Techniques for Characterization of Carbohydrate Structure of Polysaccharides

August 5-9, 2019

Room 1036 (first floor of building)

LABORATORY EXERCISE

Section I

Alditol acetate (AA) composition

Section II

Methyl glycosides (TMS) composition

Section III

Partially methylated alditol acetate (PMAA) linkage

Lab Instructors: Zhirui Wang, Sara Porfirio, and Ian Black

Monday, August 5, 2018

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

12:15 p.m. – 1:15 p.m. – **LUNCH**

1:15 p.m. – 2:15 p.m.

Neutralization and drying of AA sample

Acetylation of AA sample

2:15 p.m. – 3:45 p.m.

Lecture – *"Different Forms of D-Glucose"*

Dr. Geert-Jan Boons

3:45 p.m. – 4:00 p.m. – **Break**

4:00 p.m. – 5:00 p.m.

Drying, extraction, and injection of AA sample

Overnight methanolysis of methyl glycosides (TMS) sample

Tuesday, August 6, 2018

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

11:15 a.m. – 12:15 p.m.

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

Dr. Artur Muszynski

12:15 p.m. – 1:15 p.m. – **LUNCH**

1:15 p.m. – 2:30 p.m.

Silylation of the methyl glycosides (TMS) sample

Laboratory Demonstration (Lab 1025)

Preparation of (dimsyl) potassium dimethylsuphinyl anion

2:30 p.m. – 3:45 p.m.

Lecture – "*A developing hypothesis: GAUTs synthesize pectic domains required for plant cell wall architecture: An example of how "polysaccharide" structural determination provides unique understanding"*

Dr. Debra Mohnen

3:45 p.m. – 4:00 p.m. – **Break**

4:00 p.m. – 4:15 p.m.

Lecture - *"FACES Scheduling"*

Saeid Roushanzamir

4:15 p.m. – 5:00 p.m.

Laboratory

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

Wednesday, August 7, 2018

9:00 a.m. – 9:15 a.m.

Hydrolysis of PMAA sample

9:15 a.m. – 9:45 a.m.

Demonstration – *"Lignin Analysis by Pyrolysis -Molecular Beam -MS"*

John Tang

9:45 a.m. – 10:00 a.m. – **Break**

10:00 a.m. – 11:00 a.m.

Lecture – *"Introduction to High Performance Anion Exchange Chromatography"*

Dr. Parastoo Azadi

11:00 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

Dr. Zhirui Wang, Ian Black

3:15 p.m. – 3:30 p.m. – **Break**

3:30 p.m. – 4:15 p.m.

Lecture – *Analytical tools for structural characterization of peptidoglycan*

Dr. Sara Porfirio

4:15 p.m. – 5:00 p.m.

Drying, extraction, and injection of PMAA sample

Thursday, August 8, 2018

9:00 a.m. – 10:00 a.m.

Data analysis of TMS

Dr. Zhirui Wang

10:00 a.m. – 10:15 a.m. – **Break**

10:15 a.m. – 11:15 a.m.

Lecture – *"Labeling and Separation of Carbohydrates"*

Dr. Stephanie Archer-Hartmann

11:15 a.m. – 12:15 p.m.

Data analysis for linkage

Ian Black

12:15 p.m. – 1:15 p.m. – **LUNCH**

1:15 p.m. – 1.35 p.m.

HPAEC demonstration: Oligosaccharide profiling analysis and monosaccharide composition analysis

Dr. Zhirui Wang

1:35 p.m. – 2:45 p.m.

Questions and answers for data interpretation of all the analyses

Drs. Wang and Azadi

2:45 p.m. – 3.00 p.m. – **Break**

3:00 p.m. – 4:00 p.m.

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

Dr. Breeanna Urbanowicz

4:00 p.m. – 5:00 p.m.

Lecture - *"Glycome Profiling "*

Dr. Michael Hahn

Friday, August 9, 2018

<NMR Module>

9:00 a.m. – 12:00 p.m.

Lecture – *"Introduction to NMR of Glycoproteins and Carbohydrates"*

Dr. John Glushka

12:00 p.m. – 1:00 p.m. – **LUNCH**

Afternoon:

Demonstration and data interpretation

Or <Mass Spectrometry Module>

9:00 a.m. – 12:00 p.m.

Lecture – *"Mass Spectrometry analysis of oligosaccharides"*

Dr. Parastoo Azadi

Afternoon:

Demonstration and data interpretation

Or <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