ABRF 2009:
Optimization and Application of Existing and Emerging Biotechnologies
February 7-10, 2009 – Memphis, Tennessee

SATELLITE EDUCATIONAL WORKSHOP PROGRAM
(sw4) HPLC Theory and Practice
(current as of 11/28/08)

Saturday, February 7, 2009
8:00 am – 4:00 pm
Memphis Cook Convention Center.

Practical Aspects of Protein and Peptide HPLC Separations for Proteomics
Andrew Alpert, PolyLC Inc. (organizer),
and Kerry Nugent, Michrom BioResources (co-organizer)

7:00 am - 12:00 pm **REGISTRATION OPEN** - *Lobby*

7:00 - 8:00 am **CONTINENTAL BREAKFAST** - *Ballroom Foyer*

8:00 - 9:00 am **INTRODUCTION: BIOCHEMICAL HPLC**

A. Why bother? HPLC as a complement to MS for Proteomics
B. HPLC Theory Applied to Biomolecules
1. Efficiency, Resolution, Selectivity and Peak Capacity
2. General requirements for HPLC of proteins and peptides

C. Modes of HPLC for Proteins and Peptides
1. Separations by size (SEC)
2. Separations by charge (AX, CX, Mixed Bed)
3. Separations by polarity (RPC, HIC, HILIC)
4. Separations by functionality (Affinity)

9:00 - 10:00 am **THE ROLE OF SEPARATIONS IN PROTEOMICS**

A. HPLC versus Electrophoresis and Other Separation Techniques
1. Separation of intact proteins using gels, FFE or HPLC
2. HPLC or solid phase extraction for sample prep

B. HPLC as a Fractionation Tool for Comprehensive Proteomics
1. The more you fractionate, the deeper you can dig
2. Trade-offs: Degree of fractionation vs. sample throughput
3. Advantages of separation of intact proteins

C. HPLC as an Isolation Tool for Functional Proteomics
1. Affinity separations for PTMs
2. Non-affinity separations for PTM's
3. Effects of peptide orientation and sequence on selectivity for PTM's

D. Reversed Phase HPLC Coupled to MS for Proteomics
1. Top down LCMS of intact proteins or large peptides
2. Bottom up LCMS of protein digests

10:00 - 10:30 am **REFRESHMENT BREAK** - *Ballroom Foyer*

10:30 am - 12:00 pm **USING ONLY AS MUCH SEPARATION AS REQUIRED**

A. Fast Separations for Simple Samples
1. High throughput LCMS of 1D or 2D gel digests
2. Qualitative and quantitive analysis of specific proteins

B. High Resolution Separations for More Complex Samples
1. Simple proteome samples with wide range of abundances
2. Analysis of low abundance proteins in sample preps

C. Multidimensional (MD) Separations for Highly Complex Samples
1. MDLC of intact proteins
2. MDLC of complex proteome digests

D. Optimizing Speed, Resolution, Capacity, Sensitivity and Recovery
1. Choosing the proper HPLC modes for the sample
2. Optimizing column parameters (ID, Pore Size, Particles, etc.)

12:00 - 1:00 pm **LUNCH** - *Ballroom Foyer*

1:00 - 2:00 pm **DEVELOPING A PROTEOMICS SEPARATION WORKFLOW**

A. Defining the Application and Desired Results
1. What do you know about the sample?
2. What information do you want from the sample?

B. Choosing the Tools that Best Fit the Problem
1. HPLC vs. SPE
2. Strategies to maximize throughput and resolution

C. Integrating the Workflow to Maximize Results
1. Choosing complimentary modes of separation
2. Manual vs automated methods

D. Setting Up Controls to Insure Integrity of Results
1. Run standards to optimize methods and recoveries
2. Run blanks to minimize errors

2:00 - 2:30 pm **REFRESHMENT BREAK** - *Ballroom Foyer*

2:30 - 3:30 pm **PROBLEMS ENCOUNTERED IN BIOCHEMICAL HPLC**

A. Sample Solubility and Compatibility
1. Minimize protein aggregation and precipitation
2. Insure maximum recovery from HPLC columns

B. Salts, Detergents and pH
1. Useful for protein/peptide solubility
2. May interfere with LC separation and/or MS detection

C. Dynamic Range, Capacity and Recovery
1. Choose HPLC column size to fit sample mass
2. May require overload to improve dynamic range

D. Playing "Twenty Questions" in Proteomics: Troubleshooting Separations
1. Examples of proteomics HPLC separation problems
2. Examples of proteomics LCMS instrumentation problems

3:30 - 4:00 pm **ASK THE EXPERTS SESSION**
Informal discussions between presenters, organizers, and attendees