SEPARATION AND CHARACTERIZATION OF GLYCOPROTEIN AND GLYCOLIPID OLIGOSACCHARIDES

August 15-19, 2022

Monday, August 15, 2022

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. Varughese (Alex) Mulamoottil

12:00 p.m. – 1:00 p.m. – **Lunch**

1:00 p.m. – 2:00 p.m.

Lecture – "*Bacterial Glycoproteins*"

        Dr. Christine Szymanski

2.00 p.m. – 2.45 p.m.

Lab Exercise discussion

        SECTION IV - Permethylation of glycans

        Dr. Lauren Pepi/Nathan Murray

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. Varughese (Alex) Mulamoottil

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

Denature glycoprotein and start trypsin digestion

Dr. Lauren Pepi

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. Stephanie Archer-Hartmann

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

β-elimination of O-linked glycans

Dr. Bhoj Kumar

Tuesday, August 16, 2022

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**

<u>10:30 a.m. – 12:00 p.m.</u>

Laboratory

<u>SECTION II</u> – Release of N-glycans

Terminate Trypsin digestion of Fetuin

Dr. Lauren Pepi

<u>SECTION III</u> – Release of O-glycans

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

Dr. Bhoj Kumar

<u>SECTION VI</u> – TLC analysis

Sample cleaning by C18 reversed phase cartridge

Dry sample under N_2

Dr. Stephanie Archer-Hartmann

<u>12:00 p.m. – 1:00 p.m.</u> – **LUNCH**

<u>1:00 p.m. – 2:15 p.m.</u>

Lecture – *"Regulation of Notch with Glycosylation"*

Dr. Robert Haltiwanger

<u>2:15 p.m. – 3:00 p.m.</u> **– CCRC Tour**

<u>3:00 p.m. – 3:10 p.m.</u> – **Break**

<u>3:10 p.m. – 3:40 p.m.</u>

Laboratory

<u>SECTION I</u> – Monosaccharide composition analysis

Preparation of monosaccharide digests for HPAEC analysis

Dr. Varughese (Alex) Mulamoottil

<u>SECTION II</u> – Release of N-glycans

Release N-glycans with PNGase F

Dr. Lauren Pepi

<u>3:40 p.m. – 5:00 p.m.</u>

Lab exercise discussion

<u>SECTION V</u> – Detection and analysis of carbohydrates by lectin blotting

Laboratory

> **SECTION V** – Lectin blotting
>
> Blotting samples on nitrocellulose membrane, blocking
>
> > Dr. Lauren Pepi

Wednesday, August 17, 2022

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** –*"Labeling and Separation of Carbohydrates"*
>
> > Dr. Stephanie Archer-Hartmann

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. Lauren Pepi

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. Bhoj Kumar

2:00 p.m. – 2:30 p.m.

> > **SECTION I** – HPAEC Data Discussion
> >
> > > Dr. Varughese (Alex) Mulamoottil

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. Stephanie Archer-Hartmann

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

    Laboratory

        SECTION V – Lectin blotting

        Wash blots

        Probe blots with lectins

                Dr. Lauren Pepi

Thursday, August 18, 2022

    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 microquermethylation

                Dr. Lauren Pepi/ Nathan Murray

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. Lauren Pepi

<u>12:00 p.m. – 1:00 p.m.</u> – **LUNCH**

<u>1:00 p.m. – 2:15 p.m.</u>

    Laboratory

        <u>SECTION V</u> – Lectin blotting

        Wash blots

        Color development, record results

Dr. Lauren Pepi

<u>2:15 p.m. – 2:55 p.m.</u>

    Demonstration

    MALDI TOF/TOF MS demonstration   Dr. Bhoj Kumar

<u>2:55 p.m. – 3:05 p.m.</u> – **Break**

<u>3:05 p.m. – 3:45 p.m.</u>

    HPAEC and HPLC/CE demonstration

        Dr. Varughese (Alex) Mulamoottil /Dr. Stephanie Archer-Hartmann

    Discussion of data

<u>3:45 p.m. – 4:00 p.m.</u>

        <u>SECTION VI</u> – TLC results

        Analysis of TLC data   Dr. Stephanie Archer-Hartmann

<u>4:00 p.m. – 4:15 p.m.</u>

        <u>SECTION V</u> – Lectin blotting results

        Analysis of lectin blotting   Dr. Lauren Pepi

<u>4:15 p.m. – 5:00 p.m.</u>

        <u>SECTION II, III, IV</u> – MALDI TOF/TOF-MS result

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

        Dr. Lauren Pepi/ Dr. Stephanie Archer-Hartmann

Friday, August 19, 2022

<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. Lauren Pepi /Dr. Bhoj Kumar

Or <NMR Module>

9:00 a.m. – 12:00 p.m.

Lecture – *"Introduction to NMR of glycoproteins and carbohydrates"*

Dr. Christian Heiss

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