

Mass spectrometry  
 Biochemistry 660  
 Kyle Friend

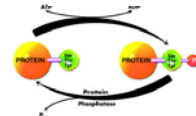
Kyle Friend  
 jkfriend@wisc.edu

### What is mass spec used for?

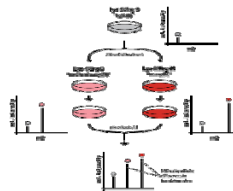
Protein identification



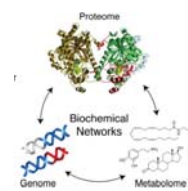
Post-translational modification



Quantitation



Proteomics and Metabolomics

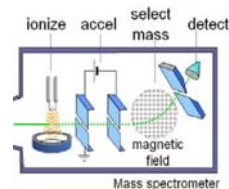


### Outline

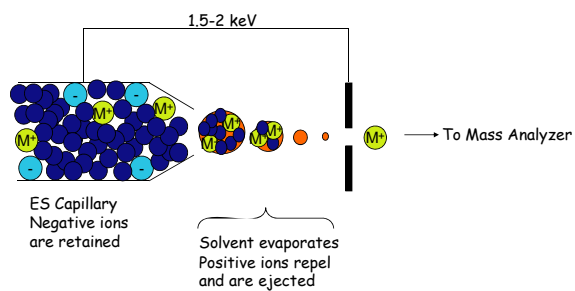
1. Mass spec components
2. Mass spec analysis
3. Applications

### Core Mass Spec Components

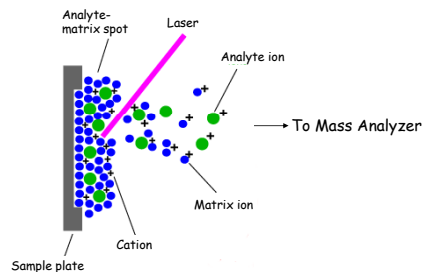
1. Ionization source
2. Mass analyzer
3. Detector



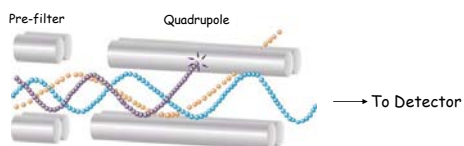
### Ionization Sources Electrospray



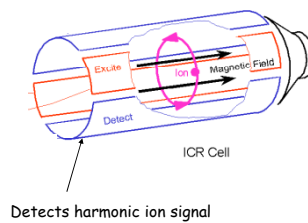
### Ionization Sources MALDI (Matrix-Assisted Laser Desorption Ionization)



### Mass Analyzers LTQ (Linear Trap Quadrupole)



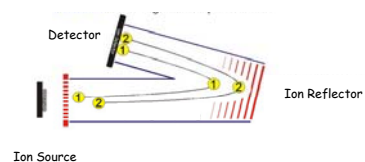
### Mass Analyzers ICR (Ion Cyclotron Resonance)



### Mass Analyzers Orbitrap



### Mass Analyzers ToF (Time of Flight)



### Ion Detector

For LTQ or MALDI:

1. Ion induces current in detector or...  
Ion strikes surface to generate current
2. Signal increased by amplification

ICR or Orbitrap:

1. Resonant ions induce oscillating signal
2. Fourier transform converts signal

### Outline

1. Mass spec components
2. Mass spec analysis
3. Applications

### Mass Analyzers Steps (Bottom-Up Mass Spec)

1. Precursor scan (gives peptide fingerprint)
2. Zoom scan (to determine charge)
3. MS/MS scan (optional - peptide sequence)

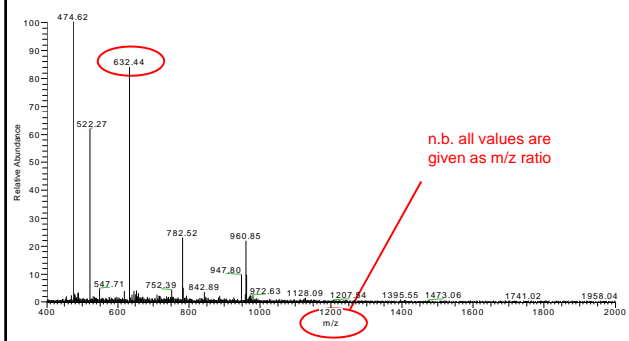
### Mass Analyzers m/z (or m/q) ratio

$$F = qB/2\pi m$$

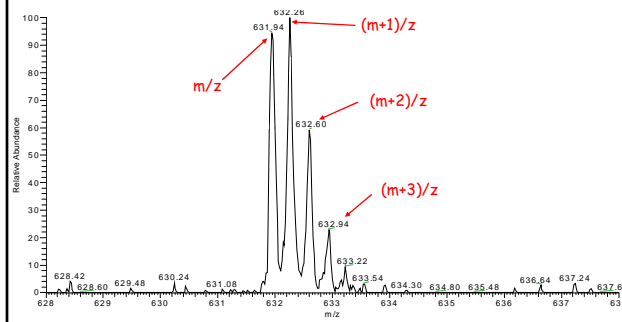
F = force  
q = charge  
B = magnetic field  
m = mass

Mass spectrometers determine an ion's m/z numerical value

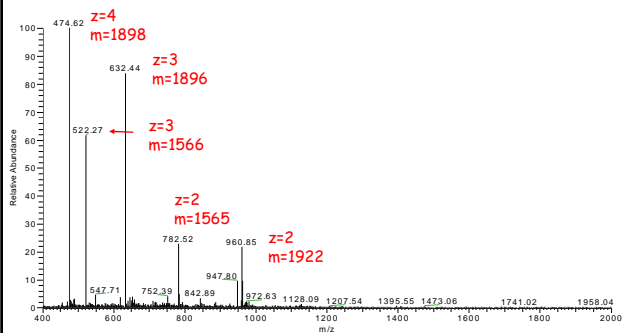
### Mass Analyzers Precursor scan



### Mass Analyzers Zoom scan

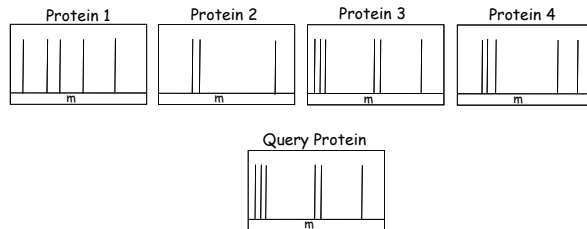


### Mass Analyzers Precursor scan (peptide fingerprint)



### Protein Identification from Peptide Fingerprint

1. Searchable database created
2. Database "digested" for peptide fragments
3. Algorithm matches query peptide fragments against the database

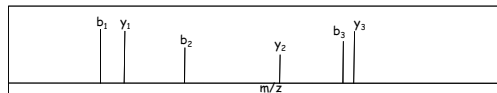
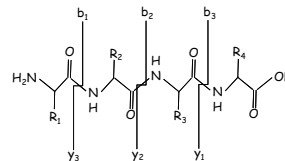


### Reasons for MS/MS

1. No genome sequence
2. Genome is complex
3. Post-translational modifications
4. Unambiguous assignments

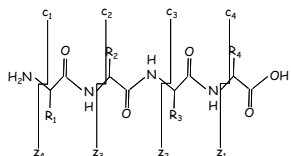
### MS/MS Analysis Collision Induced Dissociation (CID)

Peptides bombarded against inert gas ( $N_2$ )  
Collision cleaves amide bond (b and y ions)



### MS/MS Analysis Electron Capture Dissociation (ECD)

Peptides bombarded with electrons  
Captured electron generates c and z ions  
(Great for phosphorylation sites)

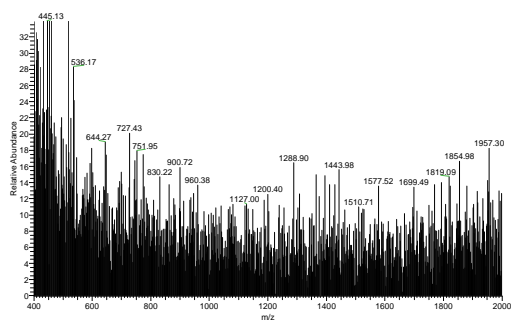


### How to use the toolkit

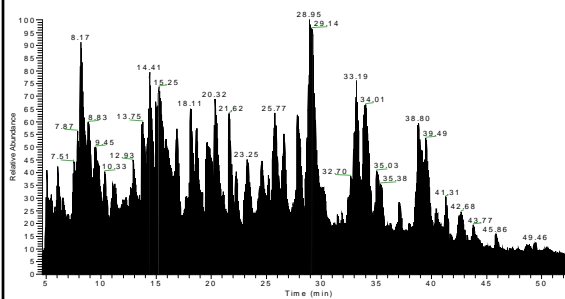
	LTK	ICR	ToF
Mass accuracy	●	●	●
Resolution	●	●	●
Sensitivity	●	○	●
Dynamic range	○	○	○
Identification	●	●	●
Quantitation	●	●	●
Throughput	●	○	○

Adapted from B. Domon and R. Aebersold Science 2006; 312: 212-217

### Unfractionated Precursor Scan



### Chromatography Separates Individual Peptides for Easier Analysis (LC-MS/MS)



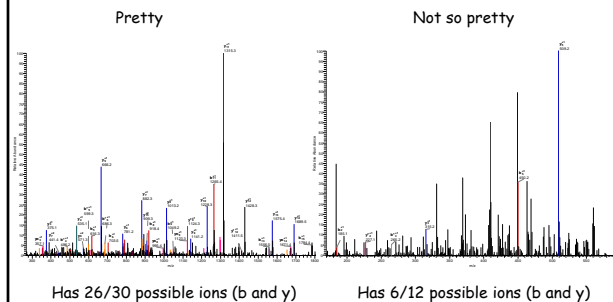
### Data interpretation Search Algorithms

1. **SEQUEST**  
Eng, McCormack, Yates (1994) *J. Amer. Soc. Mass. Spectrom.* 5: 976-989.
2. **MASCOT (FREE! On a limited basis)**  
www.matrixscience.com  
Perkins, Pappin, Creasy, Cottrell (1999) *Electrophoresis*, 20: 3551-3567.
3. **OMSSA (FREE and Open Source!)**  
Geer, Markey, Kowalak, Wagner, Xu, Maynard, Yang, Shi, Bryant (2004) *J. Proteome Res.* 3: 958-964.
4. **XITandem (FREE and Open Source!)**  
Craig, Beavis (2004) *Bioinformatics*, 20:1466-1467.
5. **Spectrum Mill**  
Chalkley, Baker, Huang, Hansen, Allen, Rexach, Burlingame (2005) *Mol. Cell. Proteom.* 4:1194-1204.

How search algorithms generally work...

1. Peptide fingerprints matched against database
2. Matching peptide(s) matched against the MS/MS scan
3. Best fit assigned score:
  - a. Number predicted ions detected
  - b. Deviation between predicted and experimental ion masses
  - c. Detected ion signal relative to background

### Data interpretation



### Outline

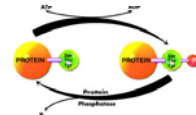
1. Mass spec components
2. Mass spec analysis
3. Applications

### Mass spec applications

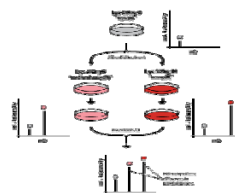
Protein identification



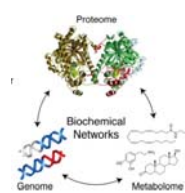
Post-translational modification



Quantitation



Proteomics and Metabolomics



### Protein identification

Single protein in solution >> Mixed proteins in solution >> Band in a gel

1. Avoid ionic detergents and all detergents if possible.

1. Avoid ionic detergents and all detergents if possible.  
2. Chromatography (LC-MS/MS) and ESI.

1. Avoid fixatives for silver staining e.g. glutaraldehyde.  
2. Consider MALDI.

Problem - hard to know whether identified proteins are correct.

Problem - peptide recovery is usually low.

What to look for in a paper...

1. How many unique peptides?
2. Sequence coverage?
3. Identity confirmed?

### Sites of post-translational modification

Phosphorylation

Lysine or Arginine modification

1. Can be difficult to i.d. using CID.  
2. ECD works very well.

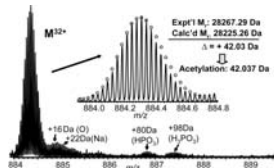
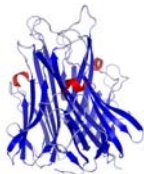
1. Consider which enzyme to use for digestion.

What to look for in a paper...

1. Mass spec resolution?
2. MS/MS data?

### Top-down mass spec

Analyze intact proteins using high resolution mass spectrometer



Exp1 M: 28267.29 Da  
Calc'd M: 28225.26 Da  
 $\Delta = +42.03$  Da  
Acetylation: 42.037 Da

1 A c M D Y R D D D D K H M P E P G K K P V S  
21 A P N K K P R S A R V T A G S A V P R E  
41 A R T H R S G V K V N M Q R R D G I S D I T  
61 A N D K Y G L A A E G K R H T L T V R D  
81 A S P D D Q G S Y A V I A G S S K V K F  
101 D L K V T E P A P P E K A S E S V A P G  
121 A P K E V P A P A T S L L R S L V S L P E  
141 G S I V S V T Q D G S A A E H Q G A F D D  
161 P I G L P L L M R P Q D G E V I T V G L S I  
181 V F S A R V A G A S L L K P P V V K W F  
201 K P K W V D L S S R V G Q H L Q L H D S  
221 Y D I A S K Y Y L F L H I T D A Q T  
241 S A G G Y R C E V S T K D K F D S C N F  
261 N L T

1 ECD: 40 x ions, 8.7' ions, 48 cleavages  
1 CAD: 8 x ions, 21 x ions, 25 cleavages

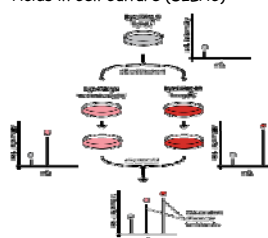
Ge Y et al. PNAS 2009;106:12658-12663

### Quantitation

Number of peptides

1. Simple
2. Unreliable

Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC)



What to look for in a SILAC paper...

1. Steps prior to mass spec analysis?
2. Number of peptides?
3. Threshold for data inclusion?

1. Compares proteins in different samples
2. Little info on splice variants, homologous peptides

### Proteomics



### Proteomics, LC-MS/MS on steroids

1. Identical to LC-MS/MS, but with additional column steps
2. Usually requires high resolution mass spec (whole genome studies)

What to look for in a paper...

1. How many unique peptides?
2. Sequence coverage?
3. Identity confirmed?

### Mass Spectrometry at UW-Madison

- **Biophysics Instrumentation Facility**
  - Darryl McClaslin
  - bifmaster@biochem.wisc.edu
- **Chemistry Dept. Mass Spectrometry Facility**
  - Martha Vestling
  - vestling@chem.wisc.edu
- **Biotechnology Center Mass Spectrometry and Proteomics Facility**
  - Amy Harms
  - harms@biotech.wisc.edu
- **Human Proteomics Program**
  - Ying Ge
  - yge@physiology.wisc.edu
- **School of Pharmacy Mass Spectrometry Facility**
  - Cameron Scarlett
  - cscarlett@pharmacy.wisc.edu

