began his career with HP/Agilent, working as a service engineer supporting LC, GC, LCMS, GCMS and ICPMS products. While working for HP, Kirk earned his MS in Analytical Chemistry from MSTU. In 2005, he left Agilent to attend the University of Cincinnati and earned his Ph.D. in Analytical Chemistry. Afterwards, he worked for the Midwest Research Institute (MRIGroup) in Kansas City as a Principal Chemist and Sr. Program Manager on DoD projects, staffing, designing and building remote laboratories for placement throughout the world. In 2014, he rejoined Agilent as a GCMS Applications Scientist focusing on forensic applications within the GCMS product line.

Monday, August 5, 2024

9:00 a.m. – 9:15 a.m.

Welcome and Introduction

Dr. Parastoo Azadi

9:15 a.m. – 9:30 a.m.

Hydrolysis of AA sample

9:30 a.m. – 10:15 a.m.

Lecture – *"Structural Characterization of Carbohydrates" (Part 1)*

Dr. Parastoo Azadi

10:15 a.m. – 10:30 a.m. – **Break**

10:30 a.m. – 11:00 a.m.

Lecture – *"Structural Characterization of Carbohydrates" (Part 2)*

Dr. Parastoo Azadi

11:00 a.m. – 12:15 p.m.

Remove AA sample from heat and dry down with nitrogen

Sodium borodeuteride reduction of AA sample

Preparation of Size Exclusion Samples

12:15 p.m. – 1:15 p.m. – **Lunch**

1:15 p.m. – 2:30 p.m.

Neutralization and drying of AA sample

Acetylation of AA sample

2:30 p.m. – 3:30 p.m.

Lecture – *"GC/MS for Beginners"*

Dr. Kirk Lokits

3:30 p.m. – 3:45 p.m. – **Break**

3:45 p.m. – 5:00 p.m.

Drying, extraction, and injection of AA sample

Overnight methanolysis of acetylated and native methyl glycosides (TMS) samples

Tuesday, August 6, 2024

9:00 a.m. – 9:30 a.m.

Dry down methyl glycosides (TMS) sample

Prepare NaOH base for permethylation (Linkage)

9:30 a.m. – 10:25 a.m.

Permethylation of the linkage sample

N-acetylation of methyl glycosides samples (TMS)

10:25 a.m. – 10:40 a.m. – **Break**

10:40 a.m. – 11:15 a.m.

Remove the CH_3I, DCM Extraction and drying (Linkage)

Drying of the N-acetylated methyl glycosides (TMS) sample after 30 min reaction

11:30 a.m. – 12:45 p.m.

Lecture – *"Chemical Properties of Mono and Oligosaccharides"*

Dr. Geert-Jan Boons

12:45 p.m. – 1:45 p.m. – **Lunch**

1:45 p.m. – 3:15 p.m.

Trimethylsilylation of the methyl glycosides- (TMS) sample

Drying, centrifugation, and injection of the methyl glycosides- (TMS) sample

Demonstration- Preparation of (dimsyl) potassium dimethylsuphonyl anion

3:15 p.m. – 4:30 p.m.

Lecture – HPAEC

Dr. Parastoo Azadi

4:30 p.m. – 4:35 p.m. – **Break**

4:35 p.m. – 4:45 p.m.

Overview of GCMS instrument

Dr. Ian Black

4:45 p.m. – 5:00 p.m.

Lecture - *"FACES Scheduling"* Saeid Roushanzamir / Brian Perkins

Wednesday, August 07, 2024

9:00 a.m. – 9:15 a.m.

Hydrolysis of PMAA sample

9:15 a.m. – 10:00 a.m.

Demonstration – Lignin Analysis by Pyrolysis -Molecular Beam -MS

Dr. Artur Muszyński

10:00 a.m. – 10:15 a.m. – **Break**

10:15 a.m. – 11:30 a.m.

Lecture – *"Isolation and Characterization of Bacterial Surface polysaccharides"*

Dr. Artur Muszyński

11:30 a.m. – 12:15 p.m.

Remove linkage sample from heat and dry down with nitrogen

Sodium borodeuteride reduction of linkage sample

12:15 p.m. – 1:15 p.m. – **Lunch**

1:15 p.m. – 2:30 p.m.

Neutralization and drying of PMAA sample

Acetylation of PMAA sample

2:30 p.m. – 3:15 p.m.

Data analysis of AA

Drs. Muszyński and Black

3:15 p.m. – 3:30 p.m. – **Break**

3:30 p.m. – 4:15 p.m.

Lecture – *"Labeling and Preparative and Analytical Size Exclusion Chromatography of Polysaccharides- a practical approach for isolation and purification of polysaccharides"*

Dr. Stephanie Archer- Hartman

4:15 p.m. – 5:00 p.m.

Drying, extraction, and injection of PMAA sample

Thursday, August 08, 2024

9:00 a.m. – 10:00 a.m.

Data analysis of TMS

Drs. Muszyński and Black

10:00 a.m. – 10:15 a.m. – **Break**

10:15 a.m. – 12:15 p.m.

Data analysis for linkage

Drs. Muszyński and Black

12:15 p.m. – 1:15 p.m. – **Lunch**

1:15 p.m. – 2:30 p.m.

Lecture - *"Cellulose: Structure, Analysis, and Application"*

Dr. Ian Wallace

2:30 p.m. – 2:45 p.m. – **Break**

2:45 p.m. – 3:00 p.m.

Demonstration - HPAEC -Oligosaccharide profiling analysis and monosaccharide composition analysis

Dr. Varughese Mulamoottil

3:00 p.m. – 3:30 p.m.

Dasta analysis for Size Exclusion Chromatography

Drs. Muszyński and Black

3:30 p.m. – 4:00 p.m.

Questions and answers for data interpretation of all the analyses

Drs. Azadi, Black, and Muszyński

4:00 p.m. – 5:00 p.m.

Lecture – "*Introduction to Xyloglucan Structure and Biosynthesis"*

Dr. Breeanna Urbanowicz

Friday, August 09, 2024

Module of your choice

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

Mass Spectrometry Module

9:00 a.m. – 12:00 p.m.

Lecture – *"Mass Spectrometry of N-linked Glycans"*

Dr. Ron Orlando

12:00 p.m. – 1:00 p.m. – **Lunch**

Afternoon:

Demonstration and data interpretation

Molecular Modeling Module

9:00 a.m. – 12:00 p.m.

Lecture and Demo– *"Introduction to Molecular Modeling"*

Dr. Lachele Foley

12:00 p.m. – 1:00 p.m. **– Lunch**

Afternoon:

Demonstration

Course summary, Course evaluation, Final Questions and Answers