Mass Spectrometry & Proteomics in Botanicals Research

Monday, September 9

7:45 am **Registration and continental breakfast**

8:15 am Dr. Stephen Barnes, Course Director, and Dr. Helen Kim, Co-Course Director:
Welcome to UAB and course objectives

- *Purdue-UAB Botanicals Center for Age-Related Disease*
- *Mass spectrometry as a versatile technique, suitable for many types of biological molecules*
- *Identification and quantification of active substances in botanicals is essential for systematic research*
- *The role of proteomics in botanicals research*

8:45 am Introduction to proteomics methodologies (*Dr. Helen Kim)*

- *2DE vs MUDPIT vs chips*

9:00 am Elements of 2D-proteomics (*Dr. Helen Kim*)

- *Sample preparation*
- *IEFsample buffer recipe*
- *How to reduce protein complexity*

9:30 am **Break**

10:00 am Sample preparation and electrospray ionization (*Dr. Stephen Barnes*)

- *What is electrospray ionization?*
- *ESI vs. HN-APCI*
- *Sample preparation and importance of desalting*
- *MS of polyphenols and peptides*

10:30 am MS-MS analysis of peptides (*Mr. Marion Kirk*)

- *Selection of parent ion*
- *Advantage of doubly charged ions*
- *Principal sites of cleavage (b and y ions)*
- *Other cleavages*

11:00 am Qualitative LC-MS and MS-MS of polyphenols (*Dr. Jeevan Prasain*)

- *LC-MS with isocratic or gradient elution*
- *Fragmentation of parent molecular ions*
- *Selection of ions for Multiple reaction ion monitoring*

11:30 am Principles of nanoelectrospray ionization and high sensitivity analysis (*Dr. Sam Wang*)

- *Concentration rules in ESI*
- *Advantages of nanoflow*
- *Miniaturizing sample absorption*
- *Application to polyphenols*

12:00 pm **Lunch**

Afternoon sessions (1 – 5 pm) involve going in turn to each of the stations in groups of 3-4. Following the break, revisit stations 1-4 depending on interest. Also visit in small groups Stations 5 and 6. Drs. Barnes and Kim will be available for informal discussions during this period. Posters concerning botanical research projects will be shown in room 637.

Station 1 Running 2D-gels (*Mr. Heath McCorkle*)

- *Rehydration of 1st D gels*
- *Starting the 1st D strip.*
- *Laying the 1st D strip on top of the 2nd D gel*

Station 2 Demonstration of Q-TOF-MS-MS of peptides (*Mr. Marion Kirk*)

Station 3 LC-MS-MS of polyphenol-containing samples (*Mr. Ray Moore)*

Station 4 Demonstration of analysis of microdialysate (*Dr. Sam Wang*)

3:15 – 3:30 pm **Break**

Station 5 Demonstration of gel scanning and protein spot selection (*Mr. Heath McCorkle*)

Station 6 Informal discussions (*Dr. Stephen Barnes and Dr. Helen Kim*)

- Student/postdoc poster presentations
- Selection of instruments to purchase
- Problem solving

Tuesday, September 10

8:00 am **Continental breakfast**

8:15 am MALDI-TOF analysis (*Dr. Stephen Barnes*)

- *Principles of MALDI and TOF-MS*
- *Large molecules*
- *Peptides*
- *Non-protein samples*

8:45 am Peptide mass fingerprinting (*Mr. Landon Wilson*)

- *Preparation of sample – clean protein/gel spot*
- *Choice of peptidase*
- *Spotting and sample purity*
- *De-isotoping MALDI spectra*
- *MASCOT*

9:15 am Bioinformatics (*Dr. Stephen Barnes and Dr. Helen Kim*)

- *So, I've discovered a protein – what next?*
- *NCBI Entrez/Swiss Protein*
- *2DE databases*
- *Blink*
- *SCOP*

9:45 am **Break**

The morning session (10 am -12 pm) involves going in turn to each of the stations in groups of 3-4.

Station 1/2 Quantitative analysis of polyphenols (*Mr. Ray Moore and Mr. Kenneth Jones*) – in lab and at various computer stations

Station 3/4 MALDI analysis of 2D-gel spots (*Mr. Landon Wilson and graduate students*)

12:00 pm **Lunch**

The afternoon session (1-3 pm) involves going in turn to each of the stations in groups of 3-4.

Station 1 Image analysis of 2D-gel (*Mr. Kiran Sarikonda)*

Station 2-4 Use of informatics (*Dr. Stephen Barnes and graduate students*)

Further opportunities to try all techniques in analysis of polyphenols and proteins, and to ask questions