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# Experimental Methods in Systems Biology

Part of the Coursera Certificate in Systems Biology

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Fall 2014, Week 3 Mass Spectrometry-Based  
Proteomics



Icahn School  
of Medicine at  
**Mount  
Sinai**

# Outline

- What is Mass Spectrometry?
- General Components of a Mass Spectrometer
  - Separation
  - Ionization
  - Mass Analyzer
  - Fragmentation
- Quantification

- Mass spectrometer: an instrument in which ions are separated according to their mass-to-charge ratio, and in which the number of ions is quantified electrically

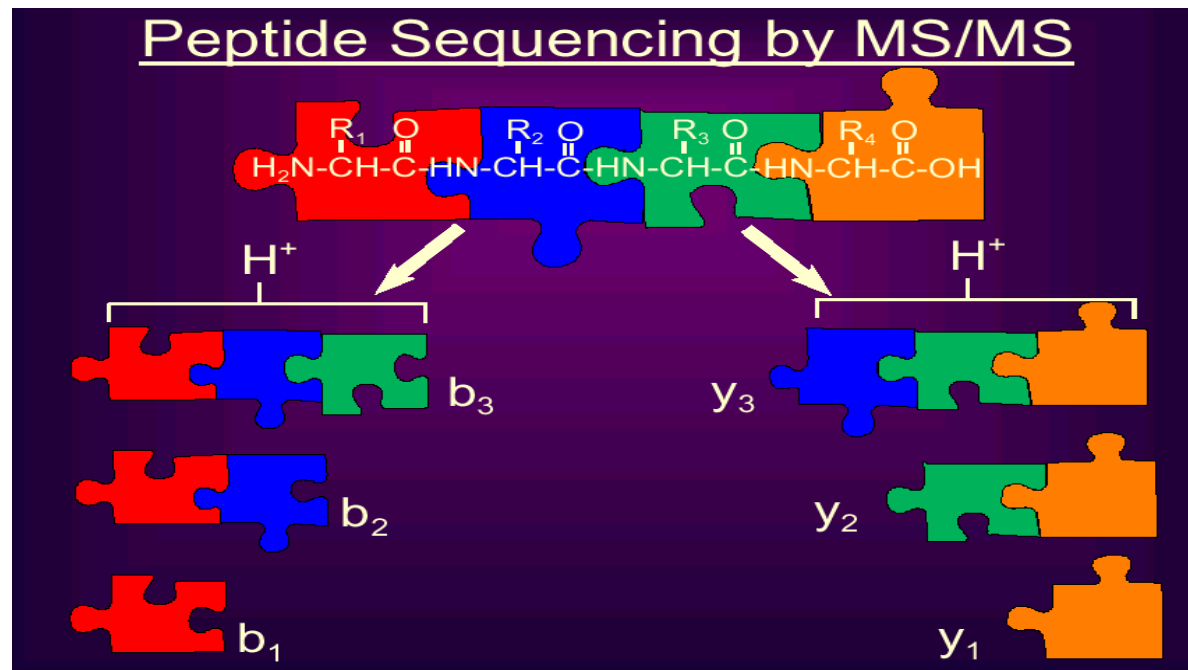
# Two MS methods for protein ID

- Peptide mass fingerprinting (PMF): based on a collection of masses of peptides derived from proteolytic digestion of the proteins
  - Benefit: quick
  - Drawback: not able to handle mixtures
- Peptide sequencing by tandem MS (MS/MS): based obtaining partial peptide sequence information in MS/MS
  - Benefit: confident sequence/PTM site identification, easily coupled with HPLC for protein complex identification
  - Drawback: higher costs, more time consuming

## Peptide Sequencing by Tandem Mass Spectrometry

- Tandem mass spectrometry: ions separated according to their  $m/z$  value in the first stage analyzer are selected for fragmentation and the fragments are analyzed in a second analyzer. It is used for protein sequencing and PTM (post-translational modifications).

# MS/MS Peptide Sequencing



# PTMs and epigenetic regulation of histones

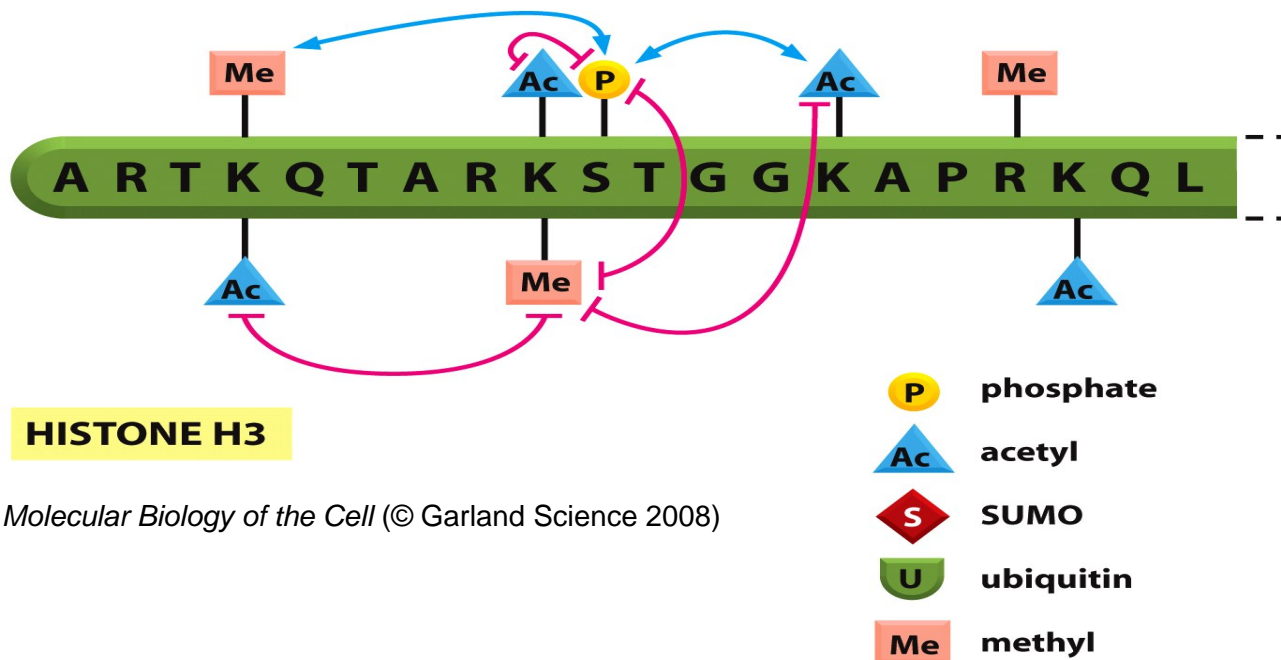


Figure 3-81b *Molecular Biology of the Cell* (© Garland Science 2008)

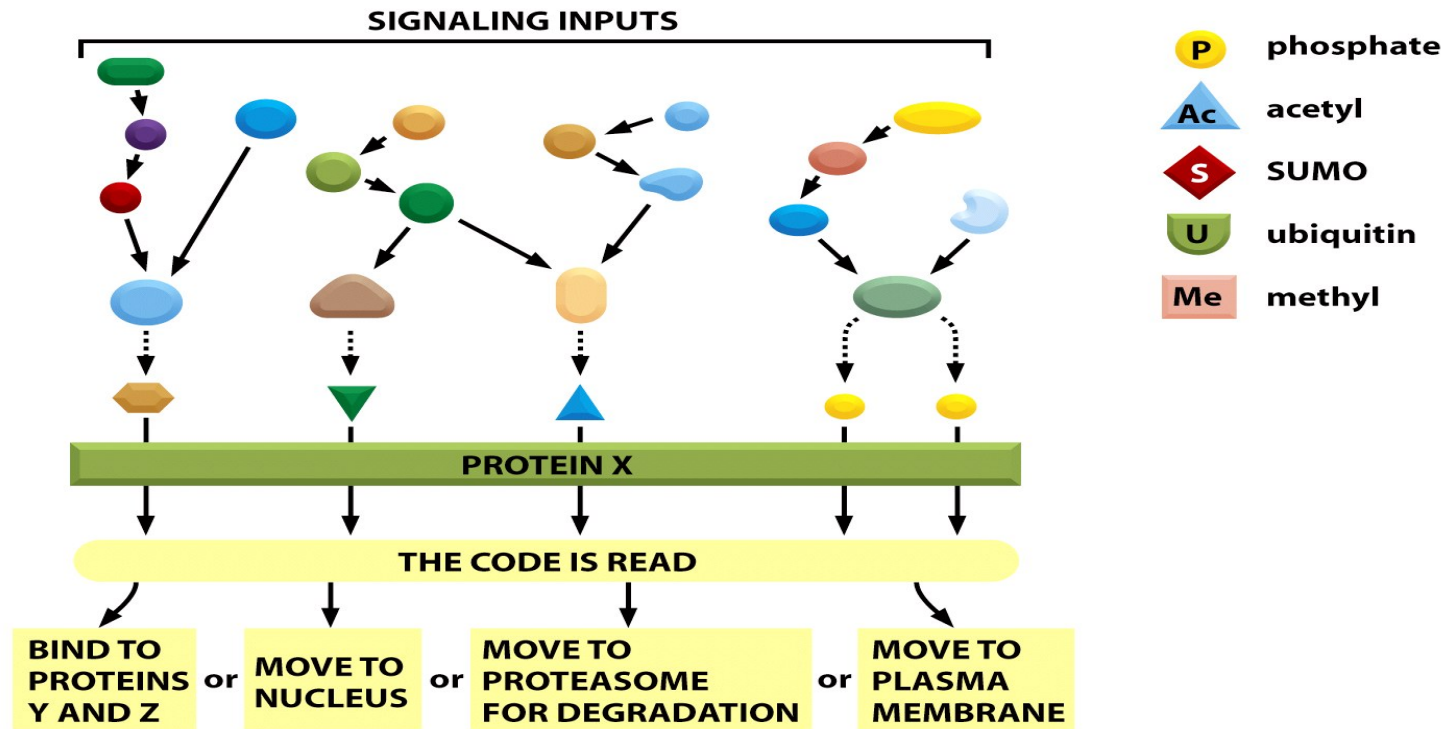


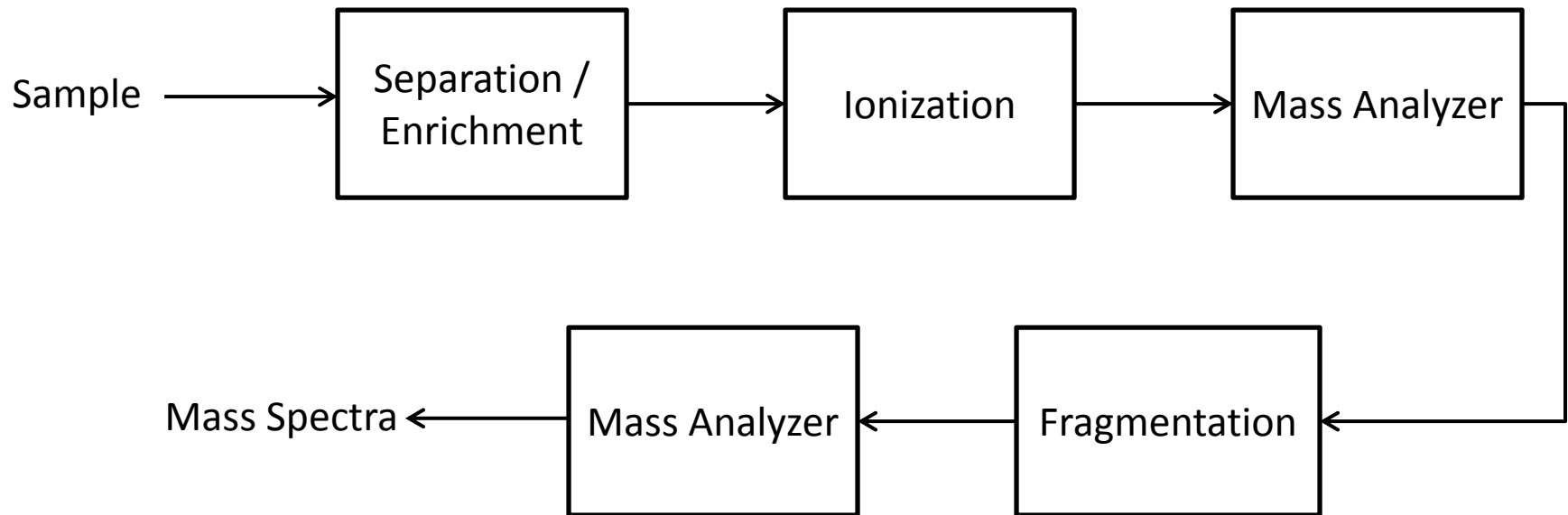
Figure 3-81c *Molecular Biology of the Cell* (© Garland Science 2008)



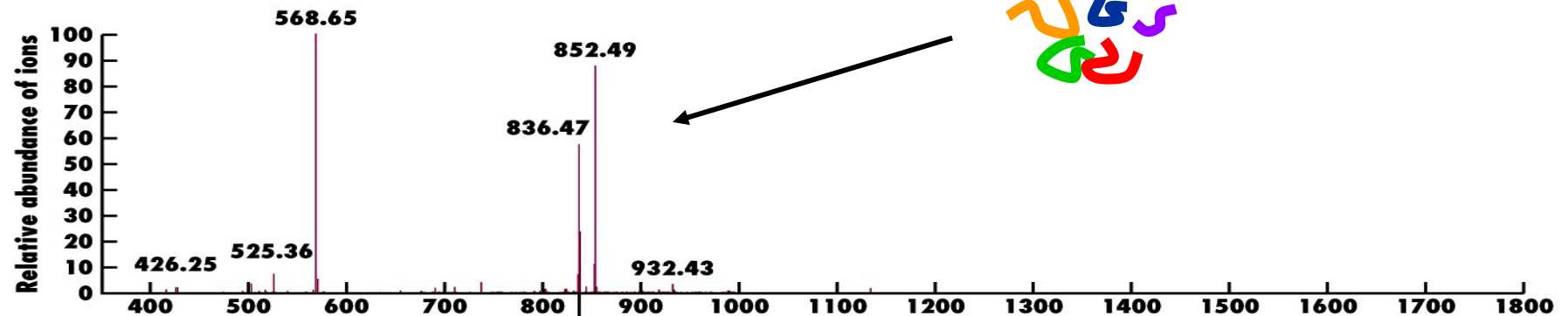
# Applications

- Compare and/or quantify:
  - Total Protein Abundance
  - Post-translational modifications
    - Phosphorylation
    - Acetylation
    - Methylation
    - Hydroxylation
    - Glycosylation
    - Etc.
  - If it has mass and can be ionized, it can likely be analyzed by mass spectrometry

# General MS/MS Setup for Proteomics



# MS/MS Peptide sequencing and PTM identification



MS/MS of  $m/z$  836.47

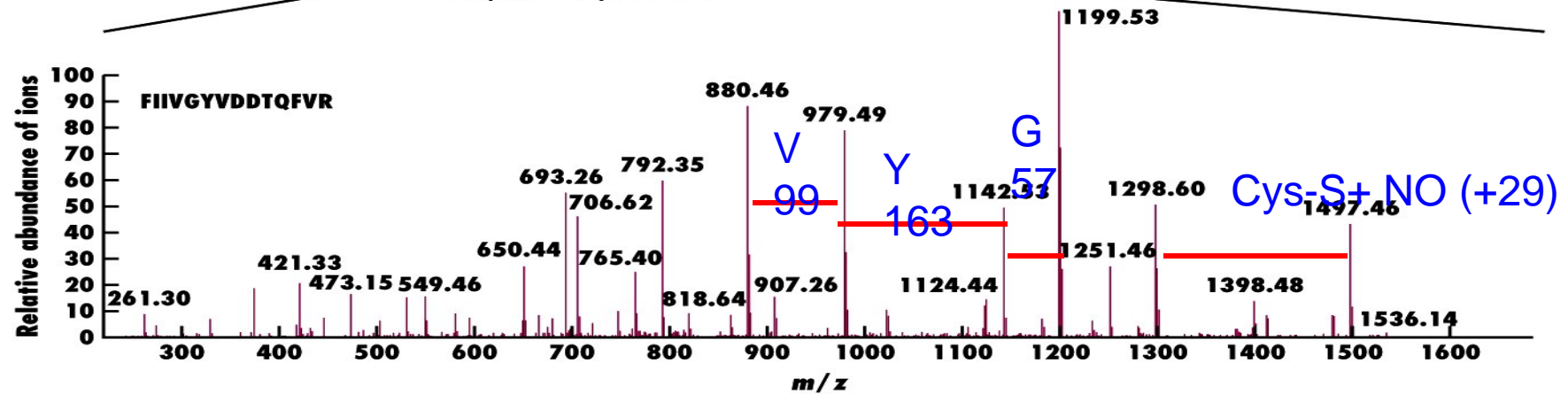


Figure 3-41b  
Molecular Cell Biology, Sixth Edition  
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