

SEPARATION AND CHARACTERIZATION OF GLYCOPROTEIN AND GLYCOLIPID OLIGOSACCHARIDES

August 17-21, 2015

Note : A 30-minute session each day is available to discuss participants' respective research with Dr. Parastoo Azadi. You may make an appointment with her or you can visit her office concerning this matter (during the coffee break, lunch time, or after the lab exercise).

Monday, August 17, 2015

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

Introduction and Welcome

Dr. Parastoo Azadi

9:10 a.m. - 10:15 a.m. - Lecture

"Overview of Glycoprotein Structures, Biosynthesis and Function"

Dr. Kelly Moremen

10:15 a.m. - 10:30 a.m. - BREAK

10:30 a.m. - 11:30 a.m. - Lecture

(continued)"

Dr. Kelly Moremen

11:30 a.m. - 12:00 p.m.

Lab exercise discussion

SECTION I - Monosaccharide composition analysis by HPAEC

Laboratory

SECTION I - Monosaccharide composition analysis

-Begin hydrolyses

Dr. Roberto Sonon

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

1:00 p.m. - 2:00 p.m. - Lecture

"Carbohydrates, Cyclooctynes and Cholesterol: How Chemistry Can Help Us

Visualize the Biology of Human Disease”

Dr. Richard Steet

2:00 p.m. - 2:45 p.m. - Lecture

“Labeling and Separation of Carbohydrates”

Dr. Stephanie Archer-Hartmann

2:45 p.m. - 3:00 p.m. – BREAK

3:00 p.m. – 5:00 p.m.

Laboratory

SECTION I - Monosaccharide composition analysis

- Remove samples from heat at appropriate times; cool on ice; centrifuge briefly; drydown hydrolysates

Dr. Roberto Sonon

Lab exercise discussion

SECTION VI - Separation of glycolipids by TLC

Laboratory

SECTION VI - TLC analysis

- Desialylation of porcine brain gangliosides

Mayumi Ishihara

Lab exercise discussion

SECTION II - Release of N-glycans from a glycoprotein

Laboratory

SECTION II - Release of N-glycans

- Heat a glycoprotein solution to denature
- Begin Tryptic digestion

Mayumi Ishihara

Lab exercise discussion

SECTION III - Release of O-glycans from a glycoprotein

Laboratory

SECTION III – Release of O-glycans

- β -elimination of O-linked glycoprotein compounds

Dr. Roberto Sonon

Tuesday, August 18, 2015

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

9:00 a.m. - 9:15 a.m.

Laboratory

SECTION III - Release of O-glycans
- Stop beta-elimination

Dr. Roberto Sonon

9:15 a.m. - 10:15 a.m. - **Lecture**

“Release and Analysis of N and O-linked Glycans from Glycoproteins”

Dr. Parastoo Azadi

10:15 a.m. - 10:30 a.m. - **BREAK**

10:30 a.m. - 11:45 a.m. - **Lecture**

“Introduction to HPAEC”

Dr. Parastoo Azadi

11:45 a.m. - 12:00 p.m.

Laboratory

SECTION II - Release of N-glycans
- Stop Tryptic digestion

Mayumi Ishihara

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

1:00 p.m. - 2:00 p.m. - **Lecture**

TBA

Dr. Michael Pierce

2:00 p.m. - 2:45 p.m.

Laboratory

SECTION III - Release of O-glycans
- De-salting of O-linked glycoprotein compounds

Dr. Roberto Sonon

2:45 p.m. - 3:00 p.m. - **BREAK**

Commented [CC1]:

3:00 p.m. – 5:00 p.m.

Laboratory

SECTION VI - TLC analysis

- Sample cleaning by C18 reversed phase cartridge
- Dry the sample under N₂

Mayumi Ishihara

SECTION I - Monosaccharide composition analysis

- Resuspend strong acid hydrolysates, transfer to autosampler vials, and load autosampler

Dr. Roberto Sonon

SECTION II – Release of N-glycans

- Release N-glycans with PNGase F

Mayumi Ishihara

Lab exercise discussion

SECTION V – Detection and analysis of carbohydrates by lectin blotting

Laboratory

SECTION V - Lectin blotting

- Put samples on nitrocellulose membrane
- Blocking

Dr. Asif Shajahan

Wednesday, August 19, 2015

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

9:00 a.m. - 10:00 a.m. - **Lecture**

TBA

Dr. Michael Tiemeyer

10:00 a.m. - 10:15 a.m. - **BREAK**

10:15 a.m. - 11:00 a.m.

Laboratory

SECTION II – Release of N-glycans

- Separation of N-linked oligosaccharides by C18 reversed phase cartridge

Mayumi Ishihara

11:00 a.m. – 12:00 p.m. – **CCRC Tour and lab exercise discussion**

12:00 p.m. - 1:00 p.m. – **LUNCH**

1:00 p.m. - 2:15 p.m.

Laboratory

SECTION III – Release of O-glycans

- Removal of Borates from O-linked glycoprotein compounds

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

2:30 p.m. - 5:00 p.m.

Laboratory

SECTION VI – TLC analysis

- Spot samples
- Develop plate
- Detection of samples

Mayumi Ishihara

Laboratory

SECTION V – Lectin blotting

- Wash blots
- Probe blots with lectins

Dr. Asif Shajahan

Thursday, August 20, 2015

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

9:00 a.m. - 11:00 a.m.

Lab exercise discussion

SECTION IV – Permethylation of released oligosaccharides

Laboratory

SECTION IV - Permethylation

- Base preparation
- Permethylation
- Extraction of permethylated glycans
- Dry the sample under N₂

Mayumi Ishihara

11:00 a.m.-11:15 a.m. - **BREAK**

11:15 a.m. - 12:15 p.m.

Laboratory

SECTION V – Lectin blotting

- Wash blots
- Overlay with antibody – AP (45 min ~ 1 h incubation) **

** Lunch during incubation

Dr. Asif Shajahan

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

1:15 p.m. – 2:15 p.m.

Laboratory

SECTION V – Lectin blotting

- Wash blots
- Color development, record results

Dr. Asif Shajahan

2:15 p.m. – 2:45 p.m.

Demonstration

–Separation and analysis of mono and oligosaccharides by HPAEC/HPLC/CE

Dr. Roberto Sonon / Dr. Dandan Zhou / Dr. Stephanie Archer-Hartmann

2:45 p.m. – 3:00 p.m. - **BREAK**

3:00 p.m. - 5:00 p.m.

Laboratory exercise discussion

SECTION I - HPAEC results

- Quantitation of monosaccharide composition

Dr. Roberto Sonon

SECTION II, III, IV - MALDI-MS result

- Analysis of N and O-linked permethylated glycans data

Mayumi Ishihara

SECTION VI- TLC results

- Analysis of TLC data

Mayumi Ishihara

SECTION V - Lectin blotting results

- Analysis of lectin blotting data

Dr. Asif Shajahan

Friday, August 21, 2015

<Mass Spectrometry Module>

9:00 a.m.-12:00 p.m. - Lecture
TBA

Dr. Lance Wells

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

Afternoon

Demonstration and data interpretation

Mayumi/Roberto/Asif

<NMR Module>

9:00 a.m.-12:00 p.m. - Lecture

“Introduction to NMR of Glycoproteins and Carbohydrates”

Dr. John Glushka

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

Afternoon

Demonstration and data interpretation

<Computer Modeling Module>

9:00 a.m.-12:00 p.m. - Lecture
TBA

Dr. Lachele Foley

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

Afternoon

Demonstration

Course summary, Course evaluation, Final Questions & Answers