LC-MS Analysis

LC-MS of Whole Protein?

- LC will deconvolute your protein mixture
- Separated proteins are then sent to MS
  - Mass/Charge
  - Relative Abundance
- Highly accurate, high-tech SDS-PAGE?
- Similar concerns as with 2D Gel-MS
- Solution: Tryptic Peptides
**Low Resolution LC-MS**

- Collect fractions from your favorite LC
- Digest sample with trypsin
- Analyze representative samples
  - MALDI-TOF
  - ESI-Quadrupole
- If fractions are complex, signal is complex
- Need better separation techniques

**In-Line HPLC-MS**

- HPLC gives high resolution separations
- Electrospray ionization is compatible with HPLC
  - Nanospray ionization
  - Orthogonal ESI
  - Effluent splitters
- HPLC→Trypsin→MS is inefficient, in-line better
- Digest entire sample with trypsin

*WHAT?!?!?*
Tryptic Peptide LC-MS

- Protein mixtures digested with trypsin
  - Cannot be whole cell extract
  - For best instruments, ~400-500 proteins MAX

- Tryptic peptides are separated by HPLC

- Peptide MW is determined by ESI-MS

- Bioinformatics strikes again
  - *In Silico* tryptic digest of all proteins by organism
  - Complex MW search to find multiple matching peaks

- On average ~200 proteins “ID’d” in LC-MS

Sample Complexity

- Sub-cellular fractionation
  - Nuclear
  - Membrane
  - Ribosome Associated
  - Mitochondrial

- SEC – Additional size info helps protein ID

- Affinity chromatography – protein enrichment

- CX/AX after trypsin – orthogonal separation
**LC-MS/MS & LC/LC-MS/MS**

- Adding additional dimensions provides:
  - Higher processing capability
  - Additional sequence information
  - Increase specificity/speed of protein ID

- Additional dimensions requires:
  - Additional processing steps
  - More automation of sample handling
  - More complicated instrumentation
  - More expensive instrumentation
  - More elaborate bioinformatics programs