

Analytical Techniques for Structural Characterization of Glycosaminoglycans

August 21-23, 2021

Course Overview:

In this 3-day course, participants will learn fundamental techniques for glycosaminoglycan (GAG) analysis including hands-on experiments in GAG compositional analysis, HPLC, acid hydrolysis and size exclusion chromatography. An introduction into general techniques for GAG isolation and characterization will be covered. Topics to be included are: Biological considerations of glycosaminoglycans, GAGs as biotherapeutics, and isolation strategies of GAGs from cells and tissue. Participants will explore various methods of the analysis of GAGs with combinations of enzymatic or chemical treatments, chromatography, and mass spectrometry (LCMS and ESI-MS/MS).

Takeaways:

- Gain an introduction to glycosaminoglycans, including their status as potent biotherapeutics, and learn relevant analytical techniques.
- Understand characterization techniques for GAG-based Drug Products, and GAG modification strategies.
- Learn strategies to release, isolate, and analyze GAGs through a variety of methods including chromatography, mass spectrometry, plate assays, and NMR.
- Participants will experience hands-on data interpretation with provided example datasets.
- Featured lectures include:
 - “*Mass Spectrometry Analysis for Glycosaminoglycans*” – Dr. Franklin Leach
 - “Glycosaminoglycans in Biomedicine” – Dr. Ryan Weiss
 - “Carbohydrates Drug Products and their Structures” – Dr. Parastoo Azadi

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 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. 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. Franklin Earl Leach – Dr. Franklin Leach received his B.Sc. in Chemistry in 2001 from Mississippi State University, and his Ph.D. in Analytical Chemistry in 2011. He currently serves as an Assistant Professor at the CCRC.



Dr. Ryan Weiss - Dr. Ryan Weiss received his B.S. in chemistry in 2008 at Point Loma Nazarene University in San Diego, CA. He then received his Ph.D. in chemistry in 2015 at the University of California, San Diego, under the supervision of Prof. Yitzhak Tor. He then moved to the Department of Cellular and Molecular Medicine at the University of California, San Diego, where he worked as a postdoctoral fellow in Prof. Jeffrey Esko's group. Dr.

Weiss started his independent career as an assistant professor at the Complex Carbohydrate Research Center at the University of Georgia starting in January 2021. His current research interests include drug discovery and using genomic tools to understand the regulation of glycosylation in human diseases.

Analytical Techniques for Structural Analysis of Glycosaminoglycans (GAGs)

2023 Schedule

Monday, August 21, 2023

9:00 a.m. – 9:15 a.m.

Introduction and Welcome

9:15 a.m. – 10:30 a.m.

Lecture – “*GAG Analysis Methods, Part 1*”

Dr. Christian Heiss

10:30 a.m. -10:45 a.m. – **Break**

10:45 a.m. – 11:15 a.m.

Lecture – “*GAG Analysis Methods*” (continued)

Dr. Christian Heiss

11:15 a.m. – 12:30 p.m.

Laboratory

Introduction to Laboratory experiments

Experiment 4 – Nitrous Acid Preparation and Digestion. *Page 9.*

Prepare samples for analysis by HPLC.

Dr. Lauren Pepi

Laboratory

Introduction to Chromatographic Methods - SAX-HPLC introduction. *Page 9.*

Dr. Stephanie Archer-Hartmann

12:30 p.m. – 1:30 p.m. – **Lunch**

1:30 p.m. – 2:30 p.m.

Laboratory

Experiment 4 – Discussion of Experiment 4 Results. Discussion of alternative methods

Dr. Lauren Pepi

2:30-3:00 p.m. -

Lecture “Considerations for GAG Isolation from Cells and Tissue”

Dr. Stephanie Archer-Hartmann

3:00 p.m. – 5:00 p.m.

Laboratory

(Pre-Experiments 1-3) – Introduction of Analysis by Enzymatic Methodologies.

Start Enzyme Digestions. *Page 3*

Dr. Stephanie Archer-Hartmann

Experiment 5 – Sulfate Analysis – Introduction

Hydrolysis. *Page 13.*

Dr. Lauren Pepi

Tuesday, August 22, 2023

8:45 a.m. – 9:00 a.m.

Questions and Discussion

9:00 a.m. – 10:00 a.m.

Laboratory

Experiment 2 – Stop Enzyme Digestions

Prepare Sample and Inject on SAX-HPLC. *Page 6.*

Experiment 3- Prepare centrifuge tube to dry down. *Page 8.*

Dr. Stephanie Archer-Hartmann

10:00 a.m. – 10:30 a.m. – **Break**

10:30 a.m. – 11:30 p.m.

Lecture – “*Mass Spectrometry Analysis for Glycosaminoglycans*”

Dr. Franklin Leach

11:30 a.m. – 12:30 p.m.

Experiment 5 – Sulfate Analysis – Plate Assay. *Page 13.*

Dr. Lauren Pepi

12:30 p.m. – 1:30 p.m. – **Lunch**

1:30 p.m. – 2:00 p.m.

Demonstration – “*Optical Analytical Techniques: Surface Plasmon Resonance (SPR) and Biolayer-Interferometry (BLI).*”

Dr. Varughese Mulamoottil

2:00 p.m. – 2:15 p.m. – **Break**

2:15 p.m. – 3:45 p.m.

Lecture – Lecture - Carbohydrates Drug Products and their Structures

Dr. Parastoo Azadi

3:45 p.m. – 5:00 p.m.

Laboratory

Experiment 3 – Introduction to GAG Disaccharide Labels

Label with AMAC (Demonstration). *Page 8.*

Dr. Stephanie Archer-Hartmann

Wednesday, August 23, 2023

8:45 a.m. – 9:00 a.m.

Questions and Discussion

9:00 a.m. – 10:30 a.m.

Lecture – “Glycosaminoglycans in Biomedicine”

Dr. Ryan Weiss

10:30 a.m. – 10:45 a.m. – **Break**

10:45 a.m. – 12:30 p.m.

Laboratory

Experiment 1 – Data Analysis and Interpretation

Dr. Stephanie Archer-Hartmann

Experiment 6 – Data Analysis and Interpretation

Dr. Lauren Pepi

12:30 p.m. – 1:30 p.m. – **Lunch**

1:30 p.m. – 2:45 p.m.

Lecture – “*Monosaccharide Composition and Linkage by GC-MS.*”

Dr. Parastoo Azadi

2:45 p.m. – 3:00 p.m. – **Break**

3:00 p.m. 4:00 p.m.

Laboratory –

Experiment 2 – Data Analysis and Interpretation

Dr. Stephanie Archer-Hartmann

Experiment 7 – Introduction to SEC: MW Determination of whole GAGs and GAG products by SEC-HPLC. *Page 15.*

Dr. Stephanie Archer-Hartmann

4:00 p.m. – 5:00 p.m.

Laboratory

Experiment 3 (DEMO) – Separation of AMAC Labeled GAGs by Capillary Electrophoresis and HPLC. *Page 8.*

Dr. Stephanie Archer-Hartmann