



# An Advanced Mass Spectrometry and Proteomics Laboratory

Instrumentation and Methods

*Departmental Seminar by  
Laszlo Prokai, Ph.D., D.Sc.*



# Outline

- What is mass spectrometry?
- Instrumentation and methods for mass spectrometry-based proteomics
  - Protein expression profiling
  - Posttranslational modifications
- Quantification by mass spectrometry



# What is a mass spectrometer (mass spectrometry)?

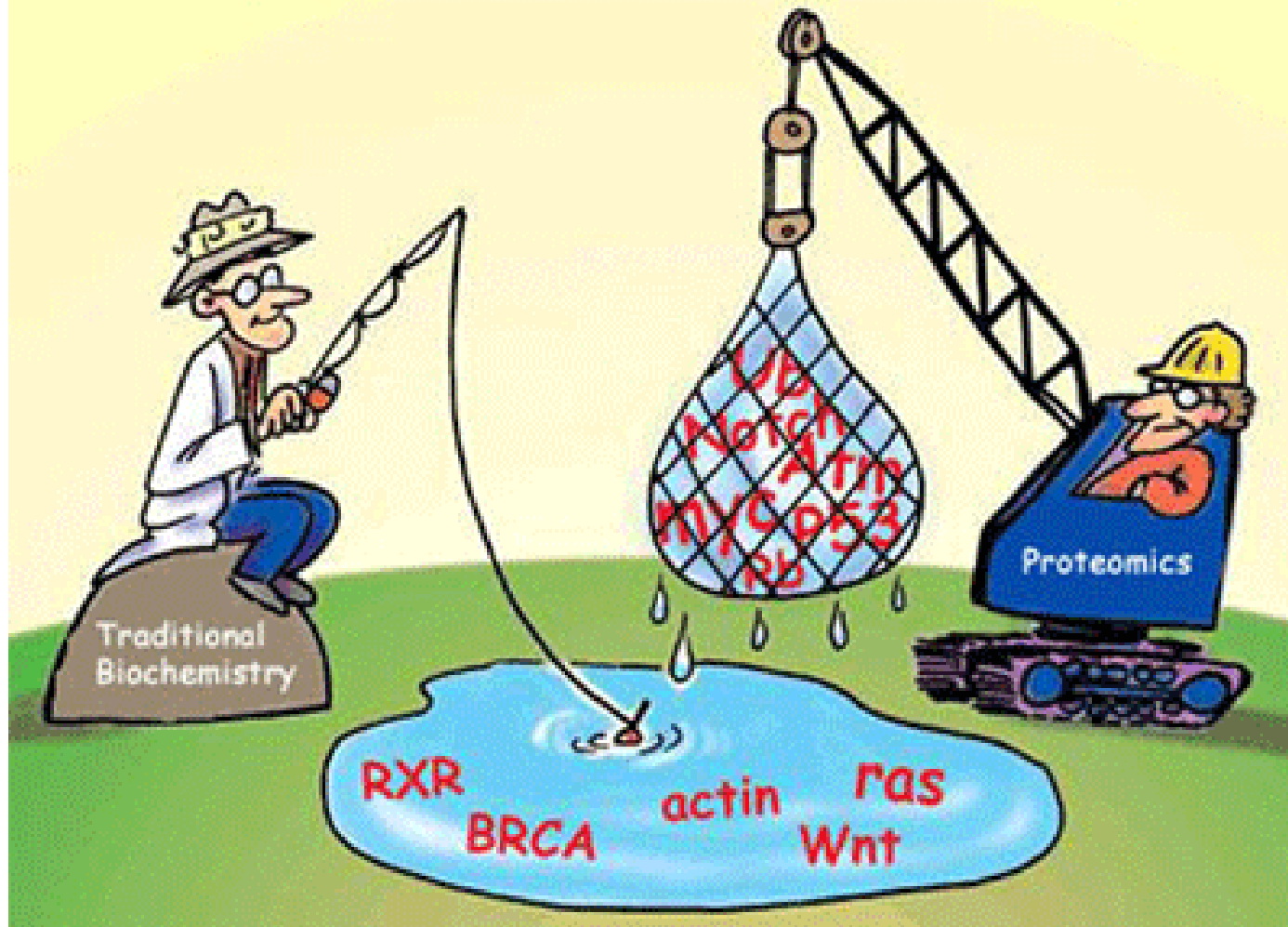
- An instrument used to weigh molecules
  - A molecular scale (measures electrically charged molecules)
    - Mass analysis
  - Identifies molecules present in solids, liquids and gases
    - Determine which atoms comprise a molecule and how they are arranged
  - Determine the quantity of each type of molecule
    - Extremely low limit of detection



# Proteomics

- The large-scale study of proteins encoded by a genome
  - Mass spectrometry is the most important tool
  - Expression profiling
    - Large databases: protein (PDB), expressed sequence tag (EST), genome for identification based on MS data
  - Post-translational modifications (PTMs)
  - Quantitative (differential) protein measurement
  - Protein-protein interactions

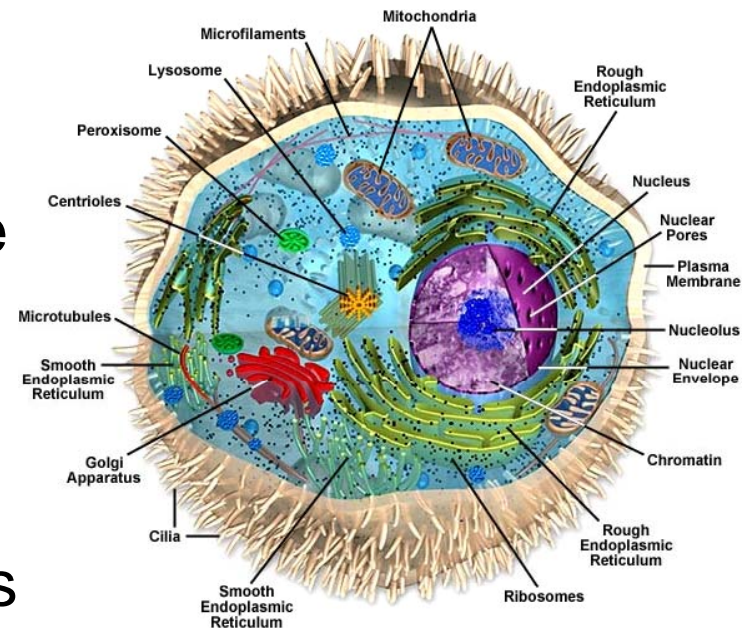
# Proteomics vs. Traditional Approach



CREDIT: JOE SUTLIFF

# Proteome

- >300,000 protein species per organism
- >30,000 protein species per cell
  - Heterogeneous molecules
  - Proteins cannot be multiplied
  - Large dynamic concentration range
  - Continuously changing in time
    - Alteration in protein biosynthesis
    - Controlled protein degradation
  - Posttranslational modifications
  - Protein-protein interactions

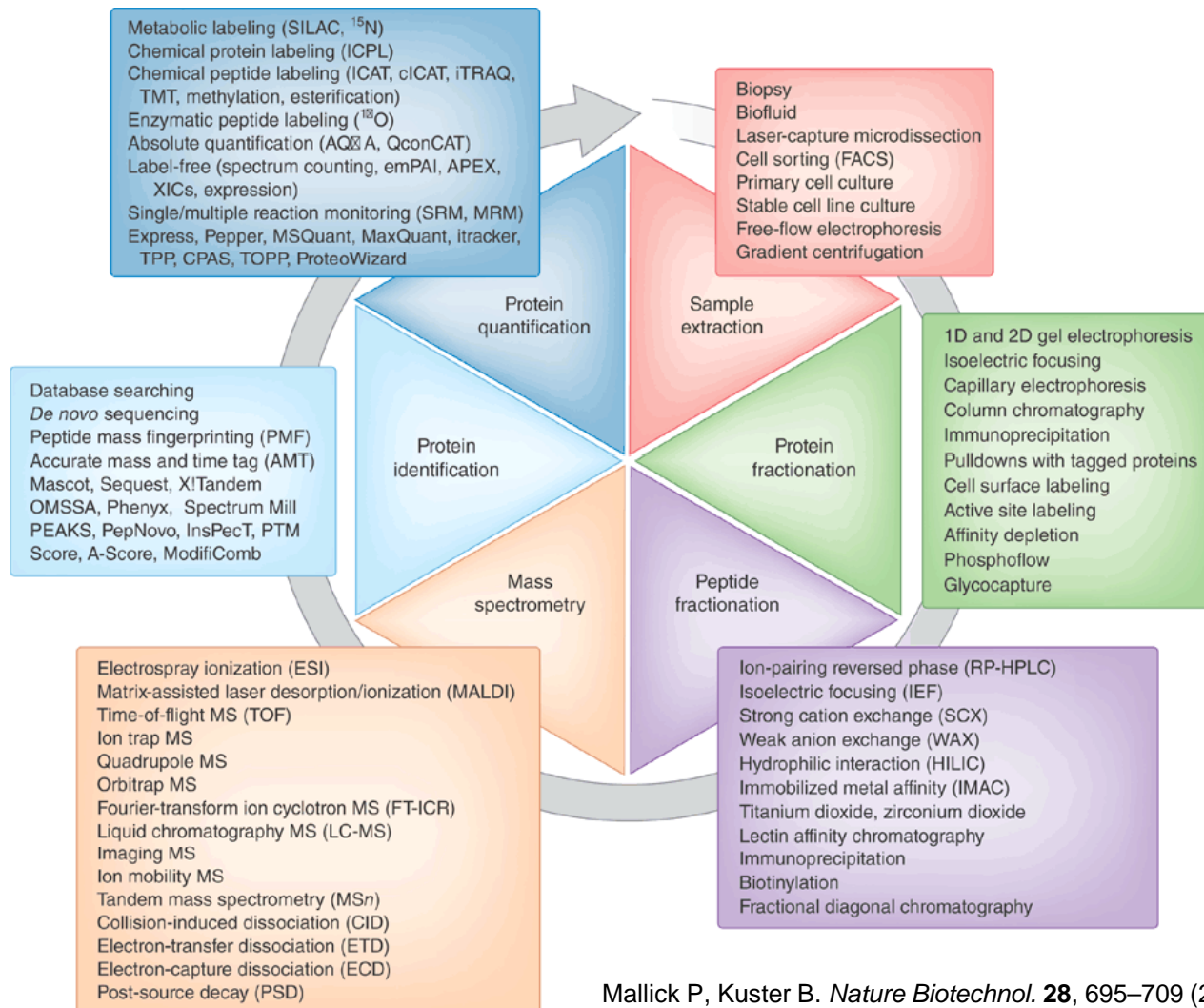




# Dilemmas About Data Collection, Storage, Access and Processing

- There may never be an effective way to analyze an entire human [*mammalian*] proteome in a high-throughput (HT) manner, but several newer technologies may help solve certain proteomics dilemmas on a case-by-case basis.
  - Key: Mass spectrometers adapted for proteomics
    - Time-of-flight (TOF), quadrupole/TSQ, ion traps (QIT & LIT), Orbitrap, Fourier-transform ion cyclotron resonance (FT-ICR) and hybrids
    - They are not necessarily more powerful and/or much faster, but they allow smarter collection strategies for proteome analyses
  - Key: Focus on knowledge generation rather than merely creating data

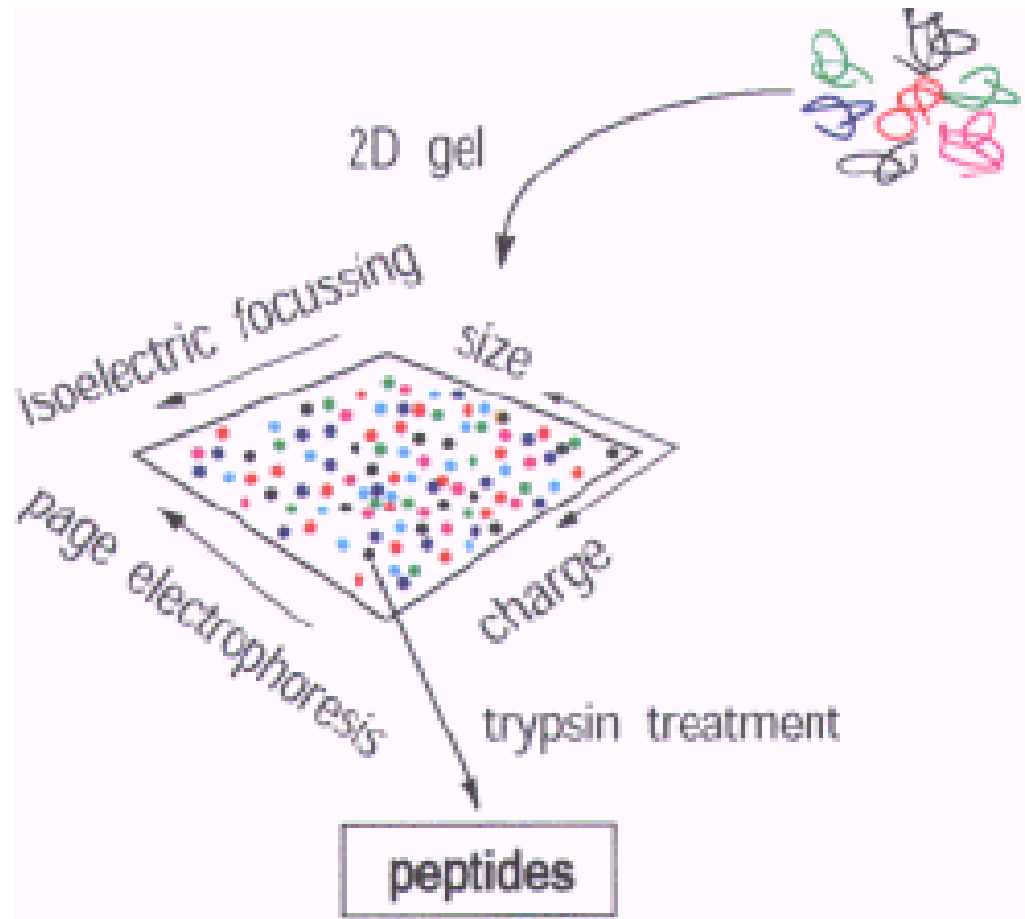
# Technologies for Proteomics



Mallick P, Kuster B. *Nature Biotechnol.* **28**, 695–709 (2010)

# Routine, Gel-Based Protein Identification

- 2-D gel electrophoresis coupled with mass spectrometry-based protein identification
  - From peptides
  - Protein database

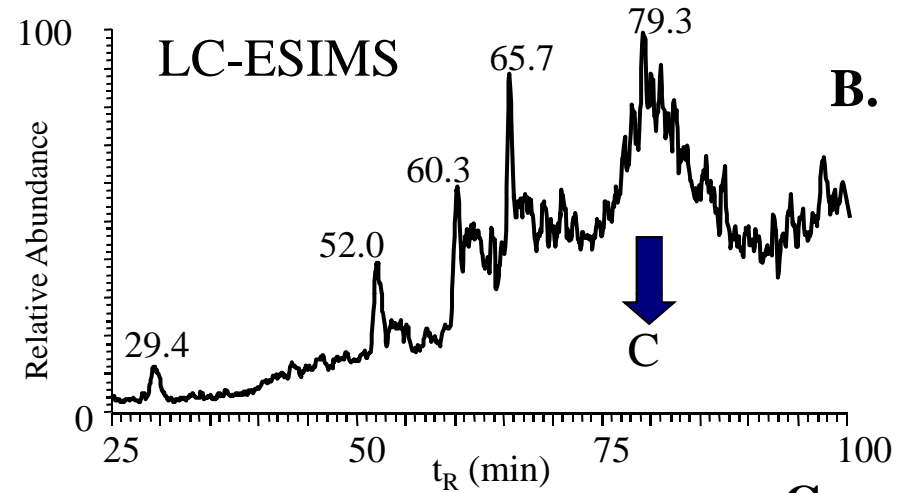
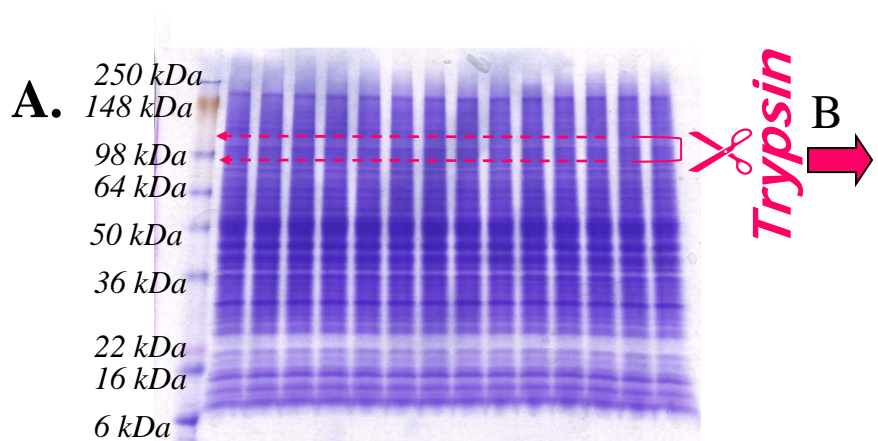




# Shortcomings of 2-D GE-based Method

- Limited capacity and resolution
  - Only high  $\mu\text{g}$  amounts of material can be loaded onto the gel
  - Individual spots from 2-D gels contain between 50 fmol to 2 pmols of protein; one spot may have more than one protein
- Limited protein coverage
  - Soluble, medium- to high-abundance (“housekeeping” proteins), etc.
  - Not suited for highly acidic/basic, membrane (hydrophobic) and low-abundance proteins

# GeLC/MS:

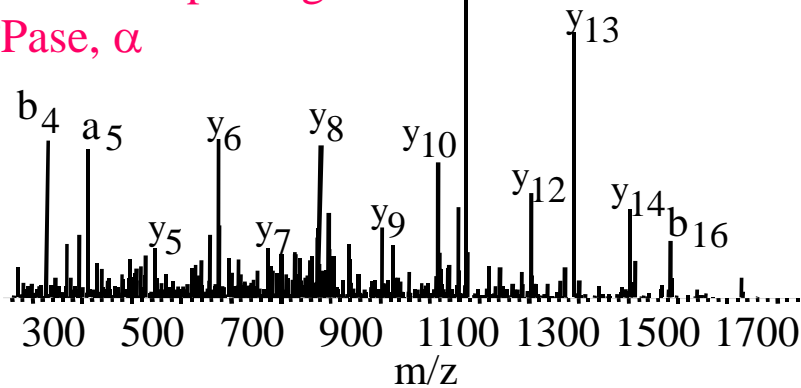


**D.** MS/MS from  $m/z$  915.5 (79.3 min)

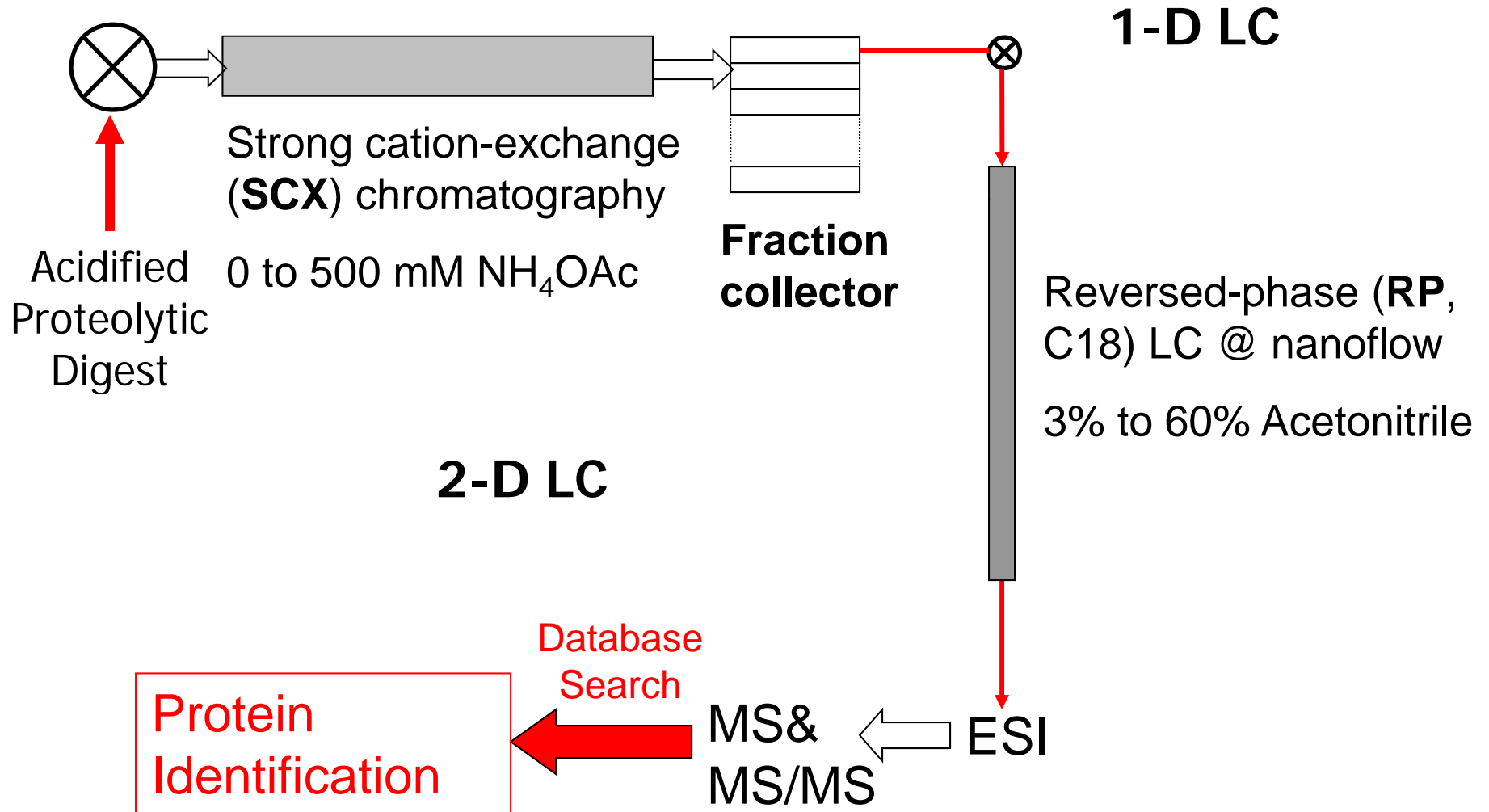
**GVGIISEGNETVEDIAAR**  $y_{11}$

$\text{Na}^+/\text{K}^+$  transporting

ATPase,  $\alpha$



# Protein Identifications: LC/ESI-MS & MS/MS (“gel-free”)

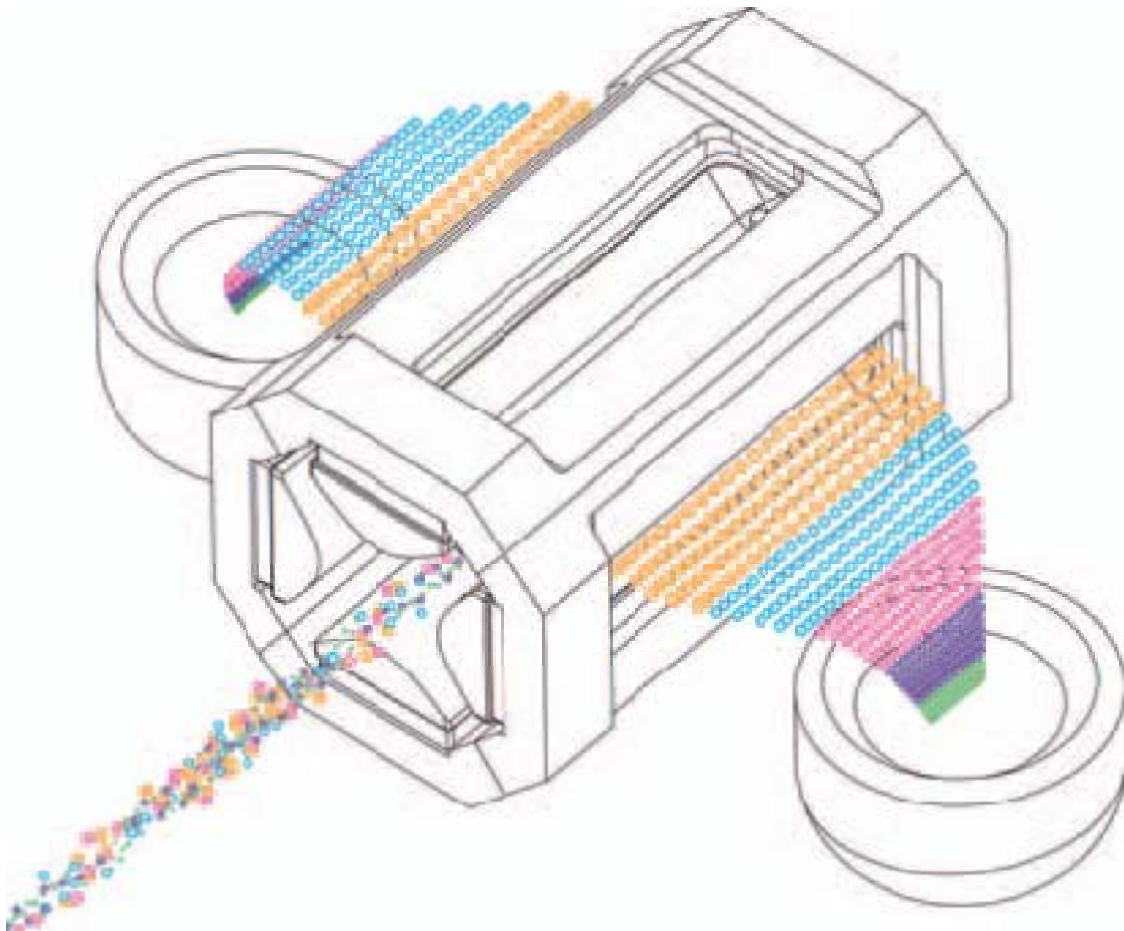


# LCQ (3-D Ion Trap)



Now open access: Ask for training, if interested.

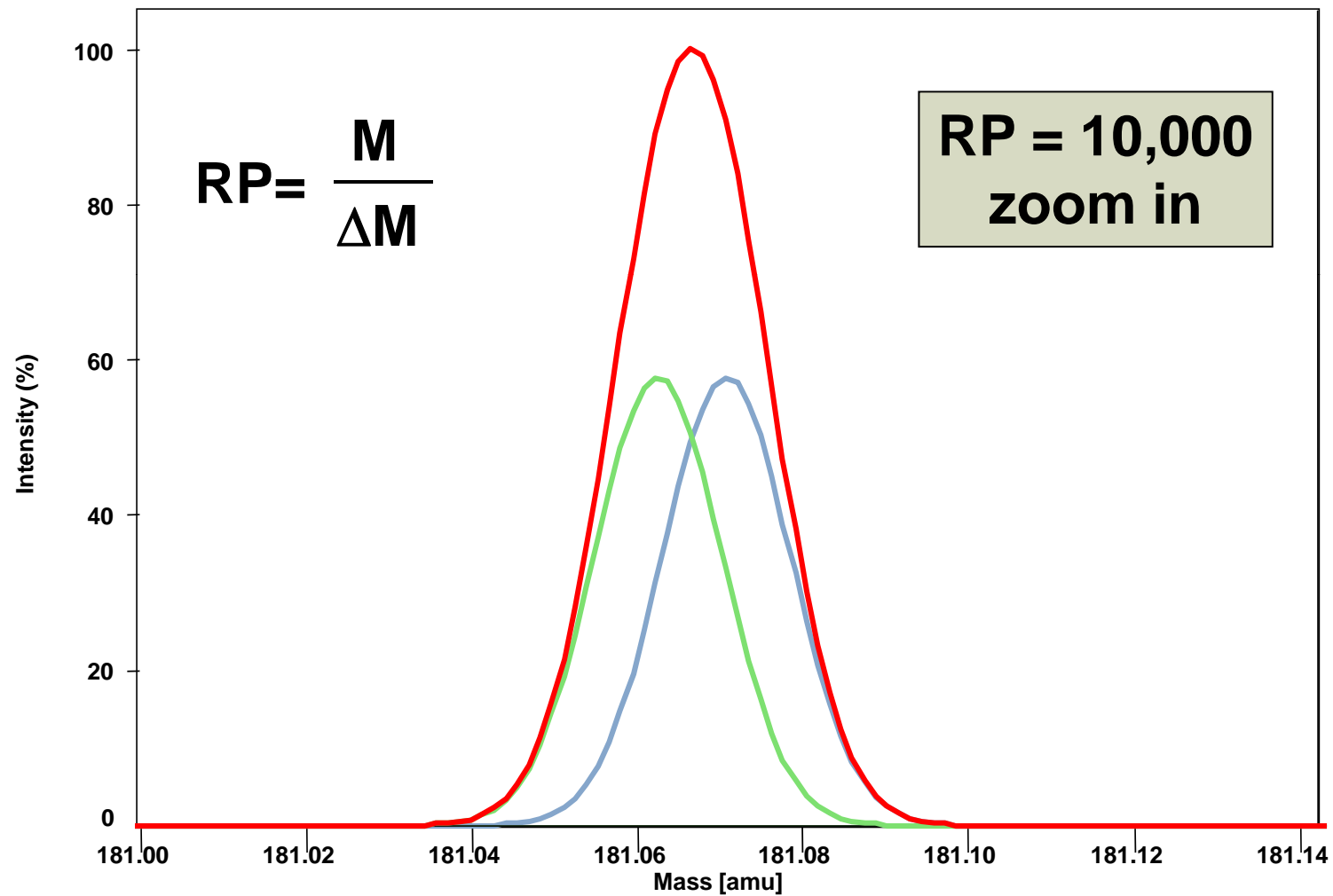
# Linear Ion Trap (LTQ)



## ■ Benefits

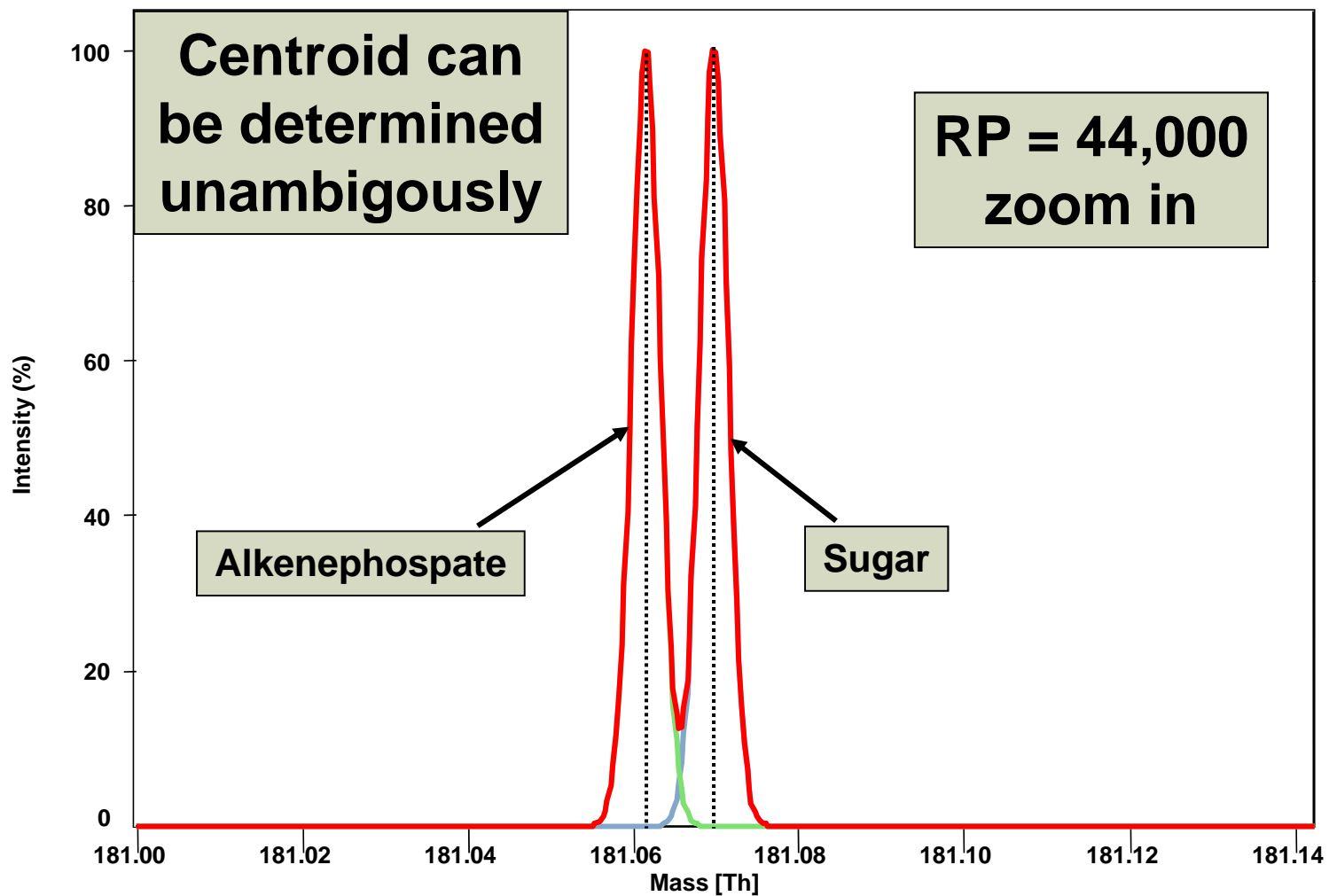
- High capacity (sensitivity)
- Fast MS, MS/MS and MS<sup>n</sup>
- However, not “accurate”

# Example for High Resolution



Credit: Thermo (A. Ziberna)

# Example for High Resolution



Credit: Thermo (A. Ziberna)

# Accurate Mass and Elemental Composition

All chemical compounds are built by combination of different Elements. With the exception of carbon (by definition the exact mass is 12.0000), all other atoms have either a negative or a positive mass defect. That means the exact mass of the atoms are not equal to nominal mass.

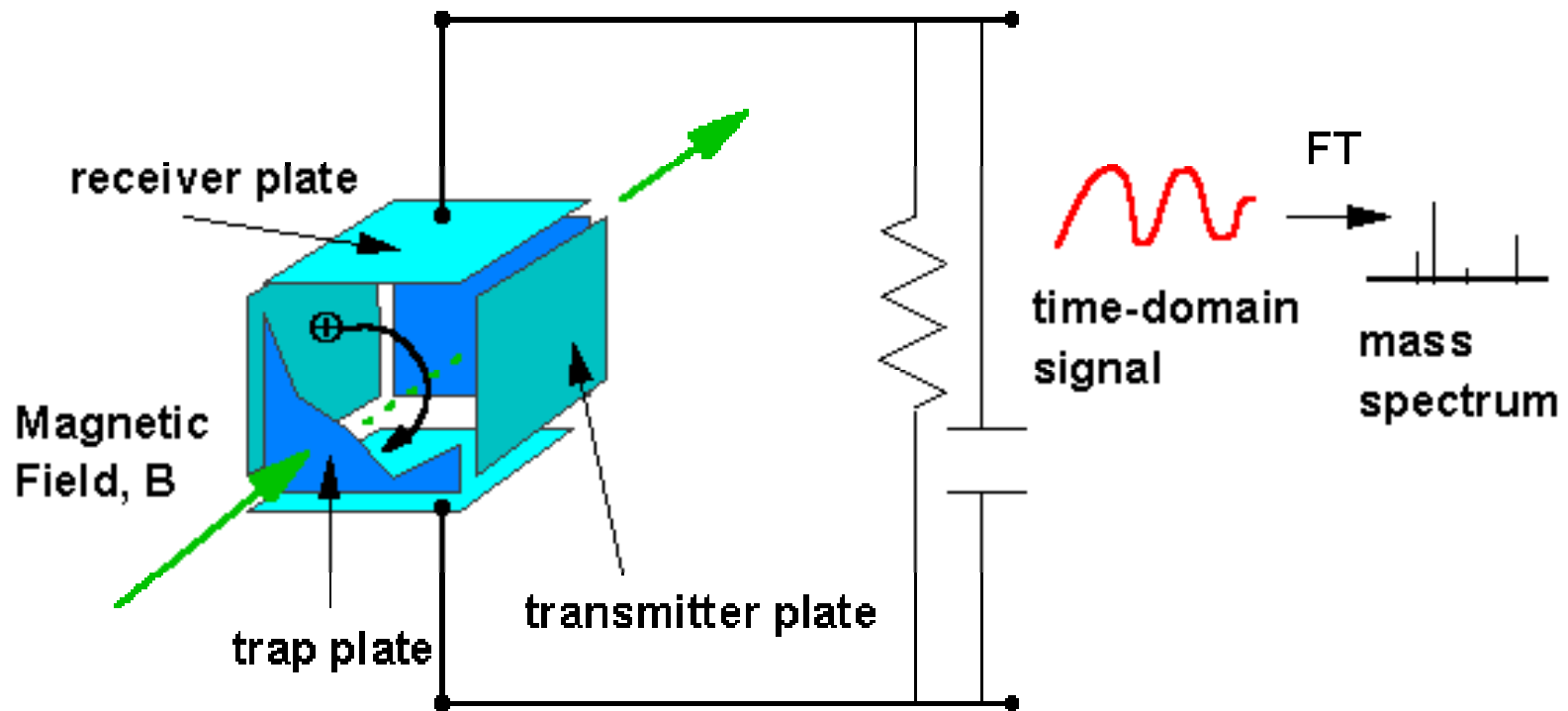
<b>Elements:</b>	<b>Carbon,</b>	<b>Hydrogen,</b>	<b>Nitrogen,</b>	<b>Oxygen</b>	<b>Sulfur</b>	<b>etc.</b>
<b>Formula:</b>	<b><math>^{12}\text{C}</math></b>	<b><math>^1\text{H}</math></b>	<b><math>^{14}\text{N}</math></b>	<b><math>^{16}\text{O}</math></b>	<b><math>^{32}\text{S}</math></b>	
<b>Exact mass:</b>	<b>12.0000</b>	<b>1.0078</b>	<b>14.0031</b>	<b>15.9949</b>	<b>31.9721</b>	



**Amino Acids, the subunit of peptides are built by the different atoms**

# Ion-Cyclotron Resonance (ICR)

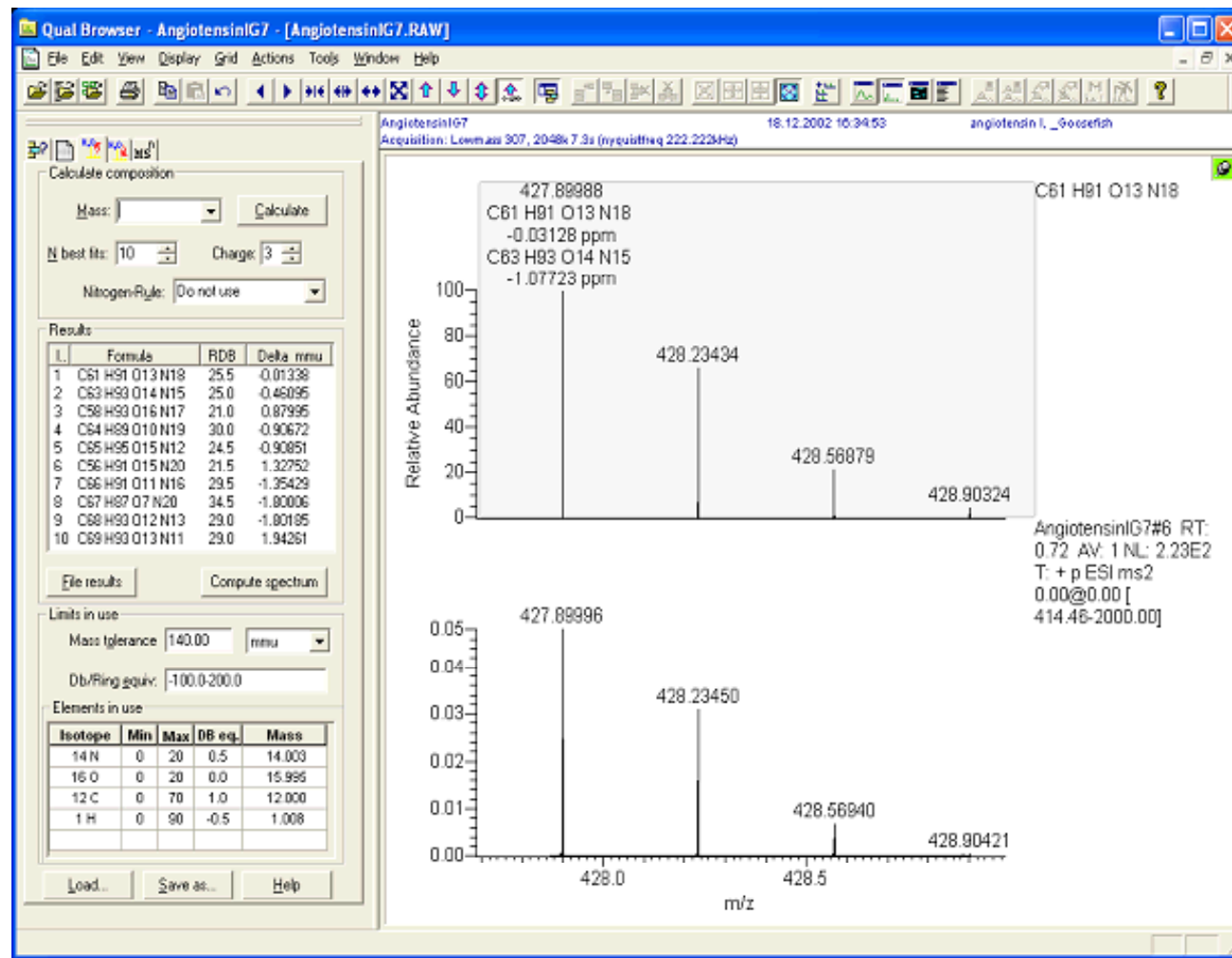
- Fourier-transform mass spectrometry
  - Accurate (high resolution, “resolving power”  $\sim$  RP)
  - However, not fast (especially for MS/MS)



# Benefits

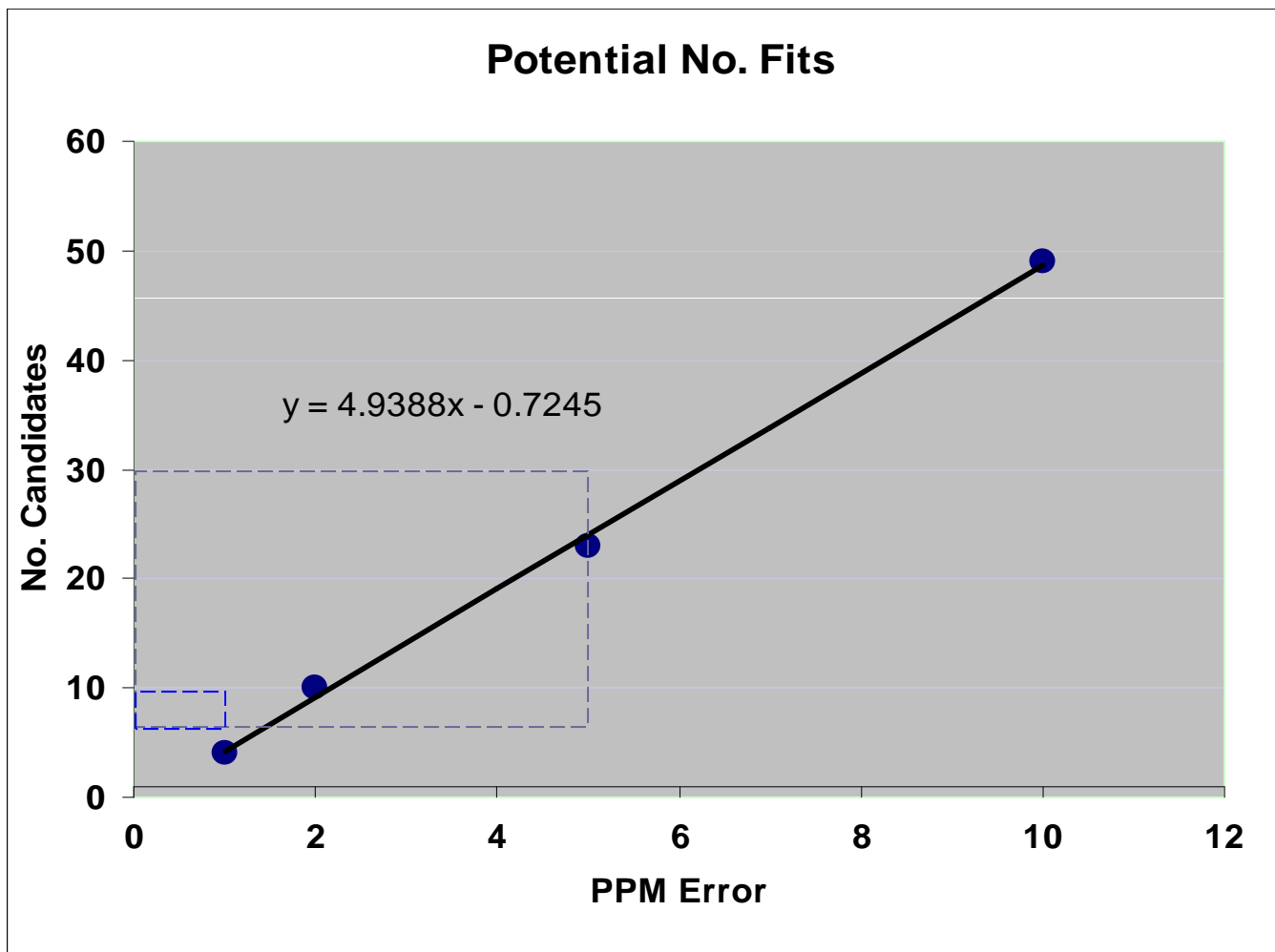
## ■ Enhanced for FTICR analysis

- Accurate mass profile data
- Resolution, charge state, noise
- Elemental composition



Credit: Thermo (A. Ziberna)

# Relationship between mass error and elemental composition



Credit: Thermo (A. Ziberna)

# Linear Ion Trap – FTICR Hybrid

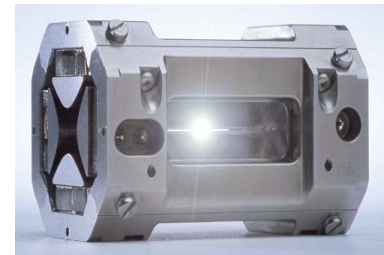
## Linear Ion Trap MS

- MS, MS/MS and MS<sup>n</sup> Analysis
- **AGC Control**
- Secondary Electron Multiplier **Detector**

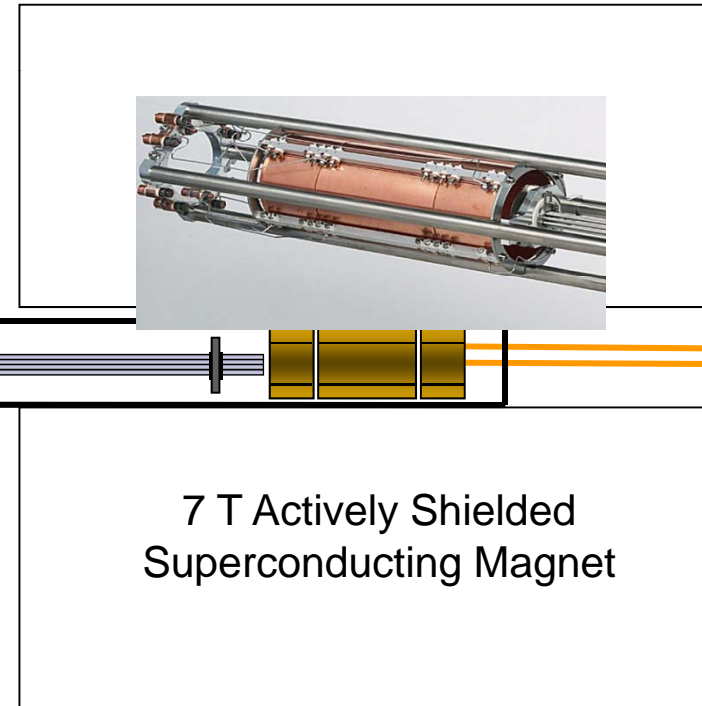
## FTICR MS

- Ion Image Current **Detector**
- Accurate Mass
- High Resolution

FTMS Data



Linear Ion Trap Data



60 m<sup>3</sup>/hr 15 L/sec 300L/sec 400L/sec 210L/sec 210L/sec  
Triple Ported Turbo Pump

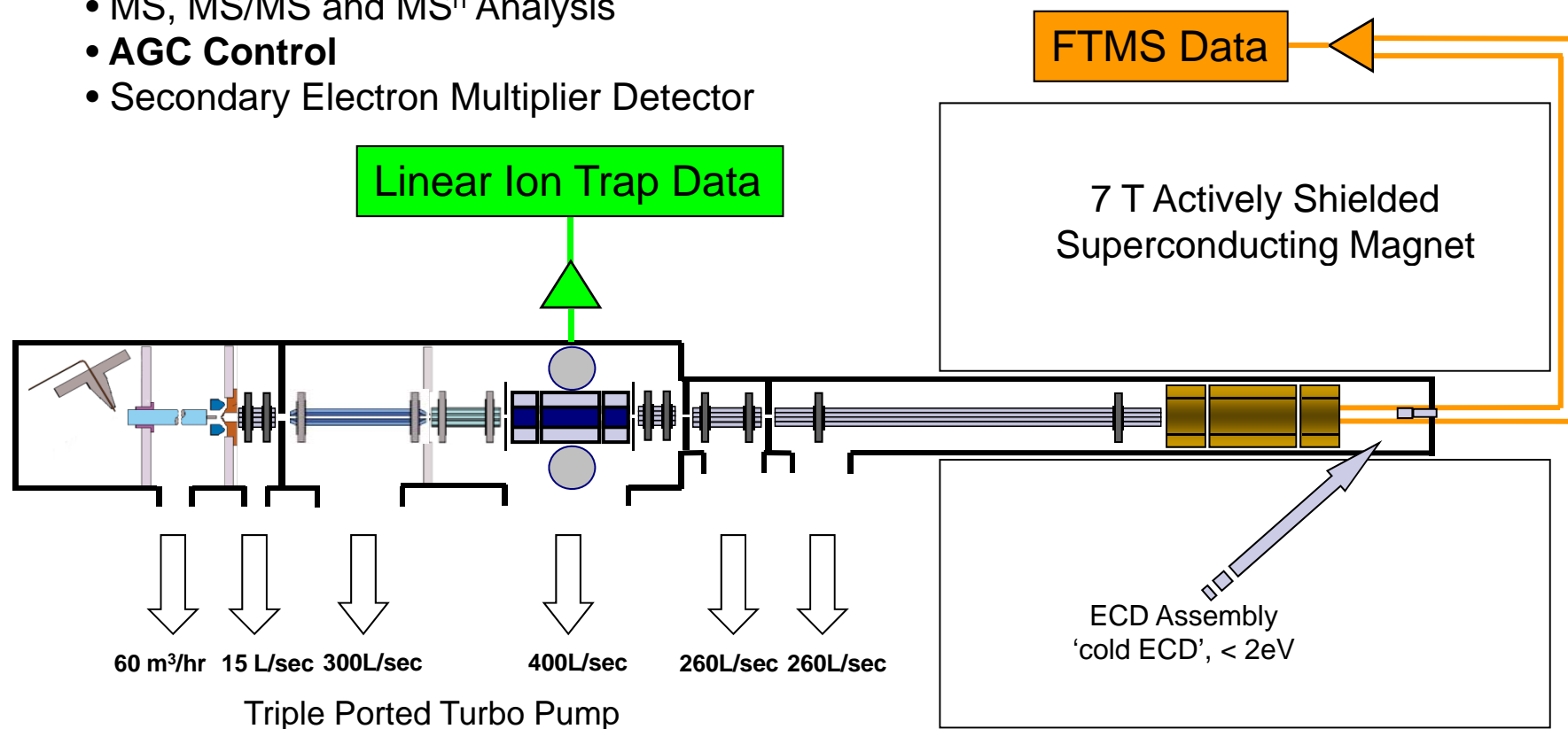
# LTQ FT: Possibilities to Use Alternative Ion Dissociation for MS/MS

## Linear Ion Trap MS

- MS, MS/MS and MS<sup>n</sup> Analysis
- **AGC Control**
- Secondary Electron Multiplier Detector

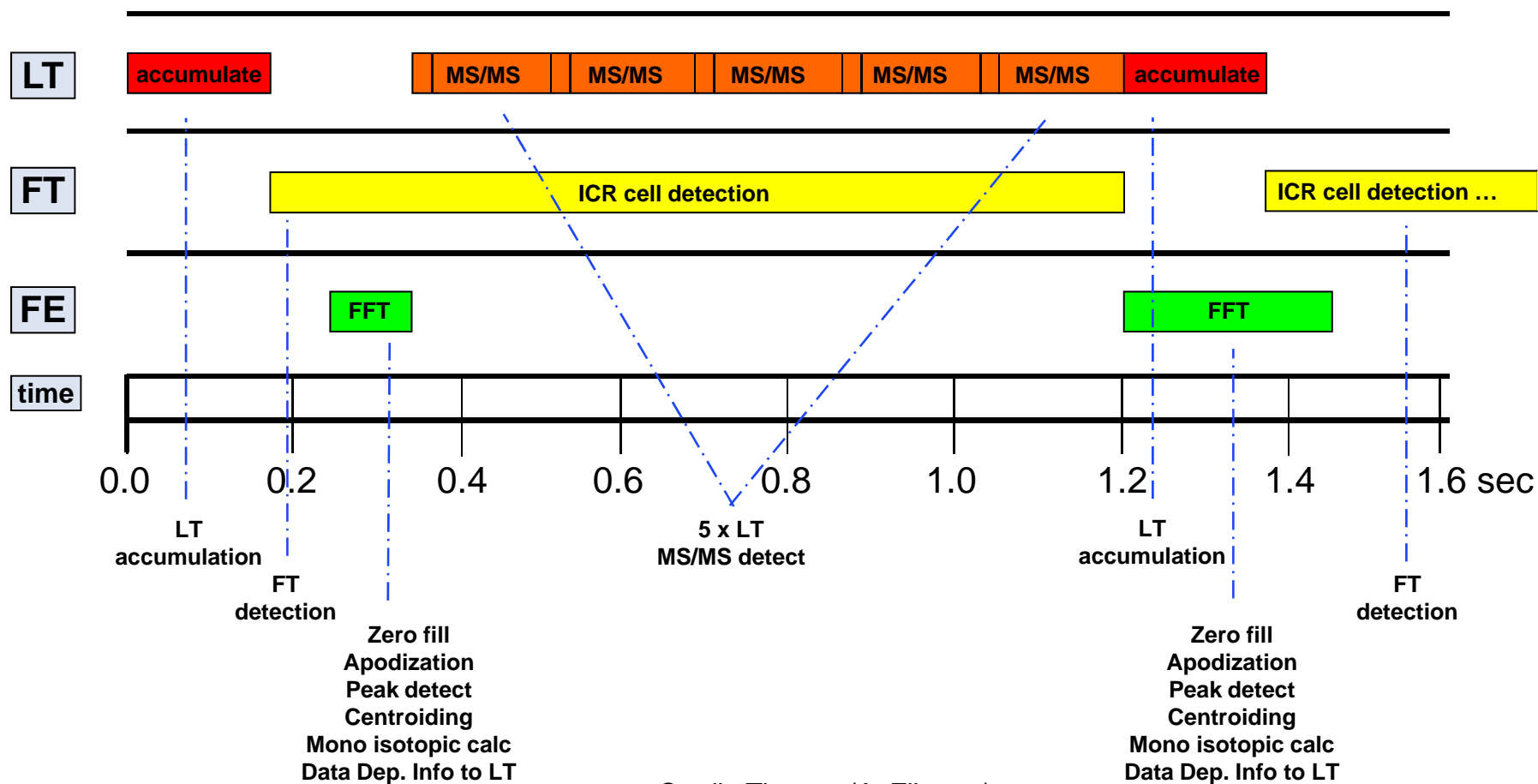
## FTICR MS

- Ion Image Current Detector
- Accurate Mass, High Resolution
- **Electron-capture dissociation (ECD)**



Credit: Thermo (A. Ziberna)

# Data-Dependent Acquisition by the LTQ-FTICR: The Essence of Discovery-Driven Proteomics



Credit: Thermo (A. Ziberna)

# Simultaneous LTQ and FTICR Operation

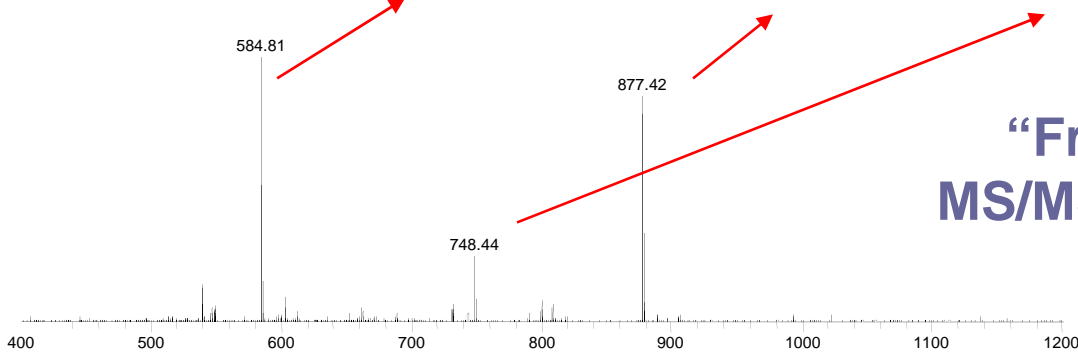
**SnapShot**  
**25k RP**  
**186 msec**

**Continue FT Acquisition**  
**100k RP**  
**560 msec**

FT



LT



**“Free”  
MS/MS Data**

# LTQ FT: Moving Proteomics to the Next Level



# An Important Addition on the Front-End



- **NanoLC-2D (Eksigent)** system that uses microfluidic flow control to generate precise gradients for liquid chromatography (LC) at nanoscale flow rate and enables two-dimensional (2D) LC
  - Increases mass spectrometer sensitivity and improves identification of low-abundance proteins

# Nevertheless, Data-Dependent LC-MS/MS Has Been Burdened by “Undersampling”

- A small fraction of the proteome identified
  - Solutions:
    - Sample fractionation (simplify!)
    - New accessory



The **TriVersa NanoMate** is a chip-based electrospray ionization (ESI) technology from Advion. It combines the strengths of liquid chromatography, mass spectrometry, chip-based infusion, and fraction collection analysis into one integrated system. It allows analysts to obtain more information from complex samples than with LC-MS alone.

# Mass Spectrometry-Based Proteomics Would be Handicapped Without Adequate Bioinformatics

- Search engines to identify peptides/proteins from database

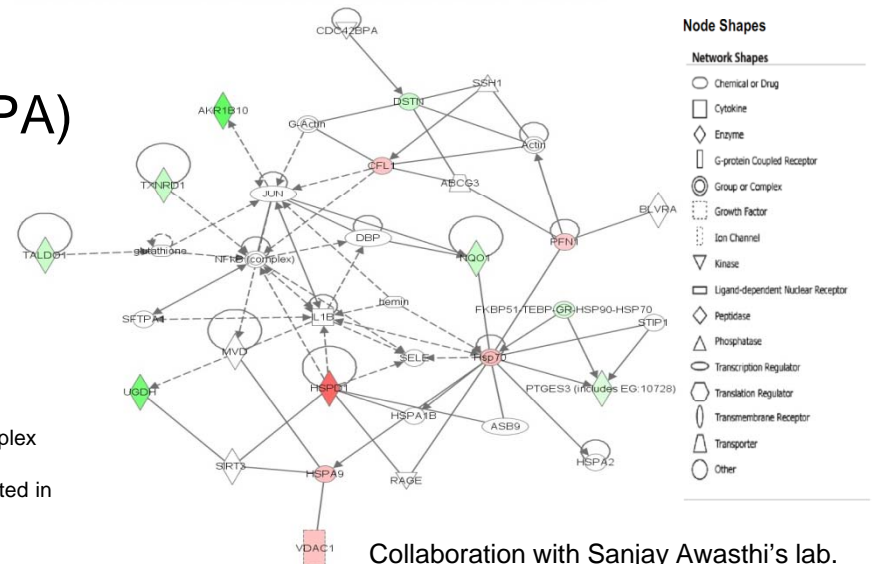
- Mascot
- SEQUEST (Proteome Discoverer)

**Scaffold:** Validation and more ...

- Turning peptide/protein identifications to biological information

- Ingenuity Pathways Analysis (IPA)

E.g., proteins with significant differential expression regulate the complex network of free radical scavenging, molecular transport and energy production: **Red**, up-regulated in *VHL*-mut RCC, **Green**, down-regulated in *VHL*-mut renal cell carcinoma.





# Context of Protein Expression Profiling

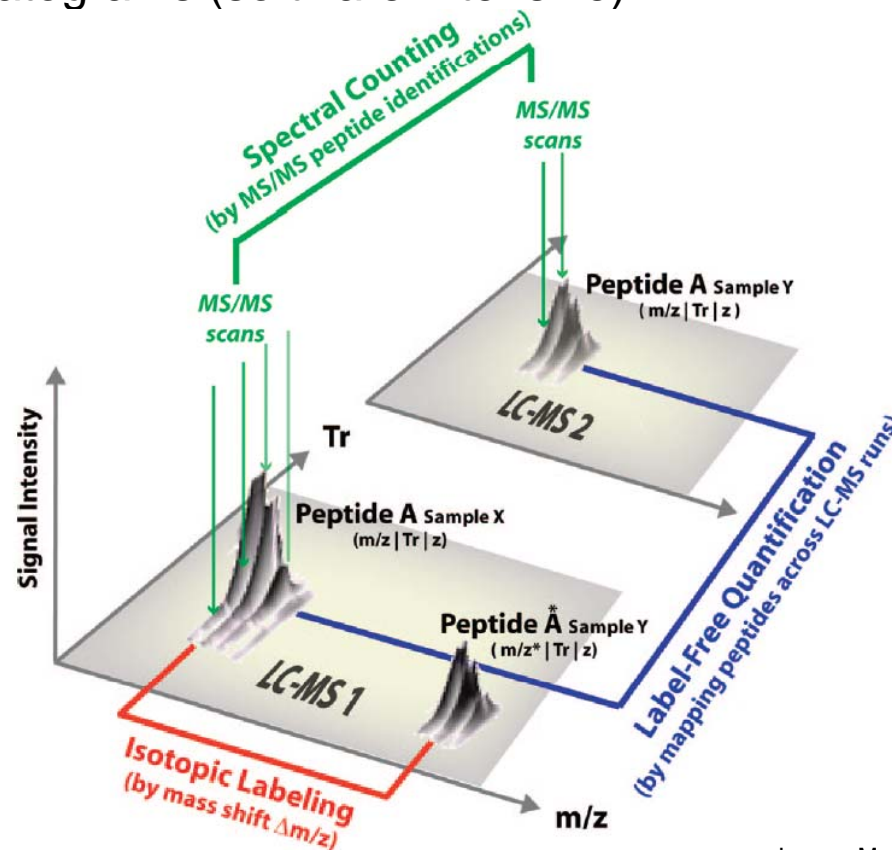
- Healthy *versus* diseased, control *versus* treated, etc.
  - Differential proteomics (obtaining relative expression differences are mostly sufficient)
    - Discovery: 2D-gel electrophoresis-based, stable isotope labeling with amino acids in cell culture (SILAC), stable-isotope labeled reagents and derivatization (mostly iTRAQ/TMT), label-free
    - Targeted: AQUA


# Label-Free Proteomics

- Best on newer tandem mass spectrometers (preferably high-resolution hybrids)
  - Spectral counting (simple) *versus* (?) reconstructed ion-chromatograms (software-intensive)



LTQ-FT



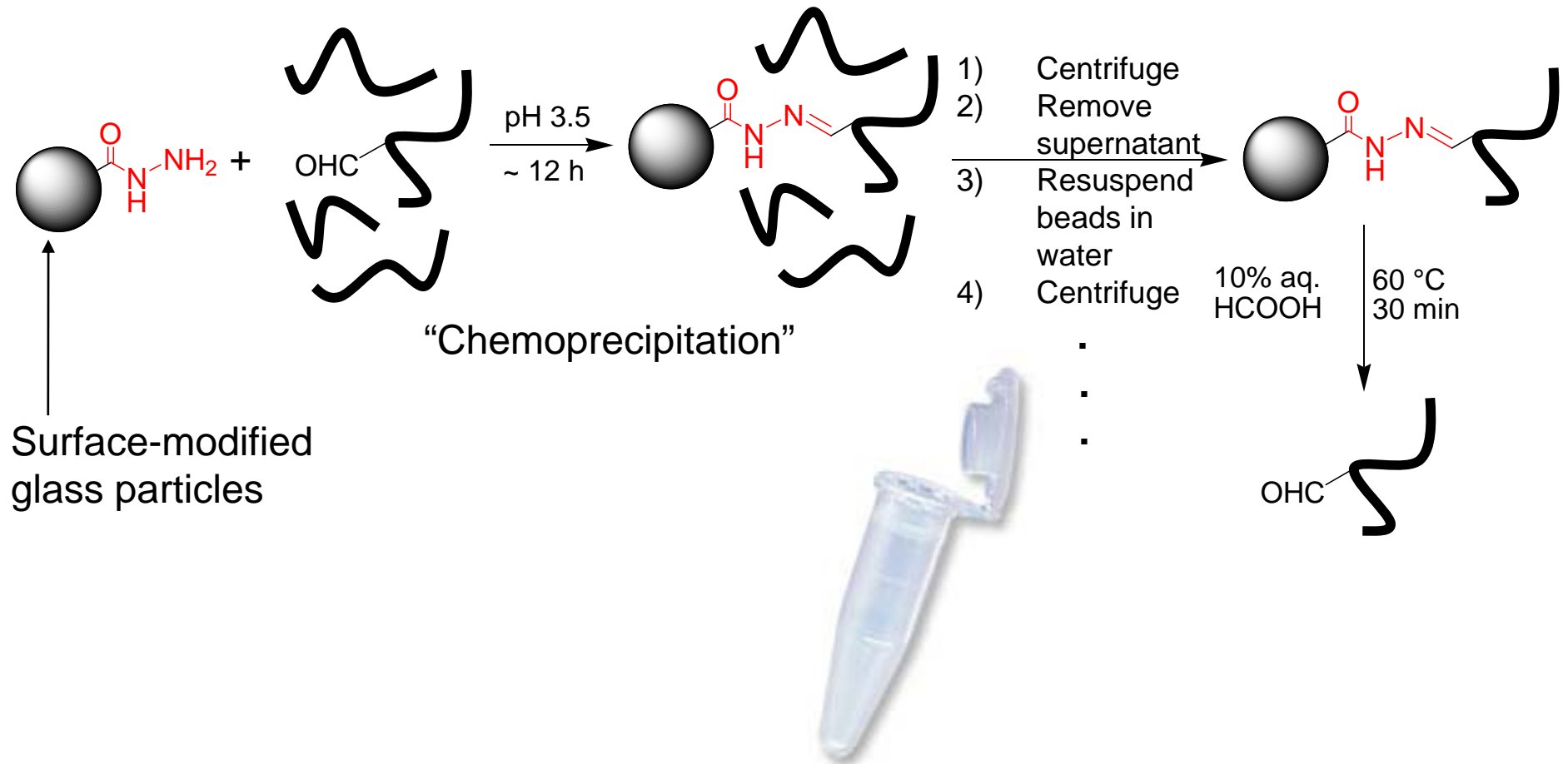


# Remember, Results Will Reflect the Quality of Your Samples

- Sample preparation is key
- Finding posttranslational modifications are challenging
  - Necessity of enrichment
    - Your trusted immunoaffinity-based method may be useless for LC-MS/MS

# Enrichment of Peptides Modified Posttranslationally by 4-Hydroxy-2-Nonenal (HNE)

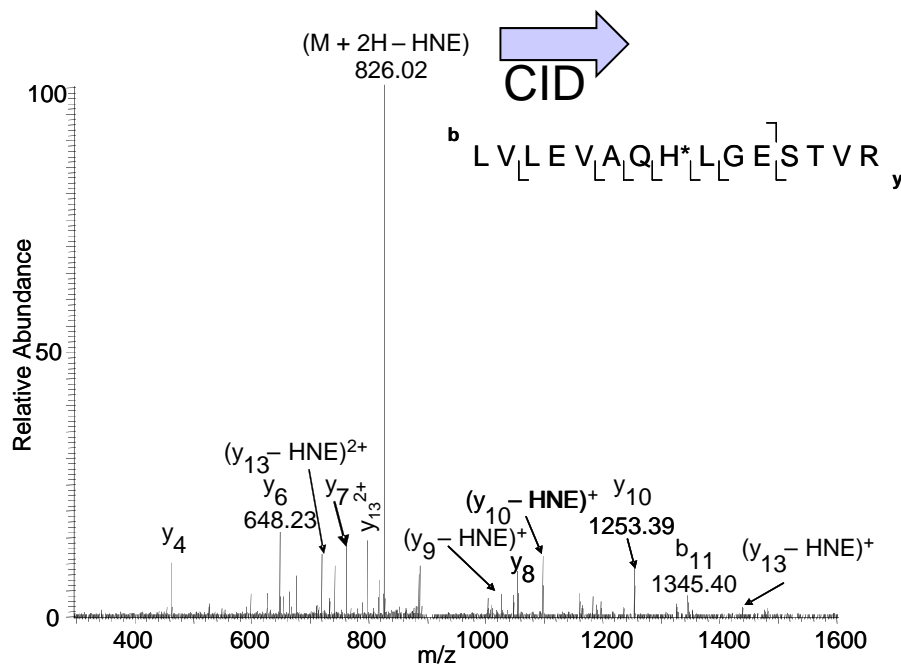
Based on solid-phase hydrazide (**SPH**) chemistry



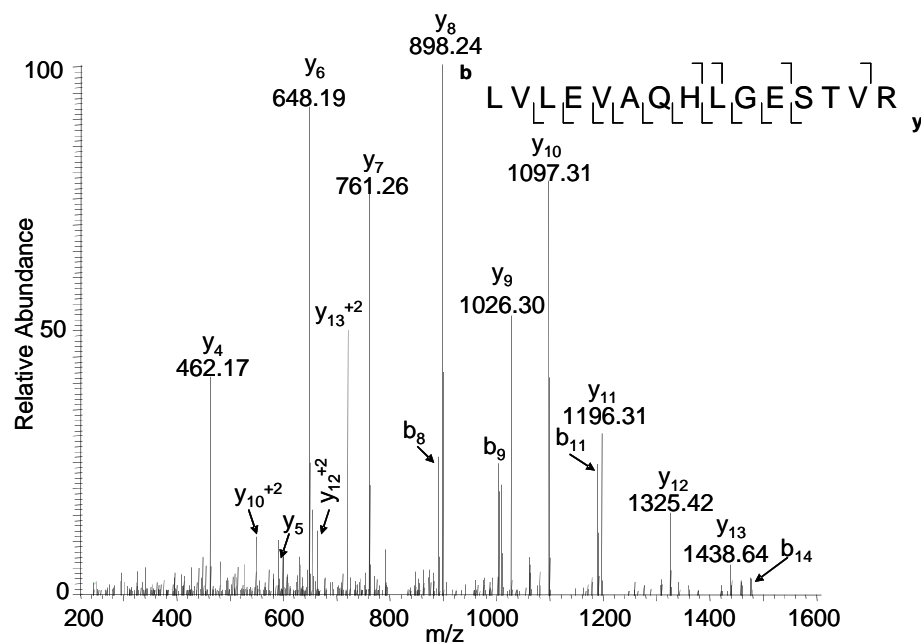
# Advanced Tandem Mass Spectrometry Is Often Required to Identify Peptides Bearing Posttranslational Modifications

## ■ LC-NL-MS<sup>3</sup>

MS/MS ( $m/z$  904.51, 2+)



MS<sup>3</sup>



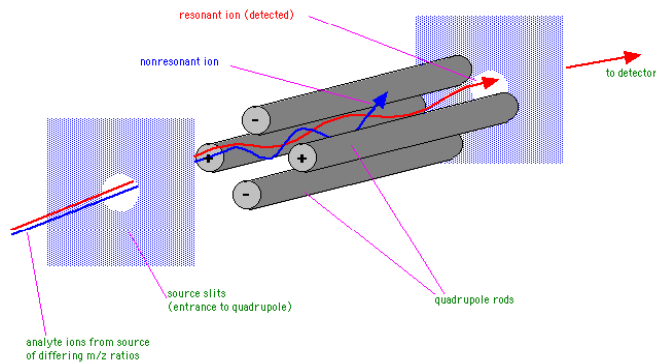


# Context of Protein Expression Profiling

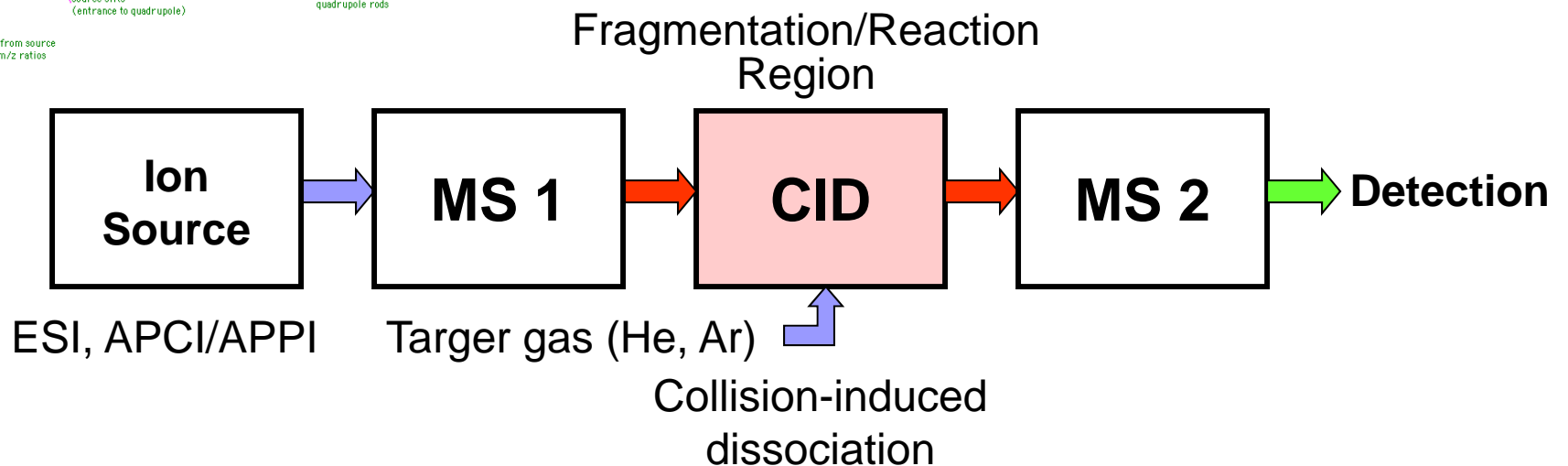
- Healthy *versus* diseased, control *versus* treated, etc.
  - Differential proteomics (obtaining relative expression differences are mostly sufficient)
    - Discovery: 2D-gel electrophoresis-based, stable isotope labeling with amino acids in cell culture (SILAC), stable-isotope labeled reagents and derivatization (mostly iTRAQ/TMT), label-free
    - Targeted: AQUA (absolute quantification)

# Triple Quadrupole

- Tandem-in-space
  - Quadrupoles: QqQ

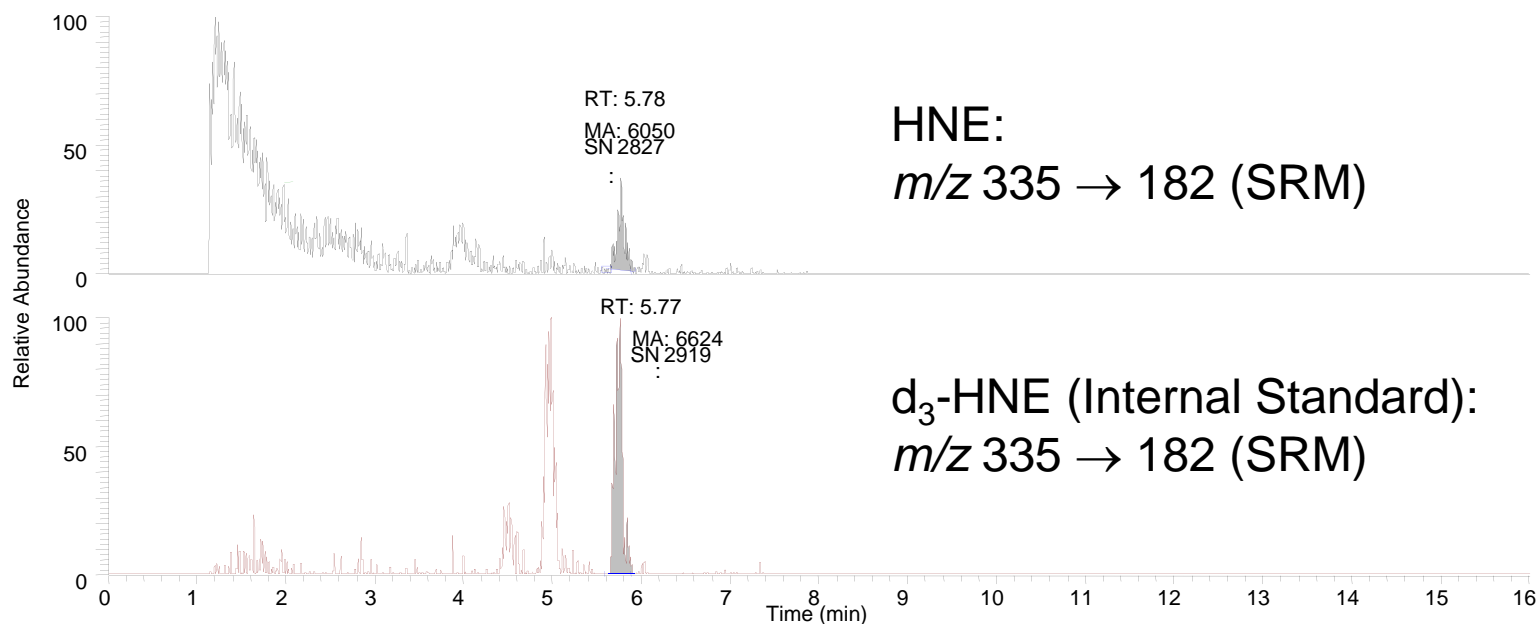


TSQ Quantum Ultra (Thermo)



# Advantages of LC-MS/MS on Triple-Quadrupole Mass Spectrometers

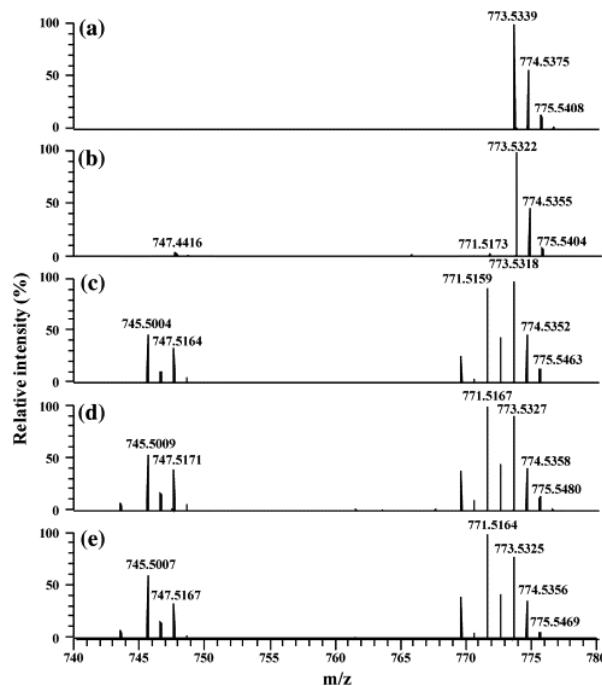
- Sensitive and selective
- Accurate quantification (with stable isotope labeled internal standards: “Isotope dilution” principle)



# There Is “Life” Outside Proteomics

## ■ Lipidomics

- “Shotgun” lipidomics: Most major and many minor lipid classes fingerprinted and quantitated directly from biological lipid extracts without chromatographic purification by (ESI-) MS



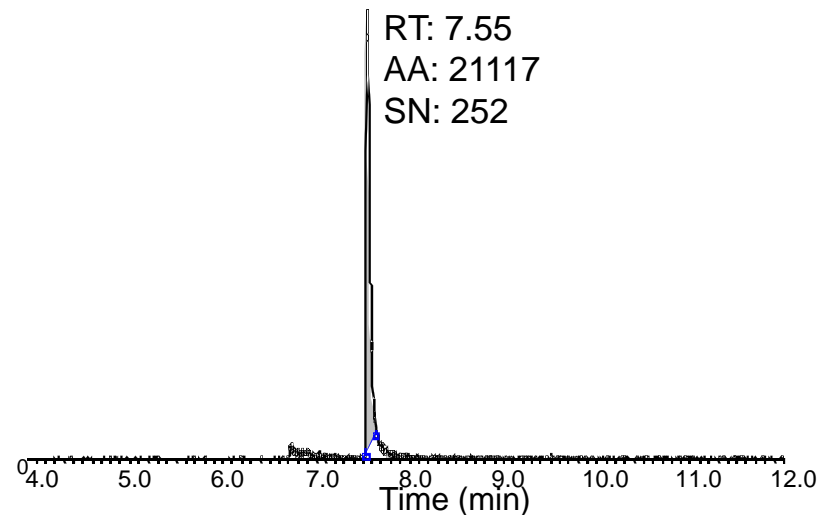
Example:  
Negative-ESI-FTICR analysis to prove remodeling of phosphatidylglycerol in *Synechocystis* PCC6803

# Additional Instrument Also Available

- GC–MS and GC–MS/MS can be used for separation of complex mixtures of small, relatively non-polar molecules



HNE analysis by GC coupled with chemical ionization (CI) mass spectrometry and selected-ion monitoring



PolarisQ (Thermo; 3D ion trap in its “heart”)



# Acknowledgments

- Research faculty (Stan Stevens, Katalin Prokai-Tatrai)
- Students (Navin Rauniyar, Jia Guo, Tatjana Talamantes, Lokesh Nagaprashantha, ...)
- Postdoctoral associates (Petr Frycak, Rui Branca, Balazs Blazics, Bettina Ughy, Attila Kiss, Xiaoquian Liu)
- Collaborators
- Funding (DoD, NIH, Welch Foundation, UNTHSC)



<http://www.hsc.unt.edu/prokai/mslab/>

<b>Service</b>	<b>UNTHSC</b>	<b>Other Universities</b>	<b>Industry</b>
<i>ESI-MS, LC/MS and MS/MS Analysis</i>	\$140/hour	\$180/hour	\$212/hour + 25% IDC*
<i>GC/MS Analysis</i>	\$50	\$70	\$100 + 25% IDC*
<i>Consultation, Interpretation, Reporting and Data Analysis (Upon Request)</i>	\$100/hour	\$140/hour	\$180/hour
<i>Custom project</i>	Inquire	Inquire	Inquire
<i>Enzymatic digestions:</i>			
Sample prep-in-gel digestion	\$20/hour	\$25/hour	\$30/hour
Trypsin digestion - simple	\$25/hour	\$40/hour	\$60/hour