

# SEPARATION AND CHARACTERIZATION OF GLYCOPROTEIN AND GLYCOLIPID OLIGOSACCHARIDES

August 12-16, 2019

## Monday, August 12, 2019

8:45 a.m. - 9:00 a.m.

Introduction and Welcome

Dr. Parastoo Azadi

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

**Lecture** - *“Overview of Glycoprotein Structures, Biosynthesis and Function”*

Dr. Kelley Moremen

10:00 a.m. - 10:15 a.m. – **Break**

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

**Lecture continued** – *“Overview of Glycoprotein Structures, Biosynthesis and Function”*

Dr. Kelley Moremen

11:15 a.m. – 12:00 p.m.

**Lab Exercise discussion**

**SECTION I** – Monosaccharide composition analysis by HPAEC-PAD

**Laboratory**

**SECTION I** – Monosaccharide composition analysis

Begin acid hydrolysis

Dr. Zhirui Wang

12:00 p.m. – 1:00 p.m. – **Lunch**

1:00 p.m. – 2:00 p.m.

**Lecture** – *“Labeling and Separation of Carbohydrates”*

Dr. Stephanie Archer-Hartmann

2.00 a.m. – 2.45 p.m.

**Lab Exercise discussion**

**SECTION IV** - Permethylation of glycans

Dr. Asif Shajahan/Dr. Nitin Supekar

2:45 p.m. – 3:00 p.m. -Break

3:00 p.m. – 3:30 p.m.

**Laboratory**

**SECTION I** – Monosaccharide composition analysis

Recover samples from hydrolysis, freeze digests and lyophilize

Dr. Zhirui Wang

**SECTION II** – Release of N-linked glycans from a glycoprotein (Fetuin)

Denature glycoprotein and start trypsin digestion

Dr. Asif Shajahan

3:30 p.m. – 4:15 p.m.

**Lab exercise discussion**

**SECTION VI** – Separation of glycolipids by TLC

**Laboratory**

**SECTION VI** – TLC analysis

Desialylation of porcine brain gangliosides

Dr. Sara Porfirio

4:15 p.m. – 5:00 p.m.

**Lab Exercise discussion**

**SECTION III** – Release of O-glycans from a glycoprotein (Mucin)

**Laboratory**

**SECTION III** – Release of O-glycans

$\beta$ -elimination of O-linked glycans

Dr. Nitin Supekar

**Tuesday, August 13, 2019**

9:00 a.m. – 9:15 a.m. – Questions and Discussion

9:15 a.m. – 10:15 a.m.

**Lecture** – *“Introduction to HPAEC”*

Dr. Parastoo Azadi

10:15 a.m. – 10:30 a.m. – **Break**

10:30 a.m. – 12:00 p.m.

**Laboratory**

**SECTION II** – Release of N-glycans

Terminate Trypsin digestion of Fetuin

Dr. Asif Shajahan

**SECTION III** – Release of O-glycans

Neutralization and de-salting of beta-eliminated O-linked glycans

Dr. Nitin Supekar

**SECTION VI** – TLC analysis

Sample cleaning by C18 reversed phase cartridge

Dry sample under N<sub>2</sub>

Dr. Sara Porfirio

12:00 p.m. – 1:00 p.m. – **LUNCH**

1:00 p.m. – 2:15 p.m.

**Lecture** – *“Regulation of Notch with Glycosylation”*

Dr. Robert Haltiwanger

2:15 p.m. – 3:00 p.m. – **CCRC Tour**

3:00 p.m. – 3:10 p.m. – **Break**

3:10 p.m. – 3:40 p.m.

**Laboratory**

**SECTION I** – Monosaccharide composition analysis

Preparation of monosaccharide digests for HPAEC analysis

Dr. Zhirui Wang

**SECTION II** – Release of N-glycans

Release N-glycans with PNGase F

Dr. Asif Shajahan

3:40 p.m. – 5:00 p.m.

**Lab exercise discussion**

**SECTION V** – Detection and analysis of carbohydrates by lectin blotting

**Laboratory**

**SECTION V** – Lectin blotting

Blotting samples on nitrocellulose membrane, blocking

Dr. Asif Shajahan/ Dr. Anne Gleinich

**Wednesday, August 14, 2019**

8:45 a.m. – 10:00 a.m.

**Lecture** – *“Glycans Linked to Lipids and Lipid Precursors”*

Dr. Michael Tiemeyer

10:00 a.m. – 10:10 a.m. – **Break**

10:10 a.m. – 11:10 a.m.

**Lecture** – *“Bacterial Glycoproteins”*

Dr. Christine Szymanski

11:10 a.m. – 12:00 p.m.

**Laboratory**

**SECTION II** – Release of N-glycans

Separation of N-glycans from O-glycopeptides/peptides by C18 sep pak

Dr. Asif Shajahan

12:00 p.m. – 1:00 p.m. – **LUNCH**

1:00 p.m. – 2:00 p.m.

**Laboratory**

**SECTION III** – Release of O-glycans

Removal of Borates from beta-eliminated O-linked glycans

Dr. Nitin Supekar

2:00 p.m. – 2:30 p.m.

**SECTION I** – HPAEC Data Discussion

Dr. Zhirui Wang

2:30 p.m. – 2:40 p.m. – **Break**

2:40 p.m. – 4:00 p.m.

**Laboratory**

**SECTION VI** – TLC analysis

Spot samples

Develop plate

Detection of samples

Dr. Sara Porfirio

4:00 p.m. - 5:00 p.m.

**Laboratory**

**SECTION V** – Lectin blotting

Wash blots

Probe blots with lectins

Dr. Asif Shajahan/ Dr. Anne Gleinich

**Thursday, August 15, 2019**

**Laboratory**

8.45 a.m. – 10:10 a.m.

**SECTION IV** – Permethylation of released oligosaccharides

(N-glycans from Fetuin or O-glycans from Mucin)

10:10 a.m. – 10:20 a.m. – **Break**

10:20 a.m. – 11:00 a.m.

**Laboratory**

**SECTION IV** – Permethylation (continued)/ Introduction to micropermethylation

Dr. Asif Shajahan/Dr. Nitin Supekar

11:00 a.m. – 12:00 p.m.

**Laboratory**

**SECTION V** – Lectin blotting

Wash blots

Overlay with antibody –AP (45 min ~ 1 h incubation during lunch)

Dr. Asif Shajahan/ Dr. Anne Gleinich

12:00 p.m. – 1:00 p.m. – LUNCH

1:00 p.m. – 2:15 p.m.

**Laboratory**

**SECTION V** – Lectin blotting

Wash blots

Color development, record results

Dr. Asif Shajahan/ Dr. Anne Gleinich

2:15 p.m. – 2:55 p.m.

**Demonstration**

MALDI TOF/TOF MS demonstration

Dr. Nitin Supekar

2:55 p.m. – 3:05 p.m. – Break

3:05 p.m. – 3:45 p.m.

HPAEC and HPLC/CE demonstration

Dr. Zhirui Wang/Dr. Stephanie Archer-Hartmann

**Discussion of data**

3:45 p.m. – 4:00 p.m.

**SECTION VI** – TLC results

Analysis of TLC data

Dr. Sara Porfirio

4:00 p.m. – 4:15 p.m.

**SECTION V** – Lectin blotting results

Analysis of lectin blotting

Dr. Asif Shajahan/ Dr. Anne Gleinich

4:15 p.m. – 5:00 p.m.

**SECTION II, III, IV** – MALDI TOF/TOF-MS result

Analysis of N-and O-linked glycans MALDI data & Data analysis

Dr. Asif Shajahan/Dr. Nitin Supekar

**Friday, August 16, 2019**

**<Mass Spectrometry Module>**

9:00 a.m. – 12:00 p.m.

**Lecture – *Glycomics & Glycoproteomics***

Dr. Lance Wells

10:15 a.m. – 10:30 a.m. – **Break**

12:00 p.m. – 1:00 p.m. – **LUNCH**

1:00 p.m. – 3:00 p.m.:

**Orbi-Fusion MS Demonstration and MSMS Data Discussion**

Dr. Stephanie Archer-Hartmann /Dr. Nitin Supekar

**Or <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 <Molecular Modeling Module>**

9:00 a.m. – 12:00 p.m.

**Lecture – “*Introduction to Molecular Modeling*”**

Dr. Lachele Foley

12:00 p.m. – 1:00 p.m. – **LUNCH**

Afternoon:

Demonstration and data interpretation

**Course summary, course evaluation final Q&A**