Techniques for Characterization of Carbohydrate Structure of Polysaccharides

August 10-14, 2015

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, Radnaa Naran, Justyna Dobruchowska and Ian Black
Techniques for Characterization of Carbohydrate Structure of Polysaccharides

August 10-14, 2015

Monday, August 10, 2015

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.-11:00 a.m.  
**Lecture:** Dr. Parastoo Azadi:  
"Structural Characterization of Carbohydrates"

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:** Dr. Geert-Jan Boons:  
"Different Forms of D-Glucose"

3:45 p.m.- 5:00 p.m.  
Drying, extraction and injection of AA sample  
Overnight methanolysis of methyl glycosides (TMS) sample

Tuesday, August 11, 2015

9:00 a.m.- 9:30 a.m.  
Dry down methyl glycosides (TMS) sample.

9:30 a.m.-10:25 a.m.  
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$_3$I, DCM Extraction and drying (Linkage)

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

11:15 a.m.-12:15 p.m. **Lecture: Dr. Debra Mohnen**

*TBA*

12:15 p.m.- 1:15 p.m. **LUNCH**

1:15 p.m.- 2:15 p.m. Silylation of the methyl glycosides (TMS) sample

Laboratory demonstration (Lab 1025)

Preparation of (dimesyl) potassium dimethylsulphinyl anion

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

2:30 p.m.- 4:00 p.m. Laboratory

Drying, filtration and injection of the methyl glycosides (TMS) samples

4:00 p.m.- 5:00 p.m. **Lecture: Dr. Artur Muszynski**

*“Isolation and Characterization of Bacterial Surface Polysaccharides”*

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**Wednesday, August 12, 2015**

9:00 a.m.- 9:15 a.m. Hydrolysis of PMAA sample

9:15 a.m.- 9:45 a.m. **Demonstration: Dr. Roberto Sonon**

*“Lignin, MBMS”*

9:45 a.m.- 10:00 a.m. **BREAK**

10:00 a.m.-11:00 a.m. **Lecture: Dr. Parastoo Azadi**

*“Introduction to High Performance Anion Exchange Chromatography”*

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 AA sample
Acetylation of AA sample

2:30 p.m.- 3:15 p.m. Data analysis of AA: Dr. Parastoo Azadi

3:15 p.m.- 3:30 p.m. BREAK

3:30 p.m.- 5:00 p.m. Drying, extraction and injection of PMAA sample

**Thursday, August 13, 2015**

9:00 a.m.- 9:45 a.m. Data analysis of TMS: Dr. Zhirui Wang

9:45 a.m.-10:00 a.m. Break

10:00 a.m.-11:00 a.m. **Lecture: Dr. Michael Hahn**

TBA

11:00 a.m.-12:15 p.m. **Lecture: Dr. Stephanie Archer-Hartmann**

“Labeling and Separation of Carbohydrates”

12:15 p.m.- 1:15 p.m. LUNCH

1:15 p.m.- 2:00 p.m. HPAEC demonstration: Oligosaccharide profiling analysis and monosaccharide composition analysis: Dr. Roberto Sonon

2:00 p.m.- 2:45 p.m. Data analysis of linkage: Dr. Parastoo Azadi

2:45 p.m.- 3:20 p.m. BREAK

3:20 p.m.- 3:45 p.m. **Lecture: Saeid Roushanzamir**

“FACES Scheduling”

3:45 p.m.- 5:00 pm Questions and answers for data interpretation of all the analyses

**Friday, August 14, 2015**

Choose one of the following:

NMR Module or Mass Spectrometry Module

**NMR Module**

9:00 a.m.-12:00 p.m. **Lecture: Dr. John Glushka**

“Introduction to NMR of Glyproteins and Carbohydrates”
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: Dr. Parastoo Azadi
                        "Mass Spectrometry of O-Linked Glycans"

12:00 p.m.- 1:00 p.m.    LUNCH

1:00 p.m.- 1:45 p.m.    Demonstration and data interpretation

1:45 p.m.- 3:00 p.m.    Demonstration - Mayumi Ishihara

**Computer Modeling Module**

9:00 a.m.-12:00 p.m.    Lecture: Dr. Lachele Foley

12:00 p.m. - 1:00 p.m.    LUNCH

Afternoon    Demonstration

Course summary, Course evaluation, Final Questions & Answers