

NIH Course II

August 4-8, 2008

August 11-15, 2008

Laboratory exercise

Section I Monosaccharide composition analysis by HPAEC

Section II Enzymatic release of N-linked oligosaccharides from a glycoprotein

Section III O-linked carbohydrates cleavage from a glycoprotein using Sodium Hydroxide and Sodium Borohydride

Section IV Oligosaccharide analysis by mass spectrometry

Section V Lectin Blotting

Section VI Separation of Glycolipids by TLC

NIH SCHEDULE

August 4-8, 2008

August 11-15, 2008

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

Monday

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

Introduction and Welcome (Dr. Parastoo Azadi)

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

Lecture - Overview of Glycoprotein Carbohydrate Structures/Problems in Analysis

(Aug 4, 11 Dr. Kelley Moremen)

10:15 a.m. - 10:30a.m. **Coffee Break**

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

Lecture continued- Overview of Glycoprotein Carbohydrate Structures/Problems in Analysis

(Aug 4, 11-Dr. Kelley Moremen)

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

Lab exercise discussion – Monosaccharide composition analysis

Laboratory - Monosaccharide composition analysis

- Begin hydrolyses.....Section I

(Dr. Roberto Sonon)

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

1:00 p.m. - 1:45 p.m.

Lecture - Release and analysis of N and O-linked glycans from glycoproteins
(Aug 4, 11-Dr. Parastoo Azadi)

1:45 p.m. - 2:00 p.m. **Coffee Break**

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

Lecture - Other carbohydrate analytical methods including glycosyl composition analysis by GC/MS, Glycosyl-linkage analysis by methylation analysis N-linked glycan profiling
(Aug 4, 11-Dr. Parastoo Azadi)

2:45 p.m. - 3:00 p.m. **Coffee Break**

3:00 p.m. - 4:00 p.m.

Laboratory - Continue monosaccharide composition analysis
- Remove samples from heat at appropriate times; cool on ice; centrifuge briefly; drydown hydrolysates.....Section I
(Dr. Roberto Sonon)

Lab exercise discussion – Enzymatic release of N-linked oligosaccharides from a glycoprotein

Laboratory – Denaturation and Tryptic digestion of glycoprotein
- Heat a glycoprotein solution to denature.....Section II
- Begin Tryptic digestion.....Section II
(Ms. Mayumi Ishihara)

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

Lab exercise discussion – O-linked carbohydrates cleavage from a glycoprotein using Sodium Hydroxide and Sodium Borohydride
Laboratory - β -elimination of O-linked glycoprotein compounds.....Section III
(Ms. Seongha Park)

4:30 p.m. - 5:00 p.m.

Lab Exercise Discussion - Desialylation, exoglycosidase treatment
Laboratory - Desialylation of bovine brain gangliosides.....Section VI
(Dr. Scott Snyder)

Tuesday

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

9:00 p.m. - 10:00 p.m.

Lecture- Introduction to Glycolipids (Aug 5-Dr. Mike Tiemeyer)
Lecture – Lectins in Carbohydrate Analysis (Aug 12-Dr. Parastoo Azadi)

10:00 a.m. - 10:15 a.m. **Coffee Break**

10:15 a.m. - 10:25 a.m.

Laboratory –Stop Tryptic digestion.....Section II
(Ms. Mayumi Ishihara)

10:25 a.m. - 11:25 a.m.

Laboratory - Neutralization and de-salting of O-linked glycoprotein
compounds.....Section III
(Ms. Seongha Park)

11:25 a.m. - 12:25 a.m.

Laboratory - Termination of sialidase treatment of bovine brain gangliosides
- Sample cleaning by C18 reversed phase cartridge.....Section VI
- Dry gangliosides on Speed-Vac.....Section VI
(Dr. Scott Snyder)

12:25 p.m. - 1:25 p.m. – **LUNCH**

1:25 a.m. - 2:25 a.m.

Guest Lecture – Introduction to HPAEC
(Aug 5, 12-Dr Val Hanco – Dionex)

2:25 p.m. - 3:10 p.m.

Laboratory - Monosaccharide composition analysis
- Resuspend strong acid hydrosylates, transfer to autosampler vials, and load
autosampler.....Section I
(Dr. Roberto Sonon)

3:10 p.m. - 3:25 p.m. **Coffee Break**

3:25 p.m. - 3:40 p.m.

Laboratory - Release N-glycans with PNGase F.....Section II
(Ms. Mayumi Ishihara)

3:40 p.m. - 5:00 p.m.

Laboratory - Separation of Glycolipids by TLC
- Spot samples.....Section VI
- Separate samples by TLC.....Section VI
- Develop plates.....Section VI
- Finish analysis and interpretation of results.....Section VI
(Dr. Scott Snyder)

Wednesday

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

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

Lecture – To be announced

(Aug 6- Dr. Lianchun Wang / Aug 13-Dr. Richard Steet)

10:00.a.m. – 10:15 a.m. **Coffee Break**

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

Lecture – To be announced

(Aug 6-Dr. Lance Wells / Aug 13-Dr.)

11:15 a.m. - 12:30 p.m.

Laboratory - Removal of Borates from O-linked glycoprotein

compounds.....Section III

(Ms. Seongha Park)

Laboratory – Separation of N-linked oligosaccharides by C18 reversed phase

cartridge.....Section II

(Ms. Mayumi Ishihara)

12:30 p.m. - 1:30 p.m. – **LUNCH**

1:30 p.m. - 2:15 p.m.

Lecture - Capillary Electrophoresis of Carbohydrates

(Aug 6,13-Dr. Christian Heiss)

2:15 a.m. - 2:45 a.m.

Lab Exercise Discussion - Sequential exoglycosidase treatment

Laboratory - Exoglycosidase treatment of bovine fibrinogen for lectin blotting

.....Section V

(Dr. Roberto Sonon)

2:45 a.m. - 3:00 p.m. **Coffee break**

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

Lab Exercise Discussion – Permethylation of oligosaccharides from a glycoprotein

Laboratory- Permethylation of O-linked glycoprotein compounds....Section IV

(Ms. Mayumi Ishihara)

Thursday

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

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

Lecture – Lectins in Carbohydrate Analysis

(Aug 7-Dr. Michael Pierce)

Guest Lecture - Introduction to Glycolipids

(Aug 14-Dr. Alfred Merrill- GA Tech)

10:00 a.m. - 12:00 p.m.

Laboratory Exercise Discussion – Lectin Blotting

Laboratory –

- Detection of glycoproteins by lectin blotting.....Section V
- Prepare dot blots.....Section V
- Block blots*.....Section V
- Wash blots.....Section V
- Probe blots with lectins (lunch during 1h incubation).....Section V

*(**Coffee Break during blocking**)

(Dr. Roberto Sonon)

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

1:00 p.m. - 1:30 p.m

Demonstration- Oligosaccharide profiling by HPLC/HPAEC (Mayumi?)

1:30 p.m. - 2:30 p.m

Laboratory - Continuation of lectin blotting experiment

- Wash blots.....Section V
- Overlay with antibody – AP.....Section V

(Dr. Roberto Sonon)

2:30 p.m. - 2:45 p.m. **Coffee break**

2:45 p.m. - 4:00 p.m

Laboratory - Completion of lectin blotting experiment

- Wash blots.....Section V
- Color development, record results.....Section V

(Dr. Roberto Sonon)

Laboratory Exercise Discussion - Lectin Blotting results

(Dr. Roberto Sonon)

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

Laboratory Exercise Discussion - HPAEC results

- Quantitation of monosaccharide composition.....Section I
(Dr. Roberto Sonon)

4:30 p.m. - 5:00 p.m.

Laboratory Exercise Discussion - MALDI-MS result

- Analysis of N and O-linked permethylated glycans
data.....Section II,III,IV
(Dr. Parastoo Azadi)

Friday,

8:45 a.m. - 9:40 a.m. Final Questions and Discussion

NMR Module or Mass Spectrometry Module or Molecular Modling Module

Molecular Modling Module

9:45 a.m. - 12:00 p.m.

Lecture – Introduction to Molecular Modling

(Aug 8, 15-Dr. Robert Woods)

10:45 p.m. - 11:00 p.m.

Coffee Break

Lecture-continued

NMR Module

9:45 a.m. - 12:00 p.m.

Lecture – Introduction to NMR of glycoproteins and carbohydrates

(Aug 8,15-Dr. John Glushka)

10:45 p.m. - 11:00 p.m.

Coffee Break

Lecture- continued

Mass Spectrometry Module

9:45 a.m. - 10:45 p.m.

Lecture - Introduction to mass spectrometry of glycoproteins and carbohydrates
(Aug 8-Dr. Ron Orlando)

Lecture – Introduction to FT-MS and analysis of oligosaccharides by FT-MS
(Aug 15-Dr. Jon Amster)

10:45 a.m. - 11:00 a.m. **Coffee Break**

11:00 a.m. – 12:00 a.m.

Lecture – Introduction to mass spectrometry of glycoproteins and carbohydrates
(Aug 8-Dr. Ron Orlando)

Lecture – Introduction to mass spectrometry of glycoproteins and carbohydrates
(Aug 15-Dr. Ron Orlando)

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

1:00 p.m. - 3:00 p.m. **Demonstration** – continue modules

Mass Spectrometry Module

- MALDI-TOF/MS and ESI-MS Analysis of Permethylated glycans
(Seongha/Mayumi).....Section V

Course summary, Course evaluation, Final Questions & Answers

Monday 8/6

	Lecture	Section I Composition	Section II N-glycan	Section III O-glycan	Section IV Permethylation MALDI, LCQ	Section V Lectin	Section VI TLC	Section VII Phenol- Sulfuric
8:00								
9:00	Azadi / Moreman							
10:00	Moreman							
11:00	Moreman	11:30 Begin hydrolysis						
12:00	Lunch							
1:00	Azadi							
2:00			2:00 Begin Tryptic digestion				~2:30 Begin Exoglycosidase digestion	
3:00	3:30 Teimeyer	Stop hydrolysis Dry down						
4:00	Teimeyer			4:30 Begin β-elimination				
5:00								

Tuesday 8/7

	Lecture	Section I Composition	Section II N-glycan	Section III O-glycan	Section IV Permethylation MALDI, LCQ	Section V Lectin	Section VI TLC	Section VII Phenol- Sulfuric
8:00	8:45 Q&D							
9:00			9:00 Stop Tryptic digestion				9:10 Stop Exoglycosidase digestion	
10:00				10:30 Stop β-elimination			C18 Dry down	
11:00	11:30 Hanko			Desalting				
12:00	12:30 Lunch							
1:00	1:30 Azadi							
2:00	2:30 Heiss						Equibrate TLC chanber	
3:00		3:15 Sample setup					3:40 TLC analysis	
4:00		Start running DIONEX	4:45 Start PNGaseF treatment				TLC analysis	
5:00		for Neutral Sugar						

