

Analytical Techniques for Structural Analysis of Glycosaminoglycans (GAGs)

August 19-22, 2019

Schedule

Monday, August 19, 2019

9:00 a.m. – 9:15 a.m.

Introduction and Welcome

9:15 a.m. – 10:00 a.m.

Lecture – “GAG Analysis Methods, Part 1”

Dr. Christian Heiss

10:00 a.m. – 10:30 a.m.

Laboratory

- Introduction to Laboratory Experiments

Dr. Stephanie Archer-Hartmann

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

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

Prepare samples for analysis by HPLC.

Dr. Zhirui Wang

12:30 p.m. – 1:30 p.m. – **Lunch**

1:30 p.m. – 2:15 p.m.

Laboratory

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

2:15-2:30 p.m. **Break**

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 6 – Sulfate Analysis – Introduction

Hydrolysis. *Page 13.*

Dr. Zhirui Wang

Tuesday, August 20, 2019

8:45 a.m. – 9:00 a.m.

Questions and Discussion

9:00 a.m. – 10:00 a.m.

Laboratory

Experiment 5 – Deacetylation and Nitrous acid: Hydrazinolysis. *Page 11.*

Dr. Zhirui Wang

10:00 a.m. – 10:30 a.m. – **Break**

10:30 a.m. – 12:30 p.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

Experiment 6 – Sulfate Analysis – Plate Assay. *Page 13.*

Dr. Zhirui Wang

12:30 p.m. – 1:30 p.m. – **Lunch**

1:30 p.m. – 2:45 p.m.

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

Anne Gleinich

2:45 p.m. – 3:00 p.m. – **Break**

3:00 p.m. – 4:00 p.m.

Lecture – “*Mass Spectrometry Analysis for Glycosaminoglycans*”

Dr. Franklin Leach

4:00 p.m. – 5:00 p.m.

Laboratory

Experiment 5 – Deacetylation and Nitrous Acid: Stop Reaction and Lyophilize.
Page 11.

Dr. Zhirui Wang

Experiment 3 – Introduction to GAG Disaccharide Labels

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

Dr. Stephanie Archer-Hartmann

Wednesday, August 21, 2019

8:45 a.m. – 9:00 a.m.

Questions and Discussion

9:00 a.m. – 10:00 a.m.

Laboratory

Experiment 5 – Deacetylation and Nitrous Acid: Nitrous Acid and Inject into HPLC. *Page 11.*

Dr. Zhirui Wang

10:00 a.m. – 10:15 a.m. – **Break**

10:15 a.m. – 10:45 a.m.

Demonstration – “LC-MS of LMW Heparins”

Dr. Zhirui Wang

10:45 a.m. – 11:30 p.m.

Laboratory

Experiment 1 - UV and DMB Assay. *Page 4*

Dr. Stephanie Archer-Hartmann

11:30 a.m. – 12:30 a.m.

Laboratory

Experiment 1 – Data Analysis and Interpretation

Dr. Stephanie Archer-Hartmann

Experiment 6 – Data Analysis and Interpretation

Dr. Zhirui Wang

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. Zhirui Wang

4:00 p.m. – 5:00 p.m.

Laboratory

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

Dr. Stephanie Archer-Hartmann

Thursday, August 22, 2019

8:45 a.m. – 9:00 a.m. – Questions and Discussion

9:00 a.m. – 10:00 a.m.

Demonstration – “*Characterization of GAGs by NMR*”

Dr. Christian Heiss

10:00 a.m. – 10:15 a.m. – **Break**

10:15 a.m. – 12:00 p.m.

Demonstration – “*NMR (continued)*”

Dr. Christian Heiss

12:00 a.m. – 12:30 p.m.

Laboratory – “NMR (continued)”

Experiment 4&5 – Data Interpretation

Dr. Zhirui Wang

12:30 p.m. – 1:30p.m. – Lunch

1:30 p.m. – 3:00 p.m.

Laboratory

Final data interpretation and analysis. Summary of experiments.

Course summary, course evaluation, and final questions and answers.