# Mass Spectrometry-based Proteomics Workshop

October 20th and 21st 2009

Supported by NSF Plant Genome Program
Instruction: Drs. Joss Rose and Ted Thannhauser and Sheng Zhang
Location: Cornell Proteomics & Mass Spectrometry Core Facility

We had another extremely successful Proteomics Workshop this year that was highly oversubscribed. The workshop comprised both lectures and practical lab-based training in proteomic analysis, using advanced mass spectrometry techniques and bioinformatics and was targeted towards undergraduate and graduate students. Based on the positive and enthusiastic feedback, a similar course will be offered next year.
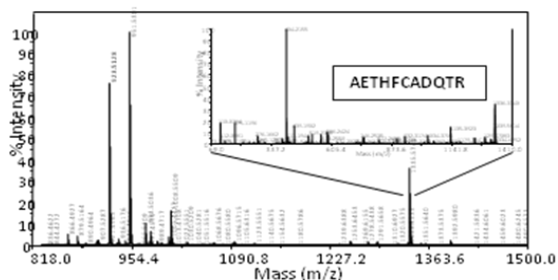
SCHEDULE

October 20th:

8:00am - 8:30am: Registration (badge, program handouts, breakfast) in room 130

8:30am - 8:35am in room 130:

   (1) Program introduction by Professor Joss Rose

8:35am - 10:00am in room 141:

(2) **Experimental 1:** Protein denaturing, reduction and alkylation of crude cell extract samples for shotgun proteomics analysis. (Attendees divided into 4 groups)

Instructors: Huiming Yan (bench 1), Yong Yang (bench 2), Bob Sherwood (bench 3) and Celeste Ptak (bench 4).

10:20am - 11:20am in room 130

(3) **Lecture 1 Presentation:** Sample preparation and separation prior to MS analysis by Dr. Ted Thannhauser

11:30am- 12:00pm in room 141:

(4) **Experimental 2:** in solution trypsin digestion.

12:00pm- 1:00pm in room 130**:** Lunch and Break

1:00pm to 2:15pm in room 130:

(5) **Lecture 2 Presentation:** MS instrument fundamentals and Applications: (proteomics case studies) by Dr. Sheng Zhang

2:30pm- 3:30pm in room 143:

(6) **Experimental 3:** Lab tour and demo, split into 3 groups (20 min/rotation)
- nanoLC-MS/MS for iTRAQ samples in 4000 Q Trap: by Dr. Celeste Ptak
- MALDI-TOF/TOF analysis for 2D gel-based protein IDs: by Bob Sherwood
- NanoLC-MS/MS analysis for 1D gel bands: by Kevin Howe

4:00pm- 5:00pm:

(7) **Experimental 4:** set up nanoLC-MS/MS overnight running for in-solution digested samples with 60-min gradient for each run.
  a. 10 samples on 4000 Q Trap by Dr. Celeste Ptak
  b. 10 samples on Synapt HDMS by Kevin Howe and Yong Yang

October 21st:

8:00am -8:30am: breakfast and lap top computer setup in room 130

8:30am -9:00am:

(8) **Experimental 5:** Database searching and file conversion for overnight LC-MS/MS files

    a. Mascot Maemon search and wiff file conversion to TMP file by Dr. Celeste Ptak in room 130

    b. PLGS 2.4-Mascot database search and generating PKL file by Yong Yang and Kevin Howe in room 143

9:30am-10:30am:

(9) **Lecture 3 Presentation:** Introduction to Mascot-based MS database search and data interpretation by Professor Joss Rose

10:30am- 10:45am: transfer PKL files and TMP files into individual laptop computers.

10:45am – 11:15am: in room 130 and room 143

(10) **Experimental 6:** Practice for generating PKL files and TMP files. Instructors: Celeste Ptak for TMP files, Huiming Yan and Kevin Howe for PKL files.

12:00pm- 1:00pm in room 130**:** Lunch and Break

1:00pm – 3:00pm in room 130

(11) **Experimental 7:** database search practice against either Mascot public search engine or in-house licensed search engine. Assistants: Celeste Ptak, Huiming Yan, Kevin Howe and Yong Yang.
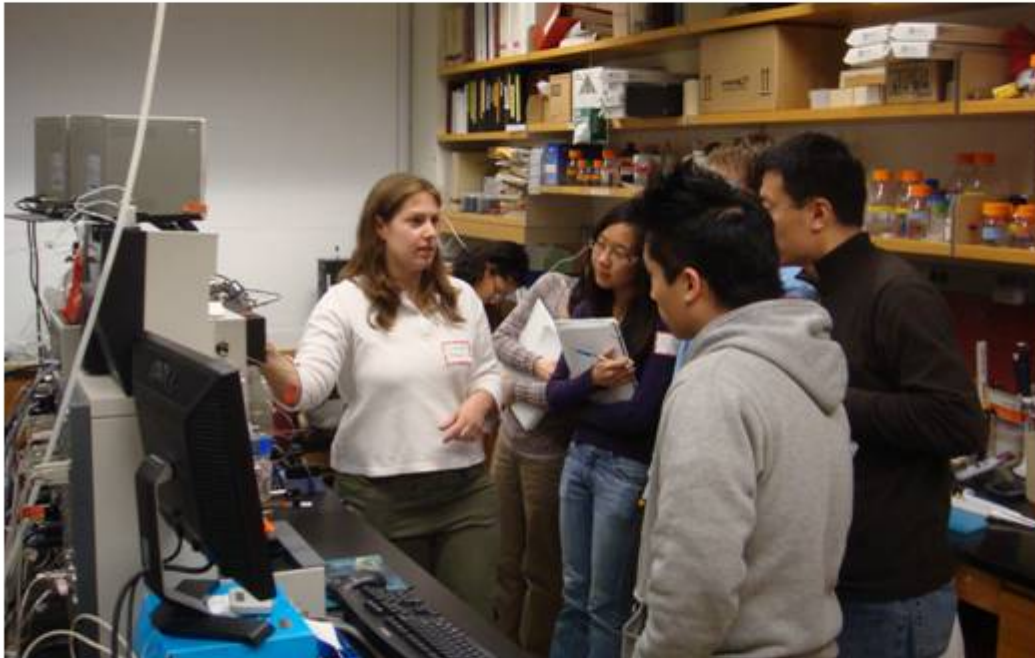
3:20pm – 4:00pm: in room 130

(12) Summary of the group results for shotgun analysis in 4000 Q Trap and Synapt HDMS. Summary of the iTRAQ demo results for quantitative proteomics by Sheng Zhang.

4:00pm – 5:00pm: in room 130

(13) Wrap-up comments, questions and suggestions

Here are some pictures of this year's workshop. Thank you all for a great time!!!

The Class of 2009…our brave band

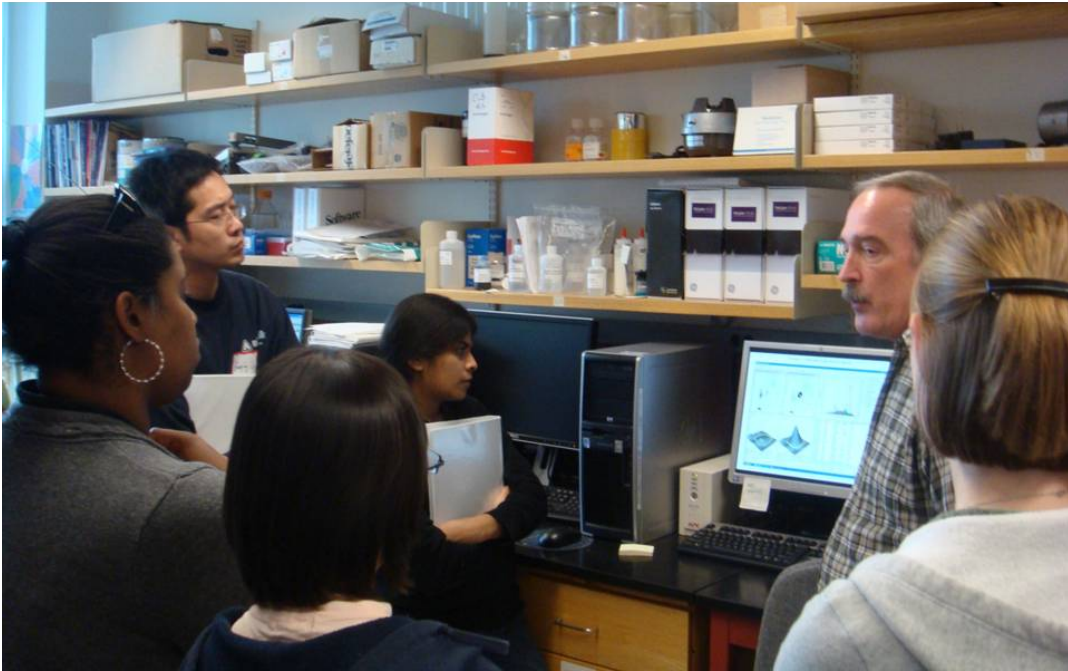Bob explaining the mysteries of the MALDI TOF-TOF

Celeste and the art of protein fractionation

Kevin revealing some of the principles and wizardry of mass spectrometry

Rapt attention as Bob demonstrates 2D gel analysis software