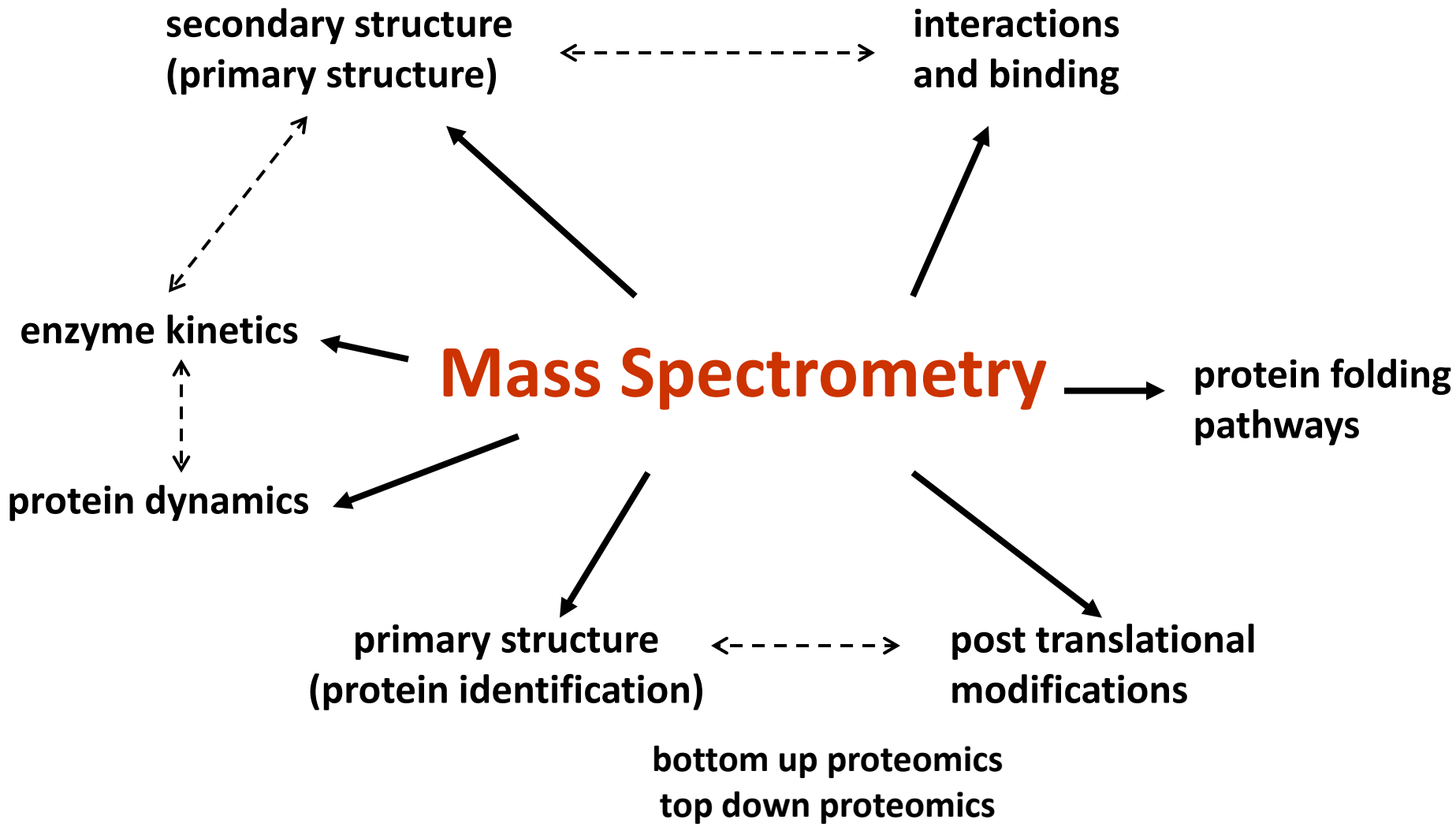
Mass Spectrometry Based Proteomics



Sample Preparation

Protein Informatics

Mass Spectrometry Based Proteomics

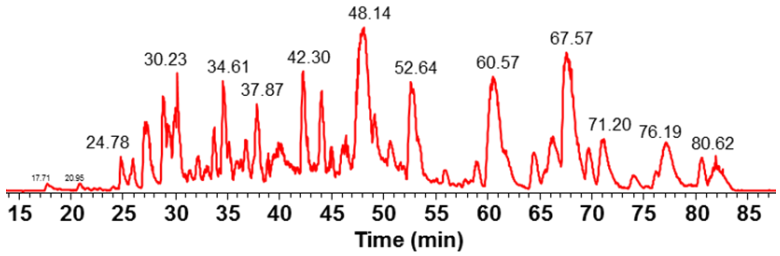
This course is concerned with……

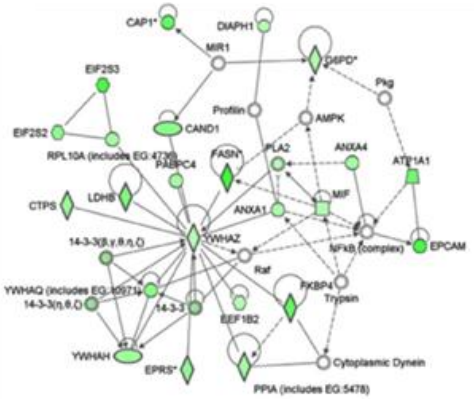
Protein primary structure sequencing

Post translational modifications

Bottom up and Top down proteomics

Individual Protein or *Complex Proteome* analysis for Identification and Characterization of *Overexpressed Proteins* or *Disease States*





Other Systems Biology Mass Spectrometry
The 'omics buzz'

Transcriptomics

Proteomics

Lipidomics

Glycomics

Metabolomics

Interactomics

Fluxomics (dynamic changes over time)

Mass Spectrometry Based Proteomics

I. Mass Spectrometers – The Basics

II. Biological Mass Spectrometry

III. Multidimensional Separations

IV. Post-translational Modification Analysis

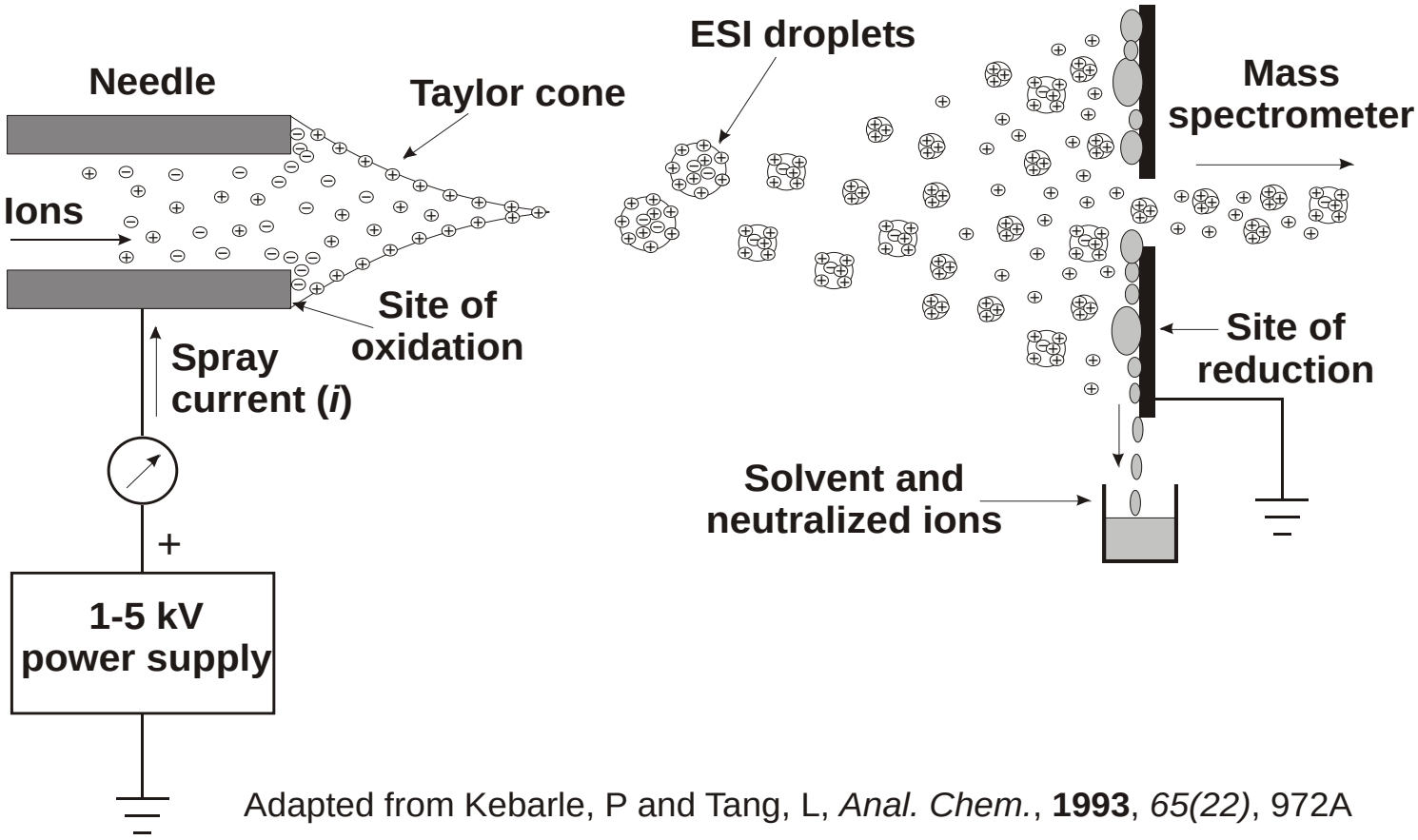
V. Protein Quantification

VI. Clinical Proteomics

Mass Spectrometry Measures *Mass-to-Charge*

m/z ***m/z*** ***m/z***

Electrospray Ionization (ESI) Process
(Positive Mode)

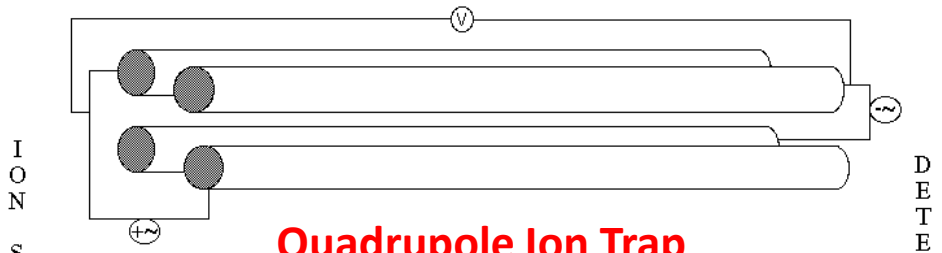


Adapted from Kebarle, P and Tang, L, *Anal. Chem.*, **1993**, *65(22)*, 972A

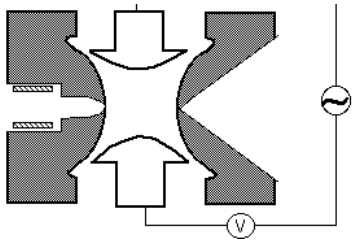
I. Mass Spectrometers – Types

Ion Traps and RF Mass Filters

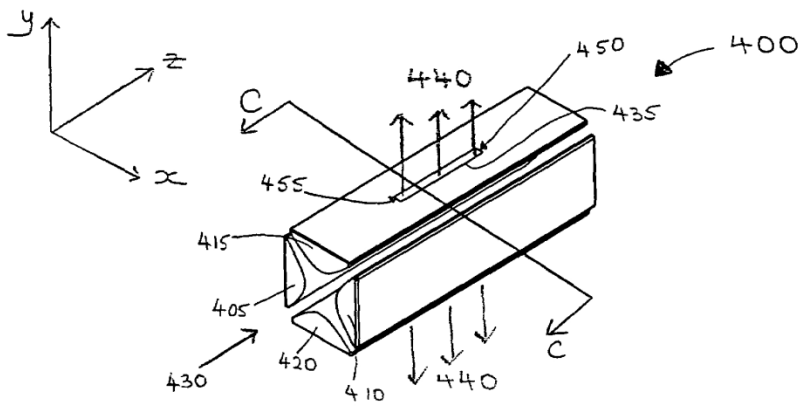
Quadrupole Mass Filter



Quadrupole Ion Trap



Linear Quadrupole Ion Trap



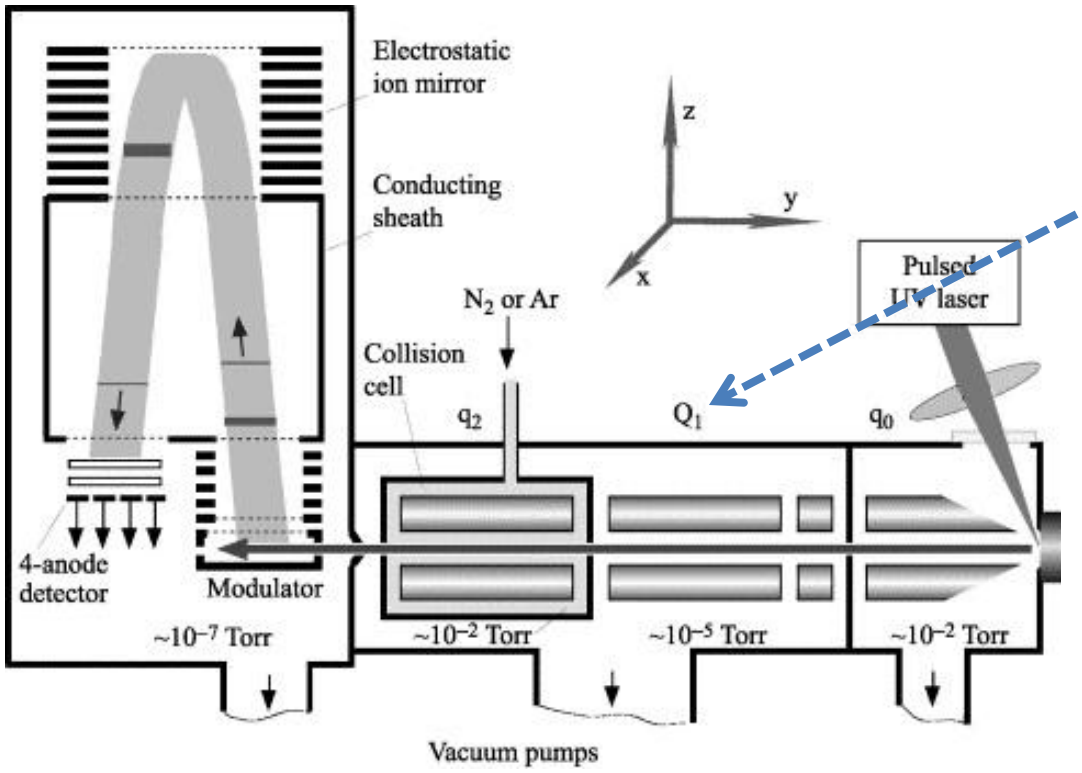
Adapted From → http://www.abrf.org/ABRFNews/1996/September1996/sep96iontrap.html

http://www.freepatentsonline.com/7180057.html

Ions of a certain *m/z range* may be trapped with certain applied voltages with *radio frequency* (RF) devices, i.e. *electric fields*.

I. Mass Spectrometers – Types

Time-of-Flight (TOF) Mass Spectrometers



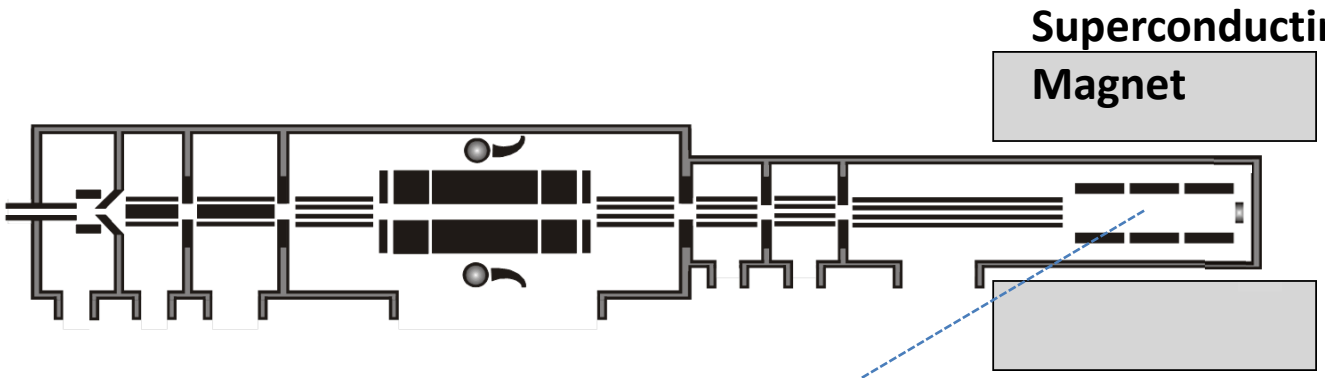
Note: Quadrupole Mass Filters are added in-line to enhance the performance of modern MS instruments. These are called "hybrid instruments".

TOF instruments separate different *m/z ions* based on the Flight Time though a field free region after normalized acceleration (i.e. kinetic energy)

Hybrid Quadrupole/Time-of-Flight Mass Spectrometers for Analysis of Biomolecules
Werner Ens; Kenneth G. Standing, Methods in Enzymology, Volume 402, 2005,
Pages 49–78 Biological Mass Spectrometry

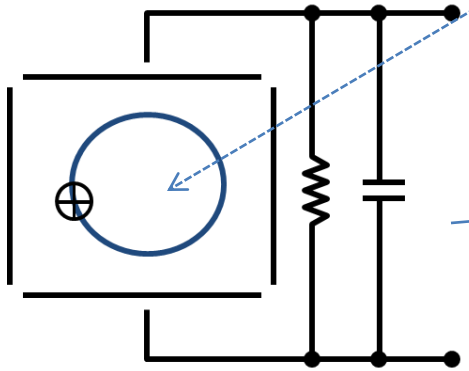
I. Mass Spectrometers – Types

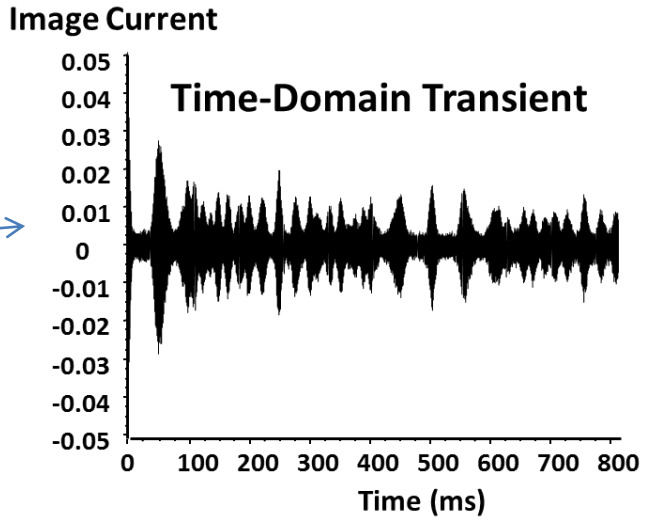
Fourier Transform (FT) Mass Spectrometers



Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

FTICR MS



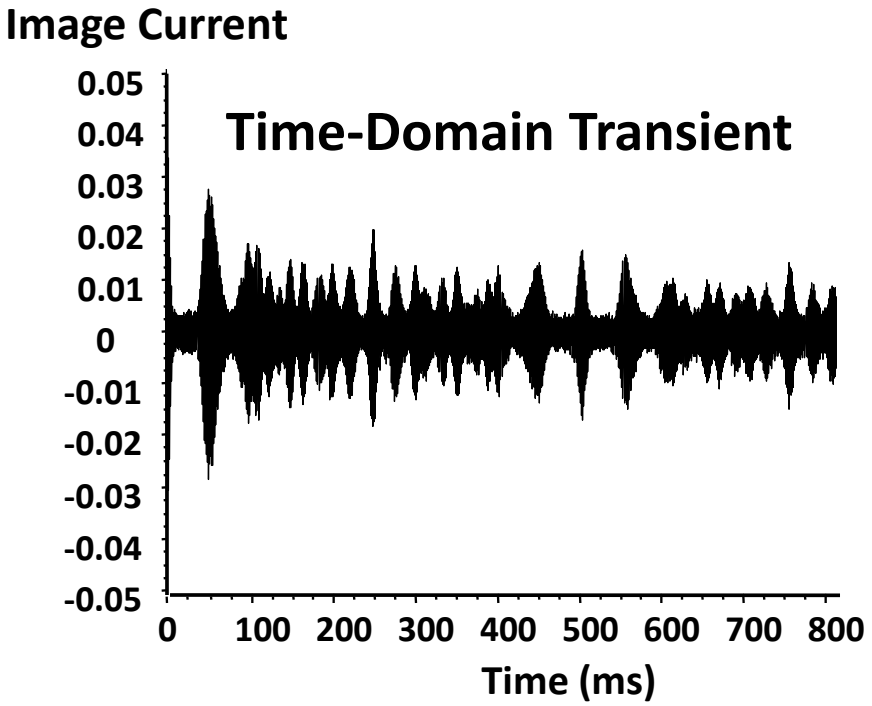


Ions go into "cyclotron frequency" once trapped in a fixed magnetic field. Each ion *m/z* has it's own "cyclotron frequency".

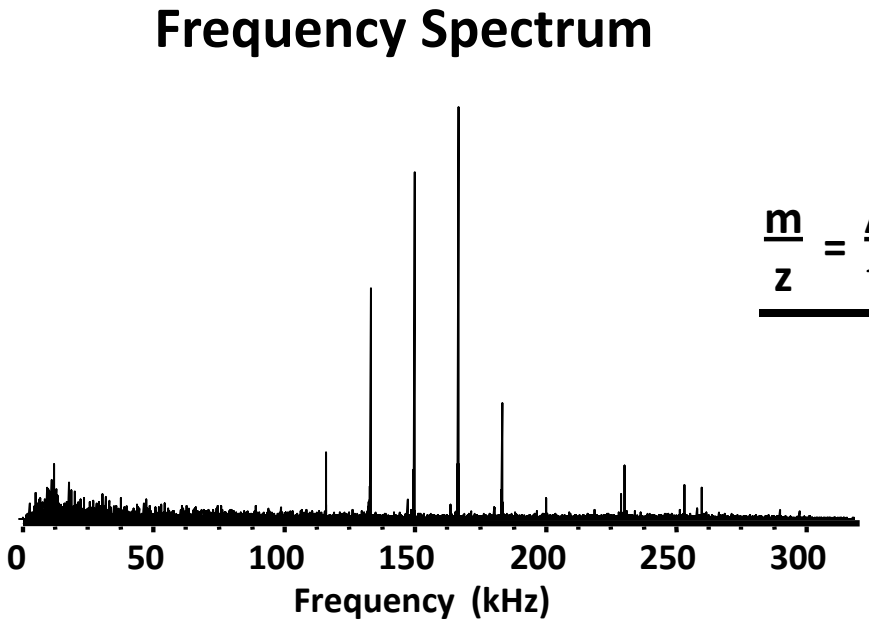
The Ions are "excited" to a higher orbit in the "ICR" cell. A second "detector plate" measures the "frequency" to which ions of a given *m/z* pass.

A Fourier Transform (FT) then converts the "time" domain signal into all the frequencies that compose the "time" signal

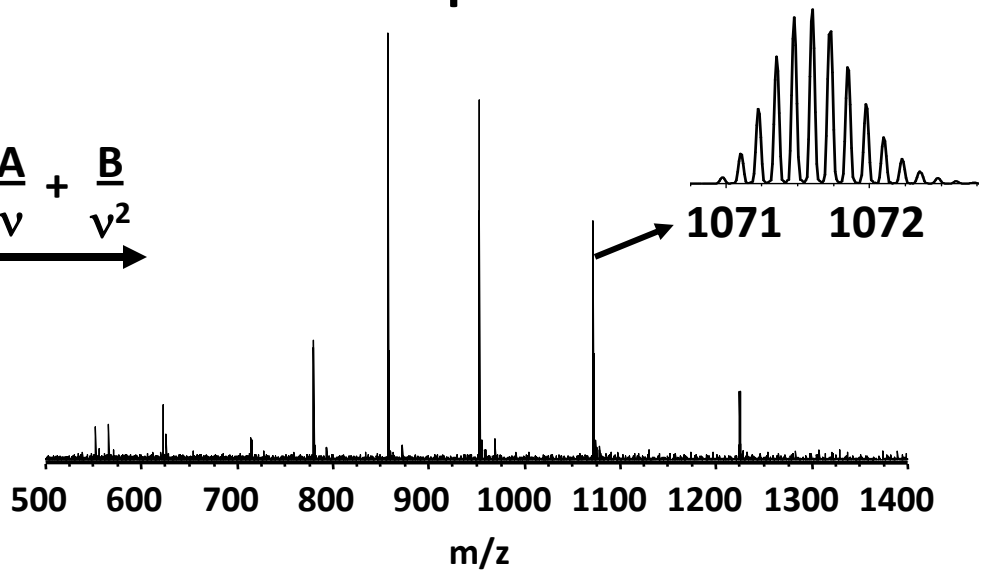
We know how frequency relates to mass, so we convert to the "Mass Spectrum"

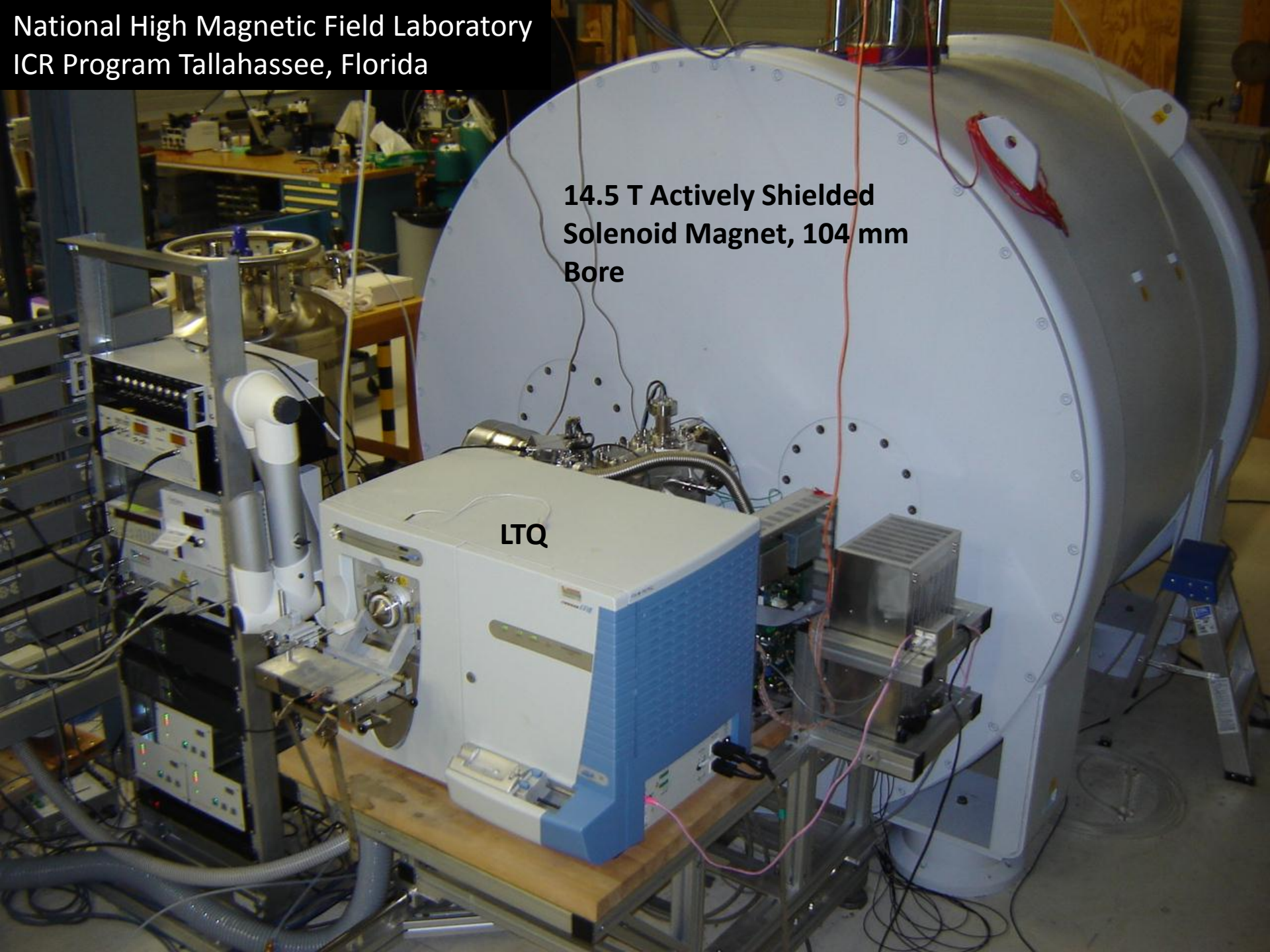


FT



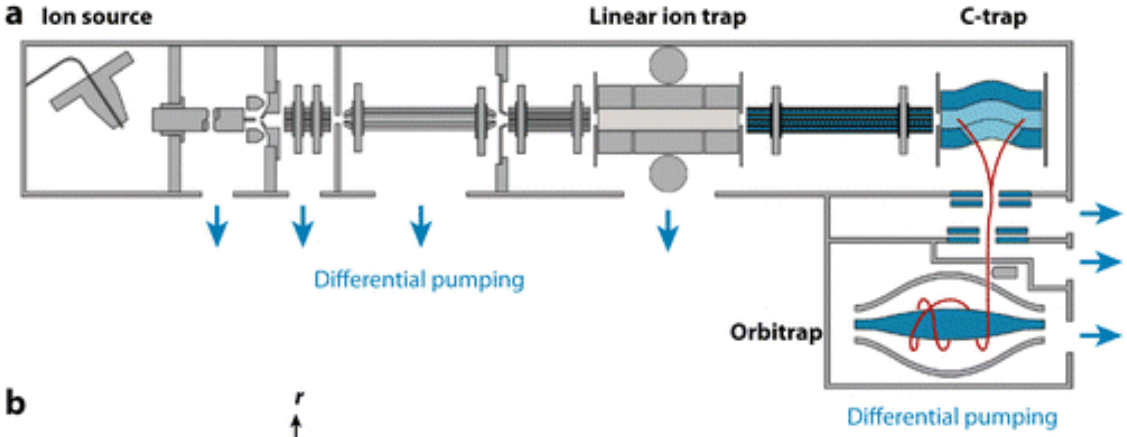
$$\frac{m}{z} = \frac{A}{\nu} + \frac{B}{\nu^2}$$



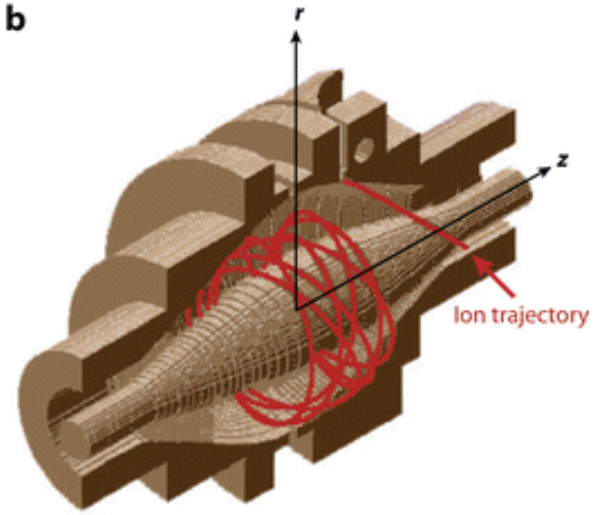

National High Magnetic Field Laboratory
ICR Program Tallahassee, Florida
14.5 T Actively Shielded Solenoid Magnet, 104 mm Bore
LTQ

I. Mass Spectrometers – Types

Fourier Transform (FT) Mass Spectrometers



LTQ-OrbiTrap



Yates JR, et al. 2009.
Annu. Rev. Biomed. Eng. 11:49–79

Operates with similar principles as FTICR MS; however without the expensive $ superconducting magnet $.

The ions are trapped in the "The Kingdon trap" *(aka.* OrbiTrap) after transfer from the C-trap. The ions then oscillate back and forth along the central spindle (aka. central electrode). Ions of different *m/z* have different frequencies of oscillation.

I. Mass Spectrometers – Types

Summary

Ion Traps (IT); Linear Ion Trap Quadruple (LTQ) and Quadruple [Mass Filters] (Q)
= measure stability of ions in an electric field

TOF = measuring the flight time or "kinetic energy" of ions

FT instruments (FT ICR and OrbiTrap) = measure "frequency" of ions moving past a detector within a magnetic or electric field.

(There are other types of mass spectrometers; however, not mentioned)

Information Content – Resolving Power

Lower Resolution

Improved Resolution

I. Resolving Power and Mass Accuracy

The mass resolving power is the measure of the ability to distinguish two peaks of slightly different *m/z*. The mass accuracy is the ratio of the *m/z* measurement error to the true m/z. Mass accuracy is usually measured in ppm or milli mass units. The mass range is the range of *m/z* amenable to analysis by a given analyzer. The linear dynamic range is the range over which ion signal is linear with analyte concentration. Speed refers to the time frame of the experiment and ultimately is used to determine the number of spectra per unit time that can be generated.

Instrument	**Can be Accurate To**	**Typical Mass Error**
FT-ICR-MS	0.0001 Da	0.2 to 1.0 ppm
FT-Orbitrap	0.0010 Da	0.2 to 2.0 ppm
High-Res-TOF	0.0025 Da	0.5 to 10 ppm
Quadrupole/Ion Trap	0.5 to 1.0 Da	> 1000 ppm

Error calculation

$$\frac{(500.0001) - (500.0000)}{(500.0000)}$$

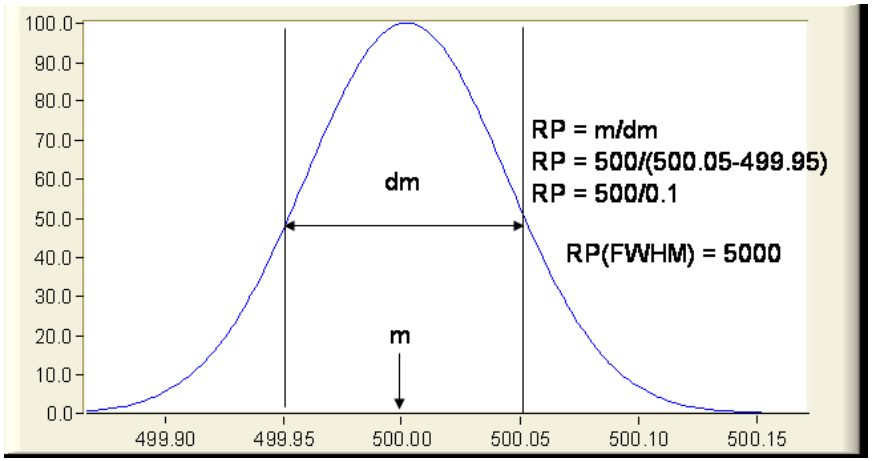
multiply by $x10^6$

= 0.2 ppm error

With *decreasing* mass error, there is a *increased* likelihood of identifying components correctly.

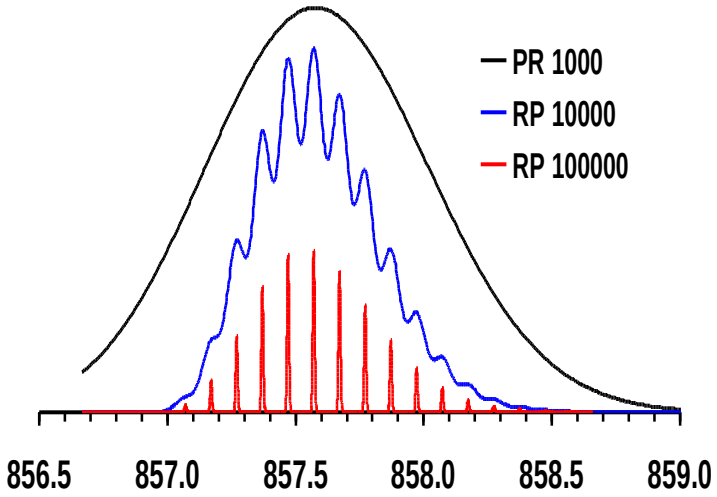
Chemical Formulas of Small Molecules can be predicted by accurate mass measurement (< 2 ppm mass error eliminates 90 - 95% of possible elemental formulas [and peptides])

I. Resolving Power and Mass Accuracy



Ubiquitin (10+ Charge State)

8560.62 (C_{378} H_{630} N_{105} O_{118} S_1)



"Routine Experiments"

Type	Resolving Power
FT-ICR-MS	1,000,000+
FT-Orbitrap	100,000
High-Res-TOF	60,000
Quadupole/Ion Trap	1,000

II. Biological Mass Spectrometry

Example Proteomics Work Flow

Proteomics is a Multistep Process

(1) Cell Lysis

(2) Protein Extraction

Focus of this course……

(3) Trypsin Digestion

(4) Multidimensional Separations (example SCX)

(5) Enrichment of PTM (Optional)

(6) nLC MS (and Tandem MS – aka. MSMS)

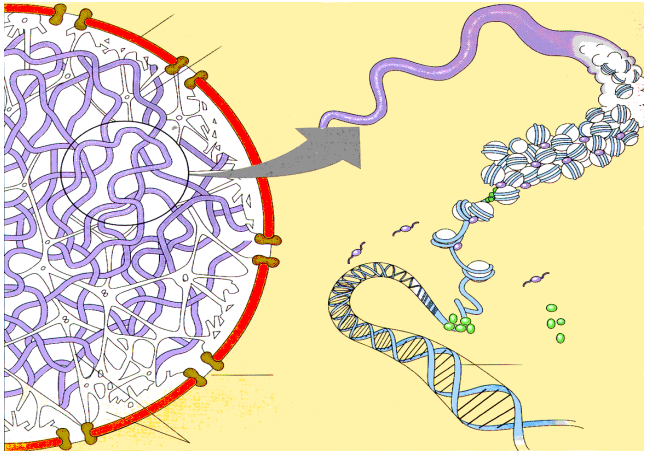
(7) Protein Informatics

(8) Pathway Analysis: Protein Regulation

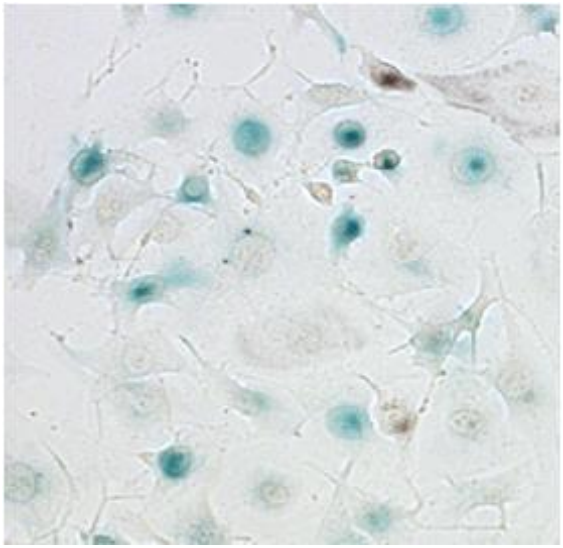
II. Biological Mass Spectrometry

Single "Complex" Proteins

Protein Families/Complexes



Whole Cell / Tissue



Characterization-Mode (off-line, targeted)

Throughput-Mode (on-line, LC-MS)

Biologics

Overexpression

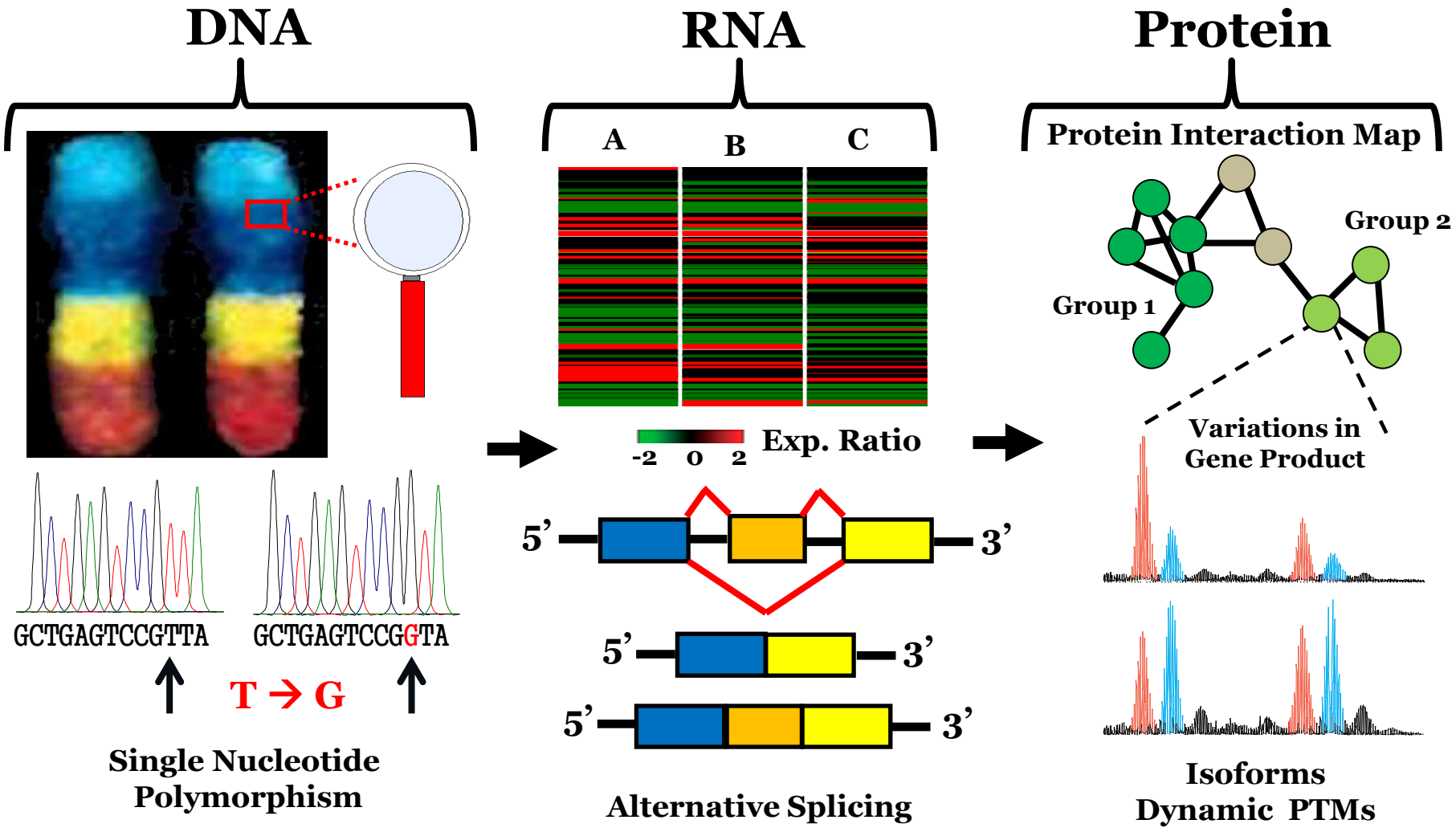
Immunoprecipitation

Protein-Protein Interactions

Organelle Analysis

Global Proteomics

II. Biological Mass Spectrometry

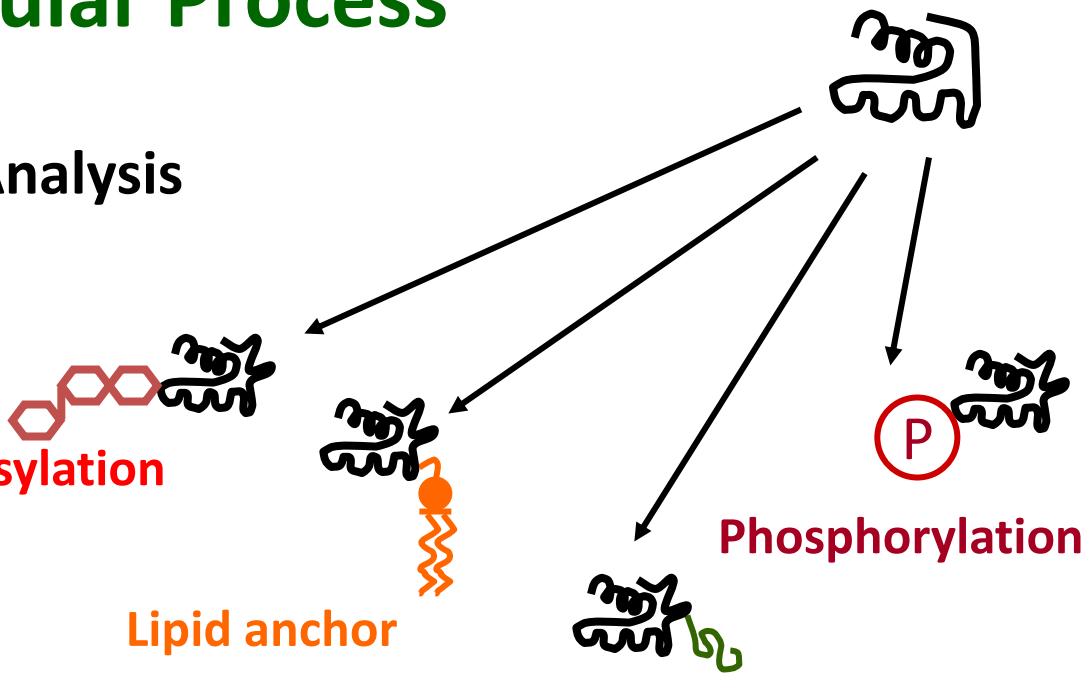


Tipton, J.D.; Tran, J.C.; Catherman, A.D.; Ahlf, D.R.; Durbin, K.R.; Kelleher, N.L. "Analysis of Intact Protein Isoforms by Mass Spectrometry" Journal of Biological Chemistry, 286 (29), 25451-25458, (2011)

Regulation of Cellular Process

High-throughput Protein Analysis

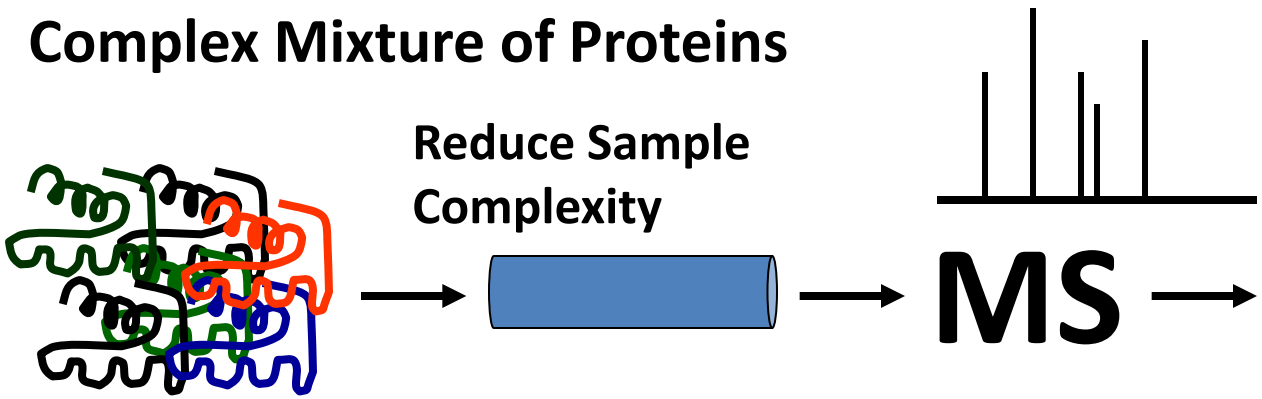
Post-translational modifications



- Splice Variants
- Isoforms

Informatics

Complex Mixture of Proteins



Bottom up and Top down Protein Analysis

Bottom-up

"Shotgun Proteomics"
Solution Phase
Trypsin Digestion

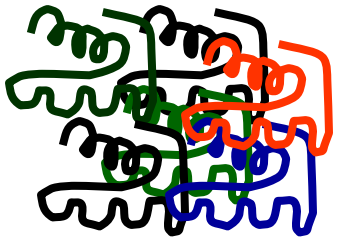
SCX, WAX
SPE, RP, NP, etc.

Gel Based
Proteomics

In-Gel Trypsin
Digestion

Reversed Phase LC MS/MS

Protein "Inference" from
Identified peptides



Top-down

Solution Phase Separation of Intact Proteins

Off-line Fractionation
GelFREE, sIEF
WAX, SCX, etc.
SPE, RP, etc.

MALDI or Desorption
Direct Infusion ESI
On-line RP ESI

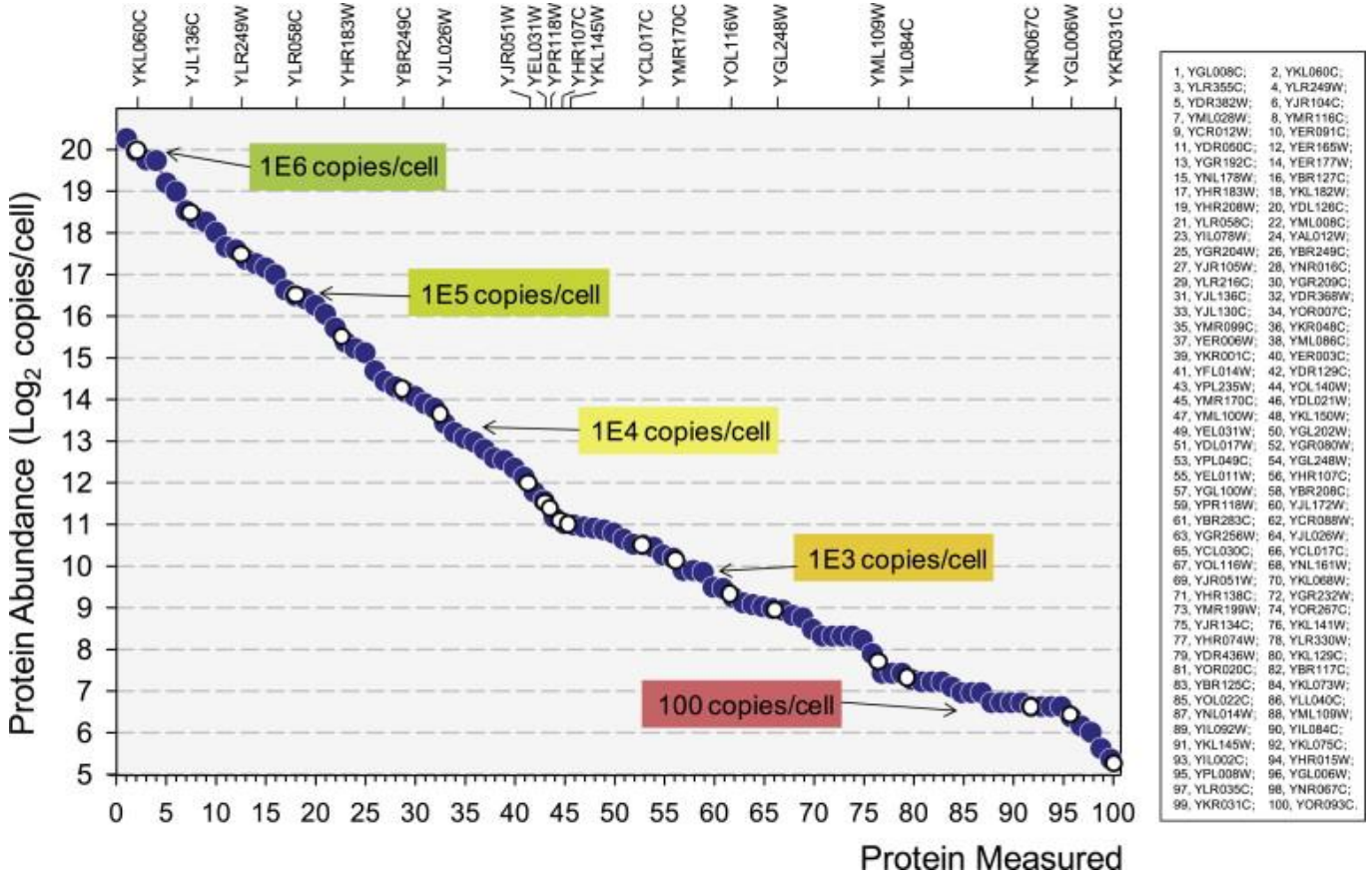
Fragmentation of intact protein in instrument

II. Biological Mass Spectrometry

Reduce Complexity of Sample

Enrichment of Cell Type, Organelle, Protein Complex
Enrichment of PTM (Protein or Peptide Level)
Orthogonal Separations, i.e. SCX – RP LC

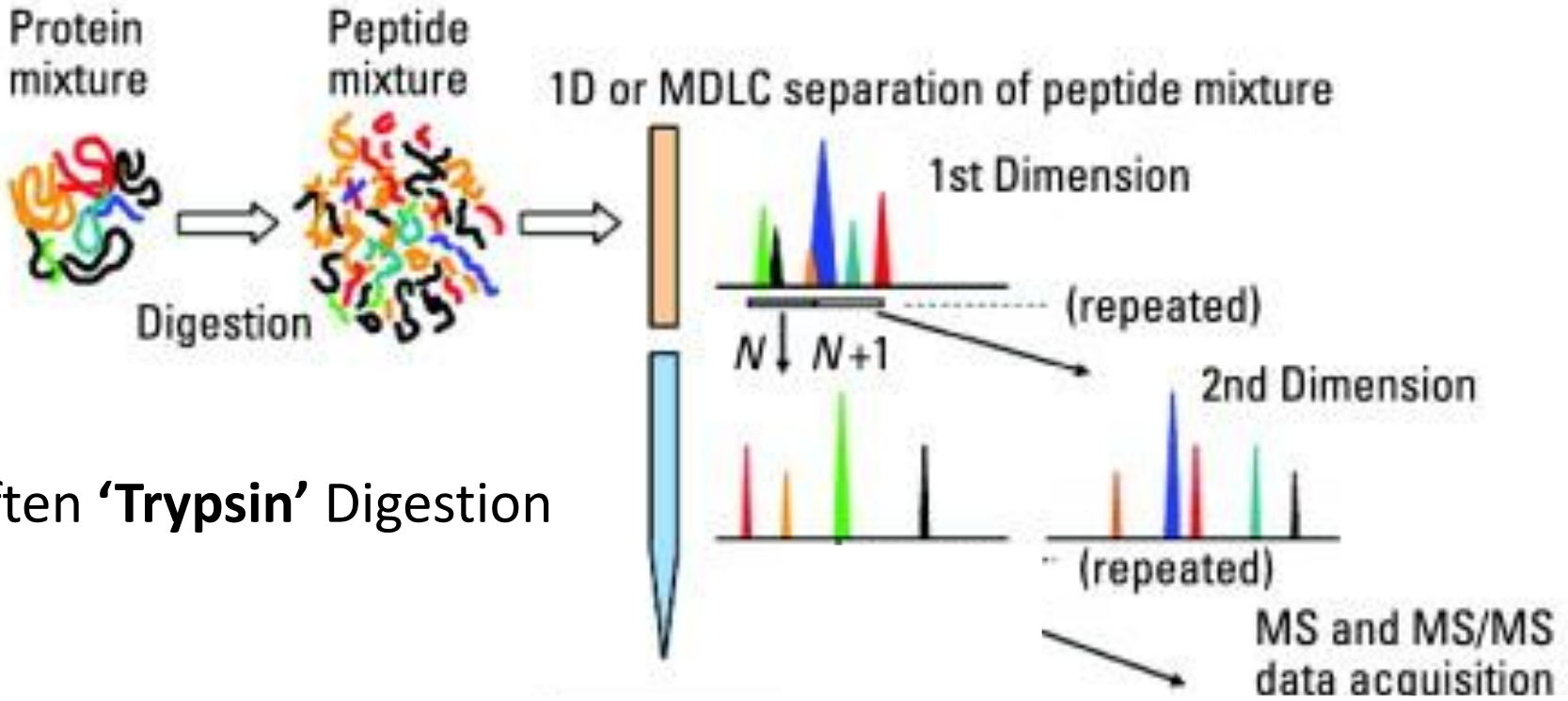
Concentrations of Proteins in Yeast – The Dynamic Range Problem



Cellular Concentrations of the Set of Measured Proteins Protein abundances are derived from Ghaemmaghami et al. (2003). Proteins detected by ***SRM assays*** are sorted by abundance to show the even distribution across the whole range of concentration (blue circles). Proteins for which the absolute abundance was measured ***using isotopically-labeled standards*** are indicated on top of the graph (open circles).

http://www.sciencedirect.com/science/article/pii/S0092867409007156

III. Multidimensional Separations



Often **'Trypsin'** Digestion

http://pubs.acs.org/cgi-bin/sample.cgi/ancham/2008/80/i19/html/ac8013669.html

(1) Strong Cation Exchange (SCX) – Reversed Phase Liquid Chromatography (RP LC)

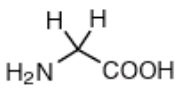
(2) Weak Anion Exchange (WAX) – RP LC

(3) hydrophilic interaction liquid chromatography (HILIC) – RP LC

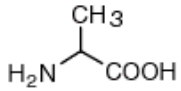
(4) ……..

III. Multidimensional Separations

Small

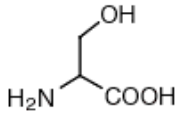


Glycine (Gly, G)
MW: 57.05

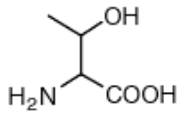


Alanine (Ala, A)
MW: 71.09

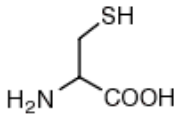
Nucleophilic



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

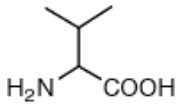


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

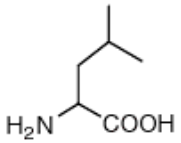


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

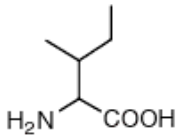
Hydrophobic



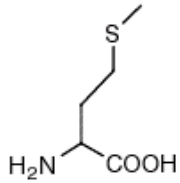
Valine (Val, V)
MW: 99.14



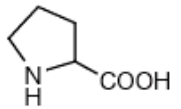
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

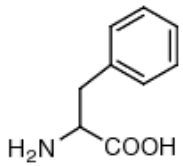


Methionine (Met, M)
MW: 131.19

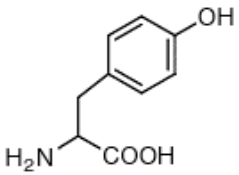


Proline (Pro, P)
MW: 97.12

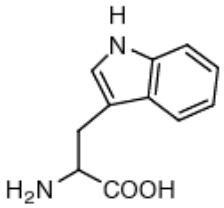
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

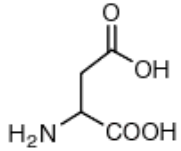


Tyrosine (Tyr, Y)
MW: 163.18

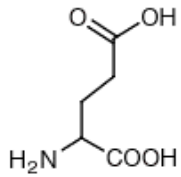


Tryptophan (Trp, W)
MW: 186.21

Acidic

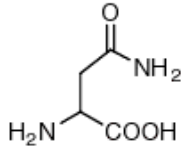


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

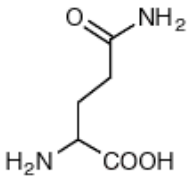


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

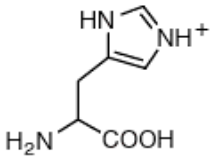


Asparagine (Asn, N)
MW: 114.11

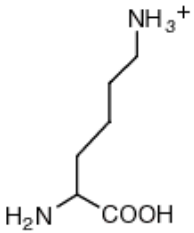


Glutamine (Gln, Q)
MW: 128.14

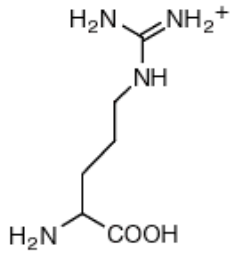
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

III. Multidimensional Separations

Trypsin Cleaves on the C-terminal Side of Lysine and Arginine
(Leaving a positively charged *amino acid side group*)

Different peptide combinations have different *physiological properties*.
(i.e. Tyrosine, tryptophan, etc. have more *hydrophobicity*)

TRYRPYTR

TEQSFTQR

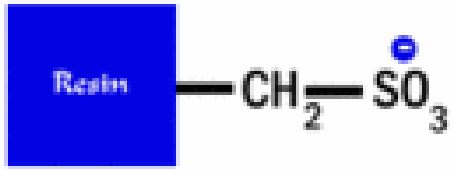
SGILATQR

ALANMARSHALNHMFLMS

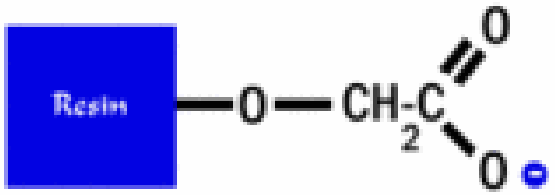
(See Previous Page For Structures of Amino Acids)

(Please Draw the Above Peptides For Homework)

III. Multidimensional Separations

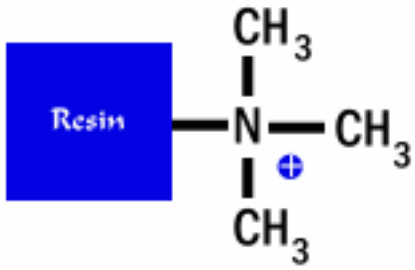


S-cation exchanger

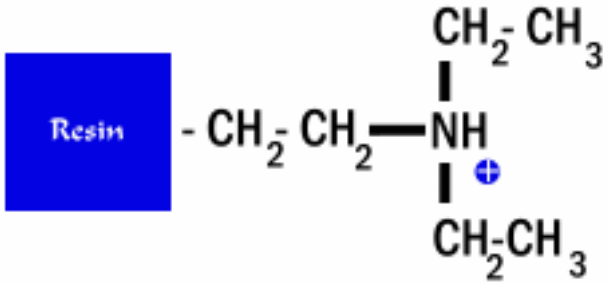


CM-cation exchanger

The first dimension is often 'ion exchange'



Q-anion exchanger



DEAE-anion exchanger

Peptides with different numbers of 'charges' will be retained under different buffer conditions. (often, increase a 'salt' concentration to obtain separations)

III. Multidimensional Separations

Ion Exchange

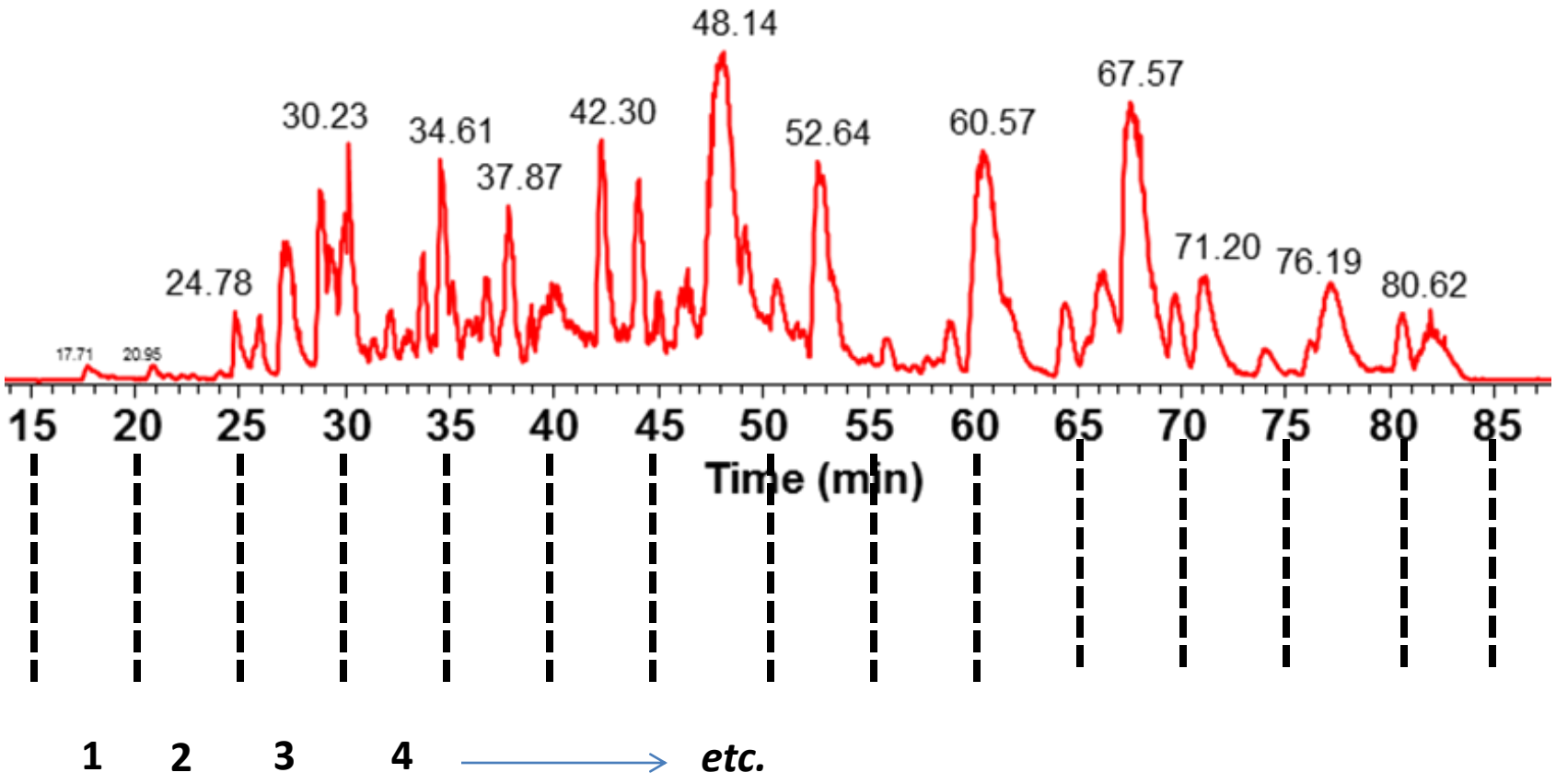
Sample amounts vary from 5 μg to 50 mg +

Pipette tips can be used, however pipette tip sample clean up is associated with *Reversed phase* Clean up

Fractionation by Column

III. Multidimensional Separations

SCX – Collecting Fractions to Reduce Complexity



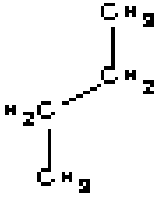
Collect each fraction → Analyze Fractions with nanoRP-LC

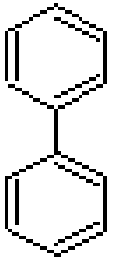
III. Multidimensional Separations

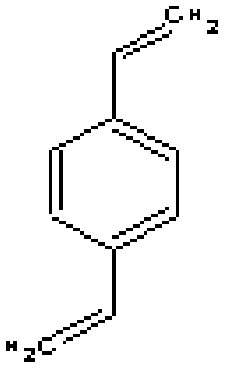
<u>Reversed Phase</u> (RP) Chromatography interacts with hydrophobic amino acid side chains.

C-18 is most common reverse phase chemistry

Common reversed phase structures



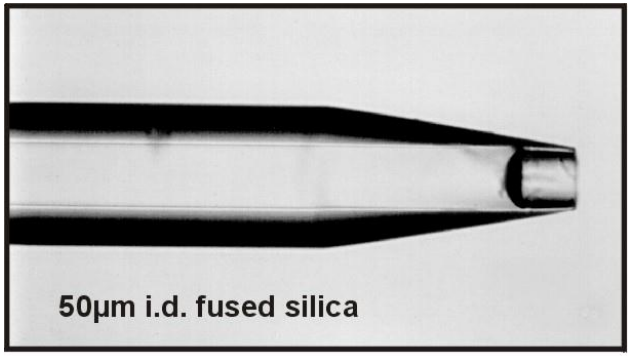


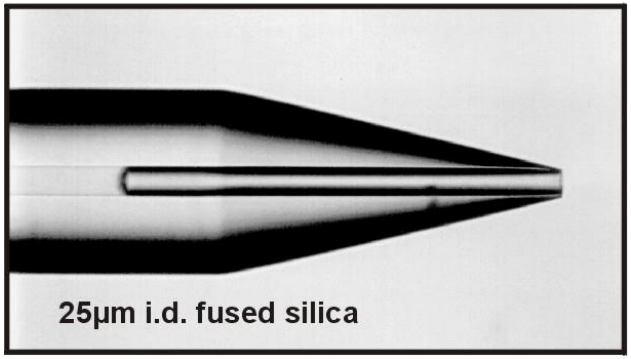


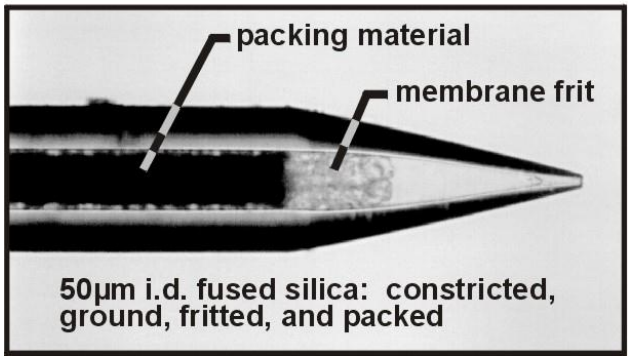
C-18 C-4 diphenyl divinylbenzene (DVB) cyano

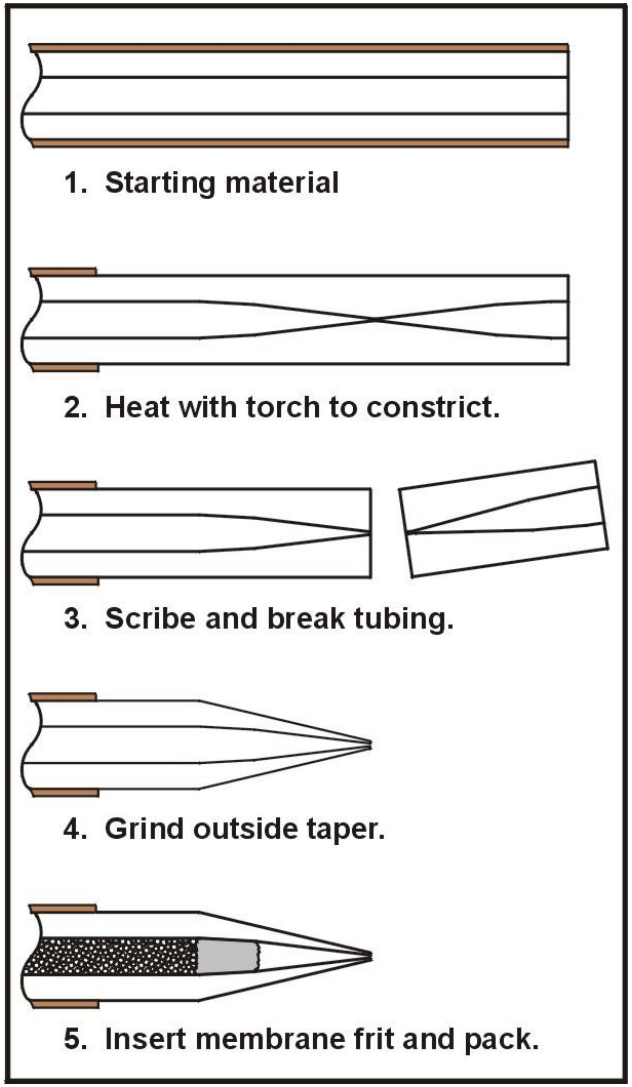
http://mach7.bluehill.com/proteinc/tutorial/rpc.html

Nano Liquid Chromatography and ESI Tips









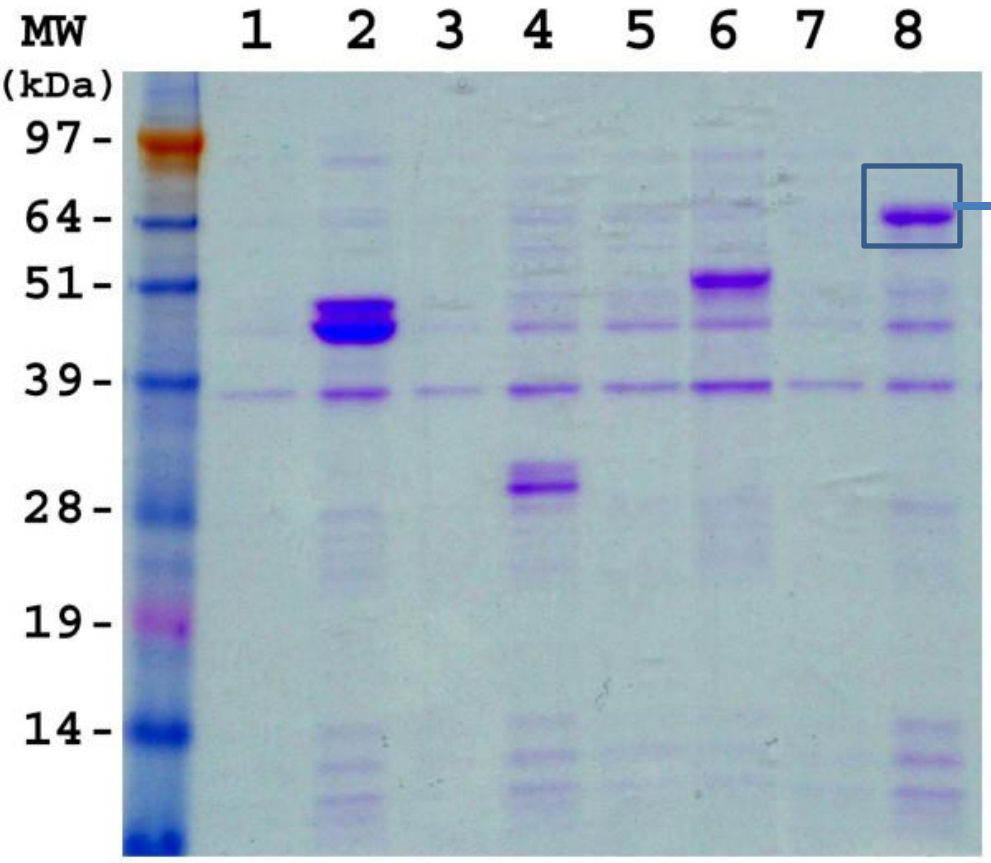
Capillary is Packed with <u>Reversed Phase</u> Material – nRP-LC

Slide: John Quinn

III. Multidimensional Separations

SDS-PAGE – In-Gel Trypsin Digestion

First Dimension of Separation is SDS-Page
(Separation by protein molecular weight)

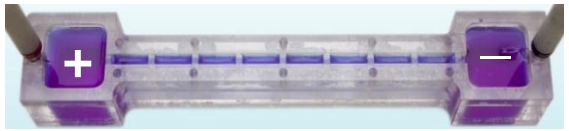


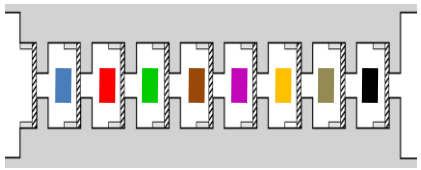
1. Coomassie Blue Stain
2. Excise Gel Band
3. De-stain
4. Reduce and Alkylate
5. Trypsin Digestion
6. Extraction of Peptides
7. Vacuum to Dryness
8. Re-suspend in MS Buffer
9. Nano RP-LC MS

III. Multidimensional Separations

3D Separations

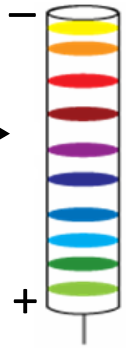
(1) Solution Isoelectric Focusing
(2) Gel-eluted Liquid Fraction Entrapment Electrophoresis (GELFrEE)
(3) nano RP LC MS

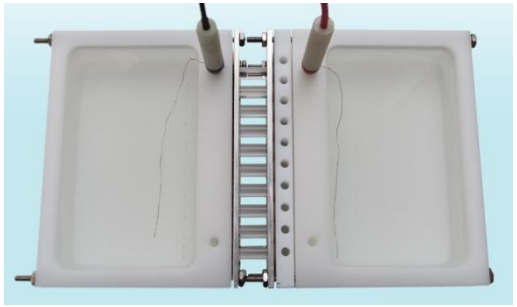




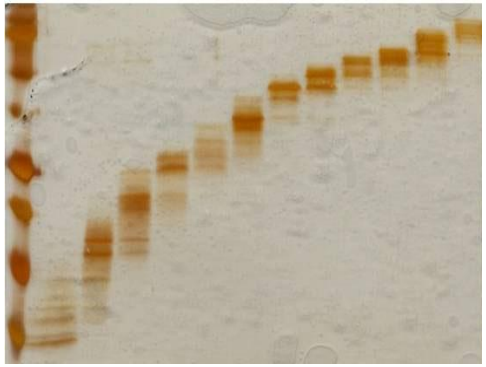
Cell lysate

Isoelectric focusing





GELFrEE – MW based separation



Proteins are retained in <u>Solution</u> (as opposed to 'fixed' in gel). Proteins elute though SDS-PAGE tube gels. A small aliquot of each timed fraction is analyzed by SDS-PAGE-Silver Stain

III. Multidimensional Separations

Quick Summary

Peptides with different amino acid combinations have different properties *(Homework. Draw Peptide Structures)*

The 'charge' and the 'hydrophobic' properties of the peptides allow for multidimensional separations – along with other chemical properties.

Three Basic Multi dimensional Separation Schemes:

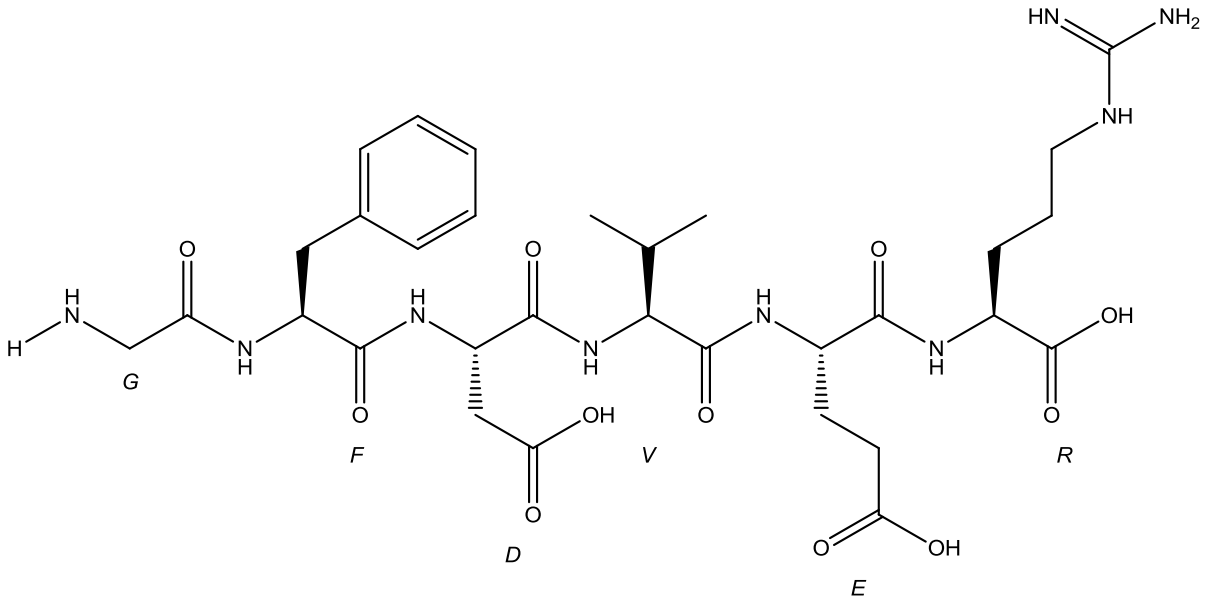
Ion Exchange + RPLC + MS

SDS-PAGE + RPLC + MS

Solution IEF + GELFrEE + RPLC + MS

IV. Post-translational Modification Analysis

What are we actually measuring with mass spectrometry?



$C_{31}H_{47}N_9O_{11}$
Exact Mass: 721.34

$[M + H^+]^+$
***m/z* = 722.34**

$[M + 2H^+]^{+2}$
***m/z* = 361.67**

Peptide: Amino Acid Letter Description

GFDVER

IV. Post-translational Modification Analysis

What are we actually measuring with mass spectrometry?

Amino acid	3-letter code	1-letter code	MW (Da)	Structure
Alanine	Ala	A	89.1	CH3-CH(NH2)-COOH
Arginine	Arg	R	174.2	H2N-C(NH2)-NH-(CH2)3-CH(NH2)-COOH
Asparagine	Asn	N	132.1	H2N-CO-CH2-CH(NH2)-COOH
Aspartic Acid	Asp	D	133.1	HOOC-CH2-CH(NH2)-COOH
Cysteine	Cys	C	121.2	HS-CH2-CH(NH2)-COOH
Glutamic Acid	Glu	E	147.1	HOOC-(CH2)2-CH(NH2)-COOH
Glutamine	Gln	Q	146.1	H2N-CO-(CH2)2-CH(NH2)-COOH
Glycine	Gly	G	75.1	NH2-CH2-COOH
Histidine	His	H	155.2	NH-CH=N-CH=C-CH2-CH(NH2)-COOH |_____|
Isoleucine	Ile	I	131.2	CH3-CH2-CH(CH3)-CH(NH2)-COOH
Leucine	Leu	L	131.2	(CH3)2-CH-CH2-CH(NH2)-COOH
Lysine	Lys	K	146.2	H2N-(CH2)4-CH(NH2)-COOH
Methionine	Met	M	149.2	CH3-S-(CH2)2-CH(NH2)-COOH
Phenylalanine	Phe	F	165.2	Ph-CH2-CH(NH2)-COOH
Proline	Pro	P	115.1	NH-(CH2)3-CH-COOH |_____|
Serine	Ser	S	105.1	HO-CH2-CH(NH2)-COOH
Threonine	Thr	T	119.1	CH3-CH(OH)-CH(NH2)-COOH
Tryptophan	Trp	W	204.2	Ph-NH-CH=C-CH2-CH(NH2)-COOH |_____|
Tyrosine	Tyr	Y	181.2	HO-p-Ph-CH2-CH(NH2)-COOH
Valine	Val	V	117.1	(CH3)2-CH-CH(NH2)-COOH

List of amino acids and masses

Individual masses used for Tandem MS Sequencing

Amino acid sequences of proteins may be predicted from Genome Database.

Individual masses used for Tandem MS Sequencing

http://www.geneinfinity.org/sp/sp_aaprops.html

IV. Post-translational Modification Analysis

What are we actually measuring with mass spectrometry?

Peptide Sequencing with Mass Spectrometry

1) **Measure the "Precursor" ion molecular weight** - ***See Slide 34***

2) **Select the "Precursor" ion for fragmentation**
   (Collisional Induced/Activated Dissociation – CAD or CID)
   (Electron Capture/Transfer Dissociation ECD or ETD)

   CAD/CID is the most common fragmentation method for proteomics and peptide sequencing.

3) **Measure the fragment ion mass to charge**

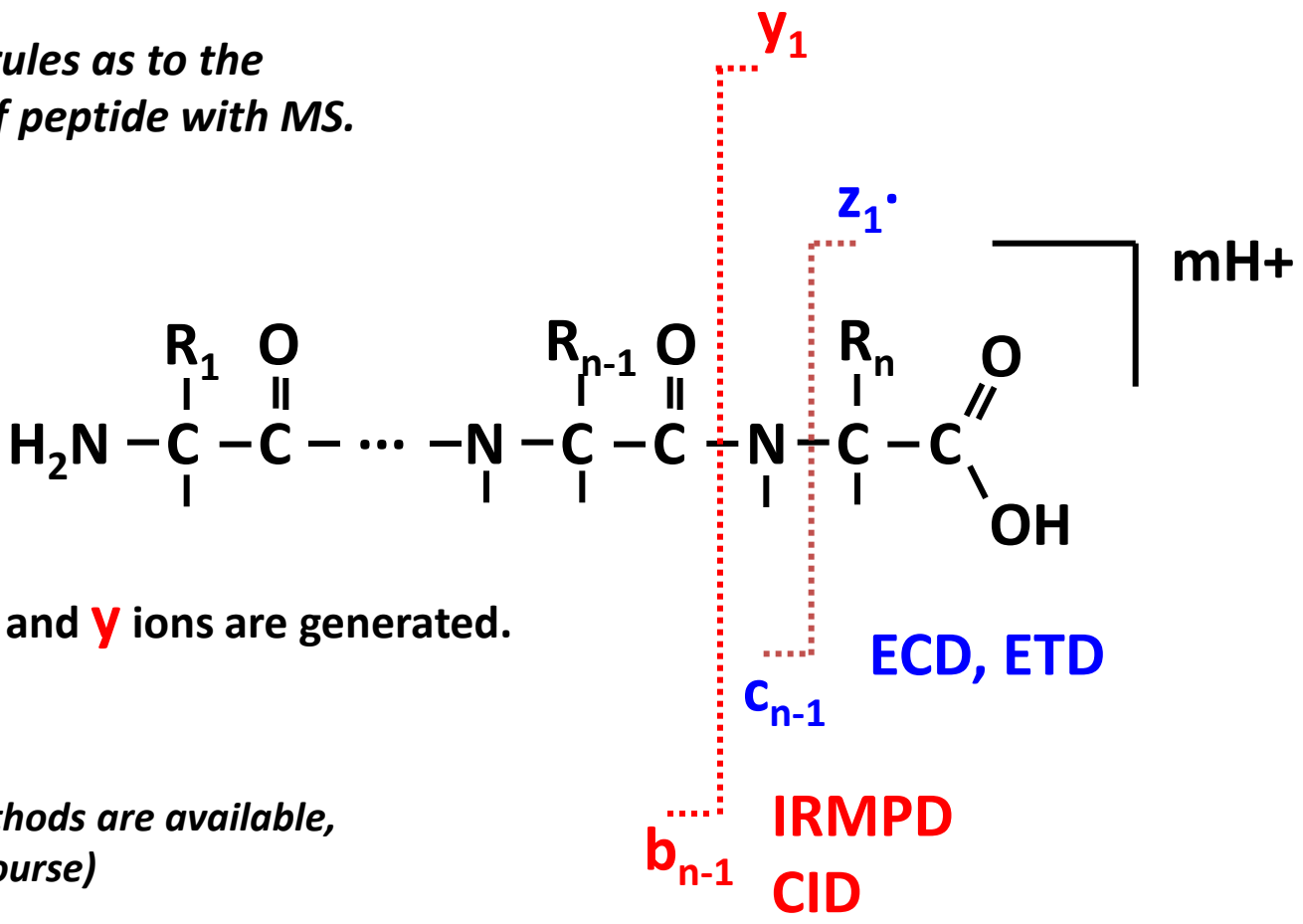
4) **Search measured precursor *m/z* and fragmentation pattern against protein sequence database.**

IV. Post-translational Modification Analysis

What are we actually measuring with mass spectrometry?

Gas Phase Fragmentation of Peptides/Proteins

There are some rules as to the fragmentation of peptide with MS.

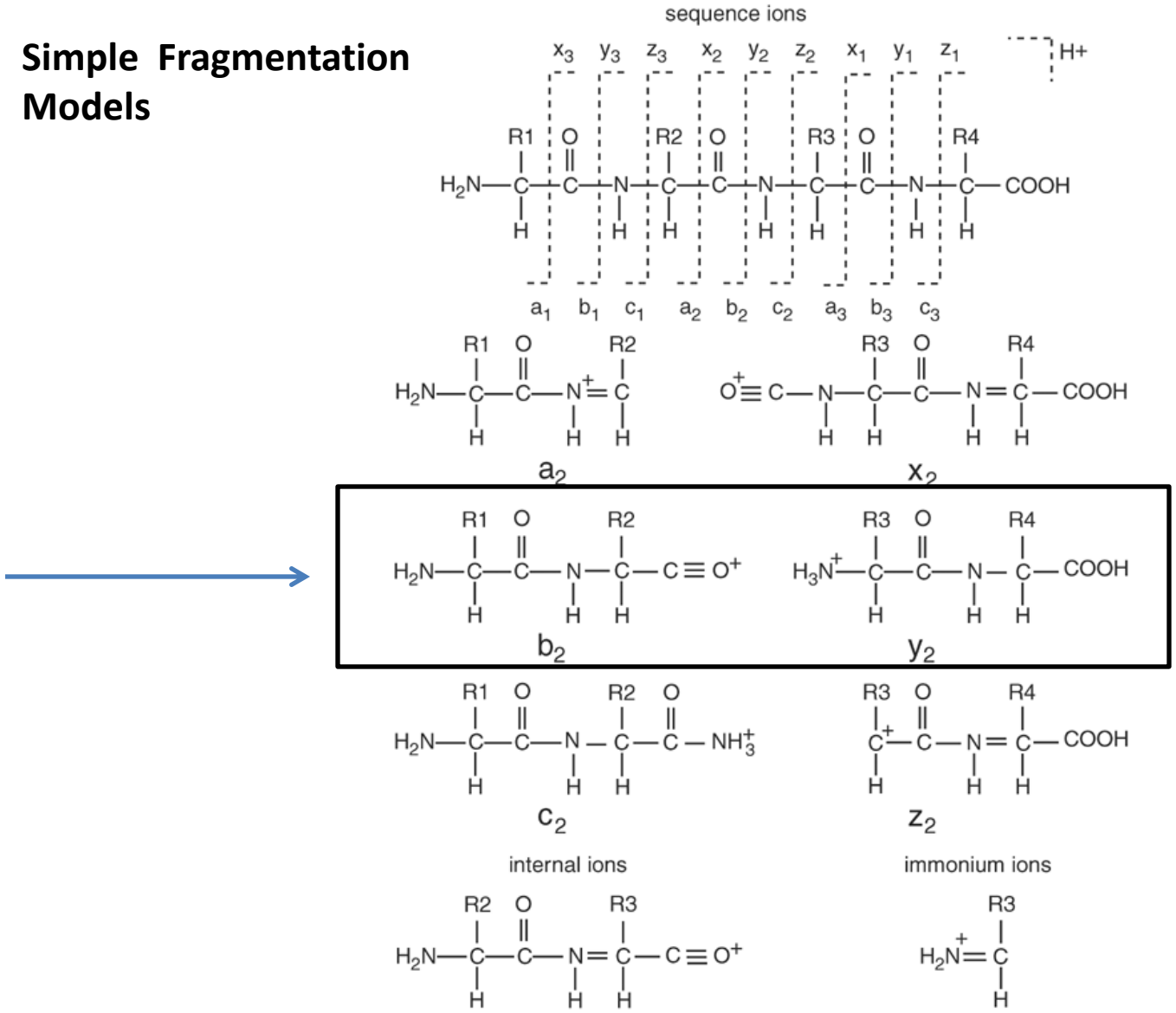


With CAD\CID, b and y ions are generated.

(Other fragmentation methods are available, but not described in the course)

IV. Post-translational Modification Analysis

Simple Fragmentation Models



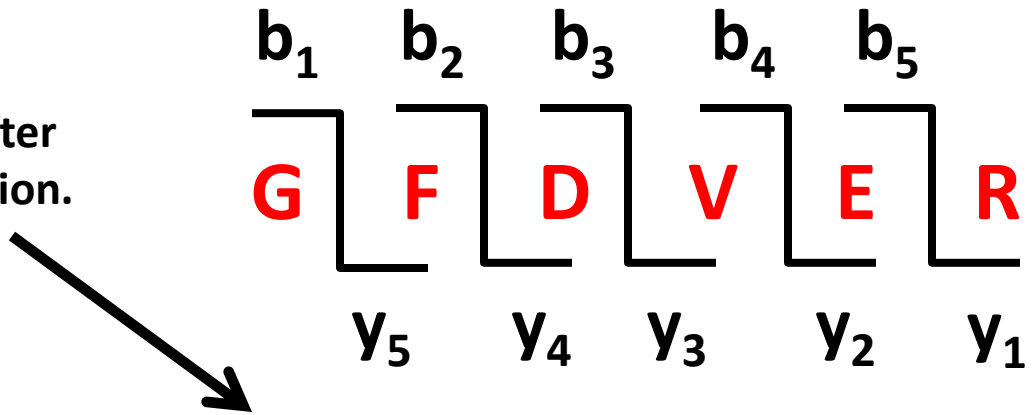
http://www.currentprotocols.com/WileyCDA/CPUnit/refId-ps1601.html

IV. Post-translational Modification Analysis

What are we actually measuring with mass spectrometry?

Example Peptide: GFDVER

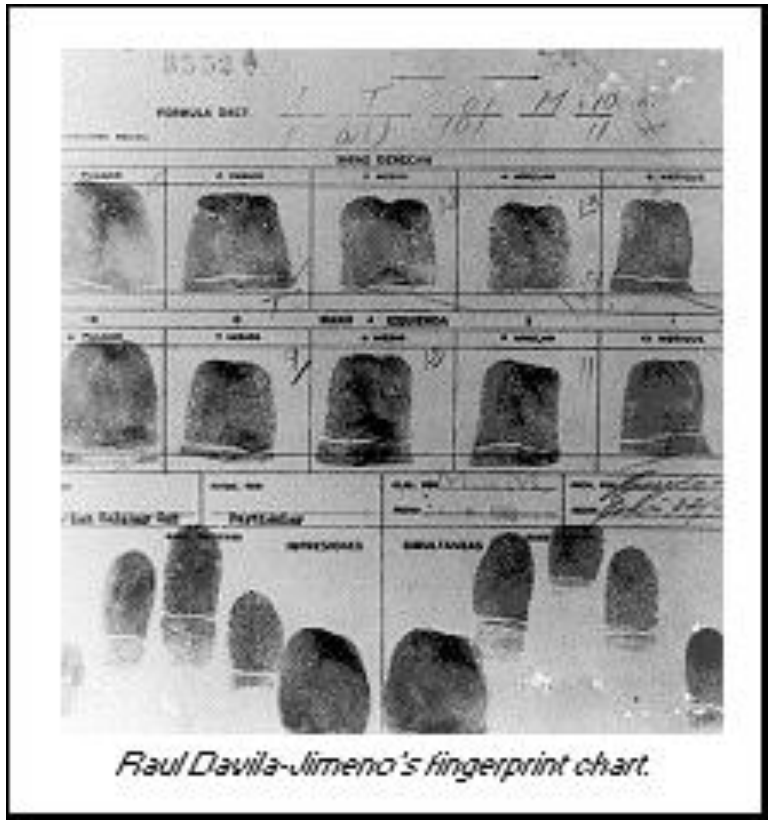
Measure after fragmentation.



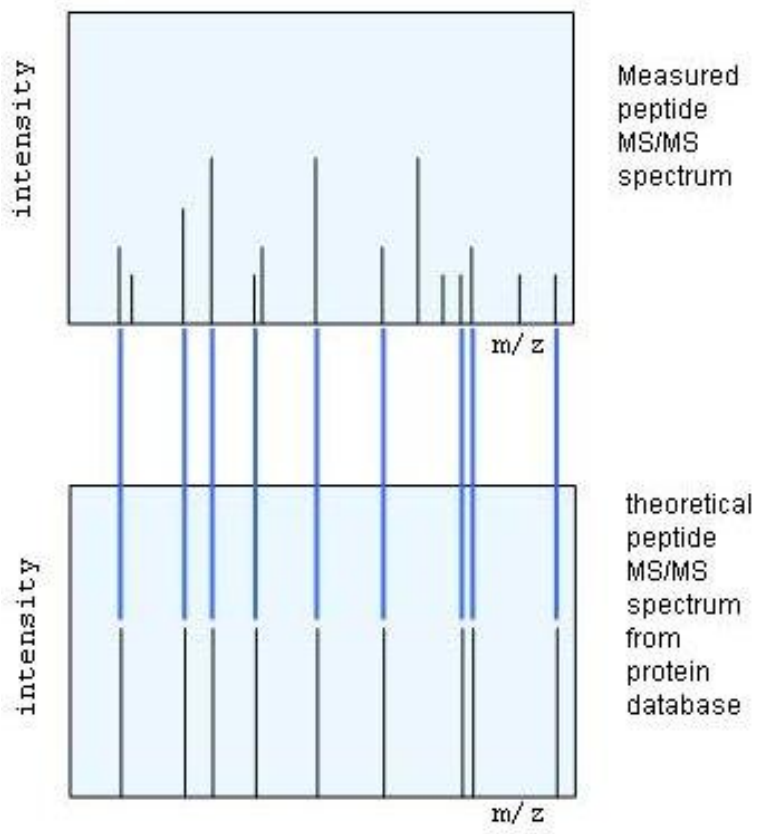
	b ion series		y ion series	
G	---	1	---	6
F	205.0972	2	665.3253	5
D	320.1241	3	518.2569	4
V	419.1925	4	403.23	3
E	548.2351	5	304.1615	2
R	---	6	175.119	1

IV. Post-translational Modification Analysis

Peptides Have a "Finger Print"
Which can be Matched to a Database


Raul Davila-Jimeno's fingerprint chart.

b and y ions
are matched



Protein identifications are inferred based on the likelihood of a peptide match to a database. (Informatics and probabilities…… another 2 week lecture)

http://www.proteomesoftware.com/Proteome_software_ed_protein_msms.html

IV. Post-translational Modification Analysis

What are we actually measuring with mass spectrometry?

Peptide = DYPVVSIEDPFDQDDWGAWQK

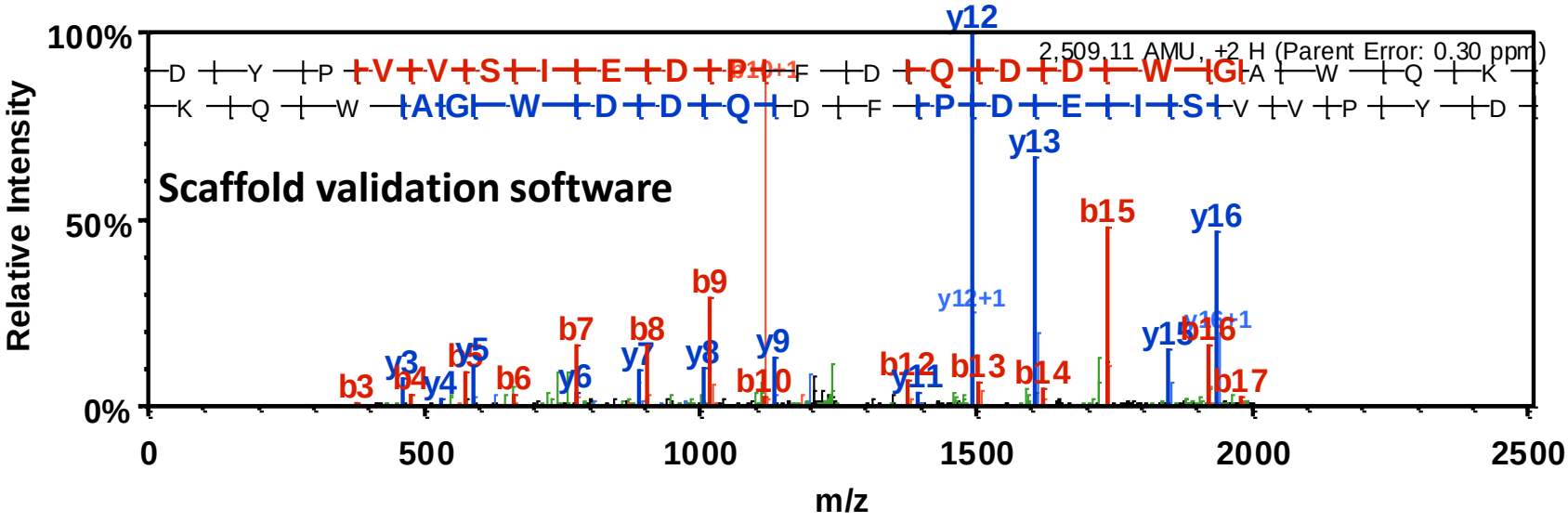
Molecular Weight = 2510.1147

$[M + H^+]^+ = 2511.1235$

Measure Precursor Ion

$[M + 2H^+]^{+2} = 1256.0650$

(Tandem Mass Spectrometry Match to Database MS/MS)



IV. Post-translational Modification Analysis

Back To Post-translational Modifications

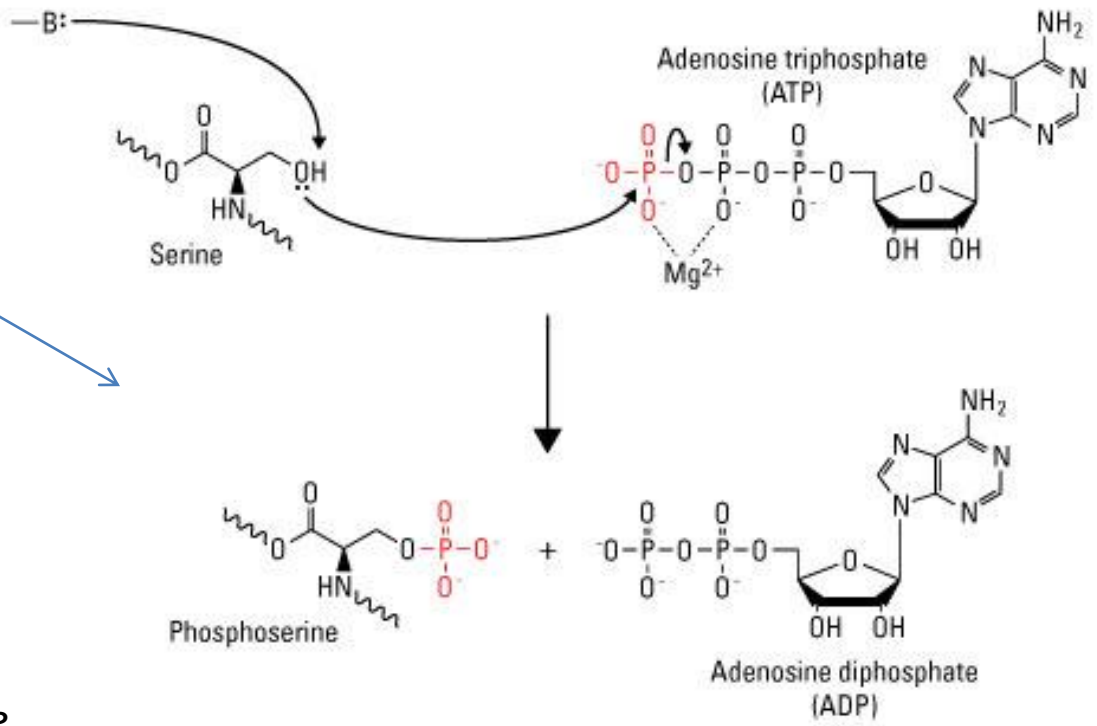
"The increase in complexity from the level of the genome to the proteome is further facilitated by protein post-translational modifications (PTMs). PTMs are chemical modifications that play a key role in functional proteomics, because they regulate activity, localization and interaction with other cellular molecules such as proteins, nucleic acids, lipids, and cofactors."

Phosphorylation is one of the most studied PTMs (by Mass Spectrometry)

More than 100 known PTMs

- Phosphorylation
- Glycosylation
- Ubiquitination
- S-Nitrosylation
- Methylation
- N-Acetylation
- Lipidation
- Proteolysis

etc. etc.



Adapted From Pierce Web Page

IV. Post-translational Modification Analysis

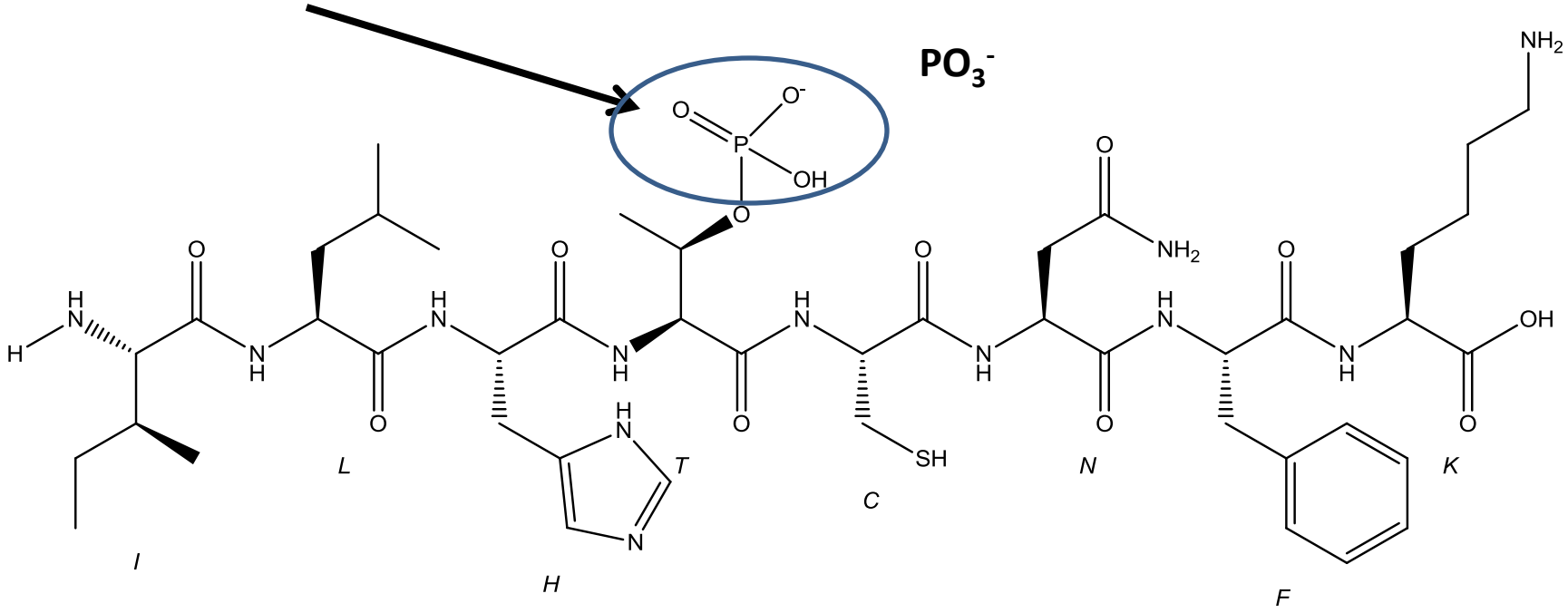
PTM type	Average MH+	Modified amino acid residue	Postion	Remarks	Reported in Pub Med (case)
Acetylation	42.04	S K	N-term anywhere	Reversible, protein stability, regulation of protein function	11,069
Phosphorylation	79.98	Y, S, T, H, D	anywhere	Reversible, regulation of protein activity, signaling	103,235
Cys oxidation			anywhere	Reversible, oxidative regulation of proteins	
disulfide bond	-2.0	C			23,538
glutathionylation	305.31	C			63
sulfenic acid	16.00	C			228
sulfinic acid	32.00	C			642
Acylation				Reversible, cellular localization to membrane	
farnesylation	204.36	C	anywhere		1,349
myristoylation	210.36	G K	N-term anywhere		644
palmitoylation	238.41	C (S, T, K)	anywhere		681
Glycosylation			anywhere	Reversible, cell-cell interaction and regulation of proteins	24,115
O-linked	>800	S, T			
(O-Glc-NAc)	203.20,				
N-linked	>800	N			
Deamidation	0. 98	N, Q	anywhere	N to D, Q to E	711
Methylation			anywhere	Regulation of gene expression, protein stability	29,889
monomethylation	14.03	K			
dimethylation	28.05	K			
trimethylation	42.08	K			
Nitration	45.0	Y		Oxidative damage	62
S-Nitrosylation	29.00	C			399
Ubiquitination		K	anywhere	Reversible/irreversible	1951
Sumoylation		K	[ILFV]K.D		104
Hydroxyproline	16.00	P		Proein stability	11,424
Pyroglutamic acid	-17	Q	N-term		710

http://bmbreports.org/jbmb/jbmb_files/%5B37-1%5D0401271834_035-044.pdf

IV. Post-translational Modification Analysis

Post-translational Modifications: Phosphorylation Example

Increase peptide molecular weight by 80 Da.



The addition of post-translational modifications increases the molecular weight of the peptide.

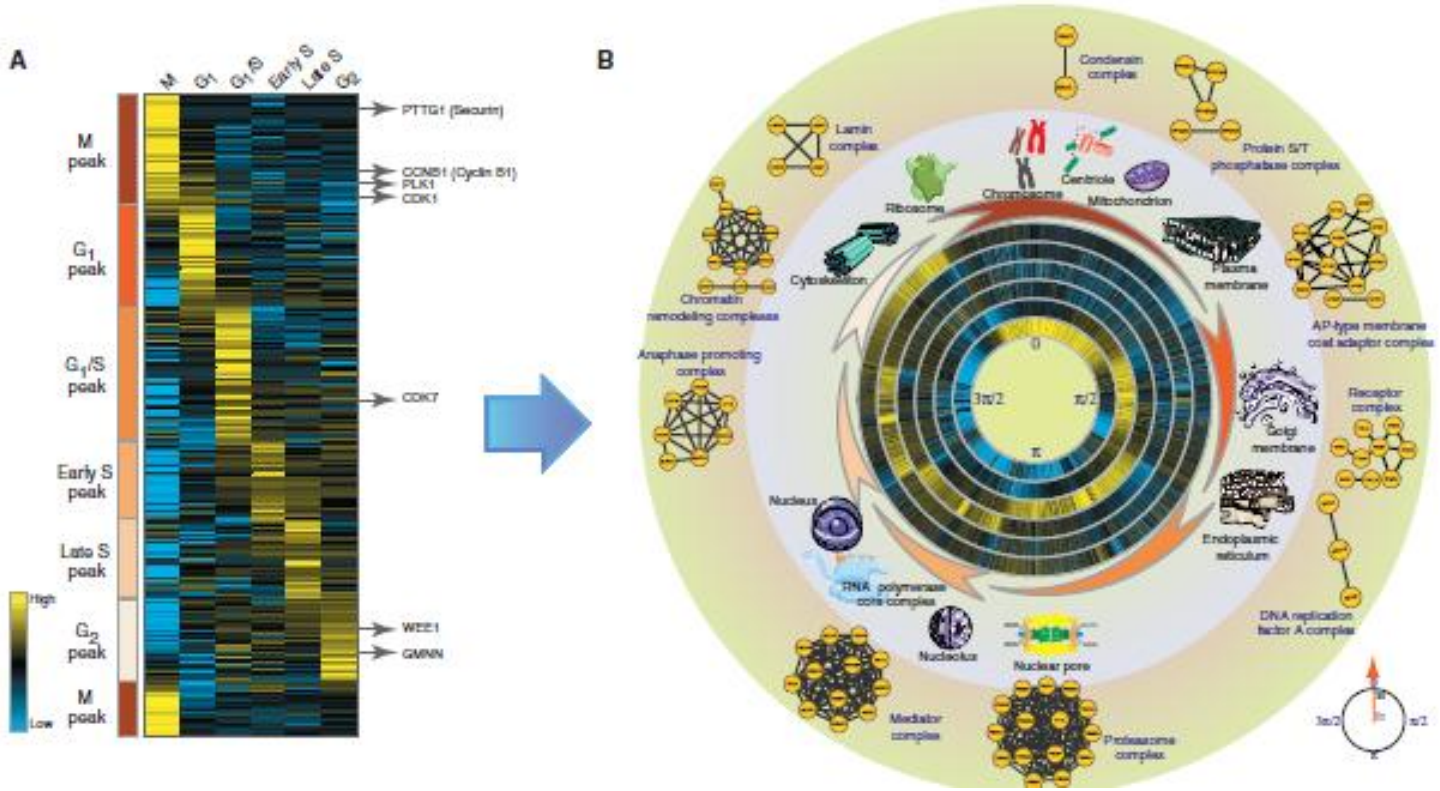
Tandem Mass Spectrometry (MS/MS) fragmentation allows for site characterization.

The amount of proteins (peptides) that contain the phosphorylation can be quantified.

IV. Post-translational Modification Analysis

Phosphorylation: Reversible protein phosphorylation, principally on serine, threonine or tyrosine residues, is one of the most important and well-studied post-translational modifications. Phosphorylation plays critical roles in the regulation of many cellular processes including cell cycle, growth, apoptosis and signal transduction pathways.

Measured the change in phosphorylation during different cell cycles.



Adapted From: Quantitative Phosphoproteomics Reveals Widespread Full Phosphorylation Site Occupancy During Mitosis, Mann, et.al.

V. Protein Quantification

Measure the differences between two (or more) proteomes.

Some Examples:

Change in the Regulation of Protein Classes
(i.e. change in Phosphatase and Kinase Abundance)

Change in the Regulation of Post translational modifications
(i.e. change in Phosphorylation)

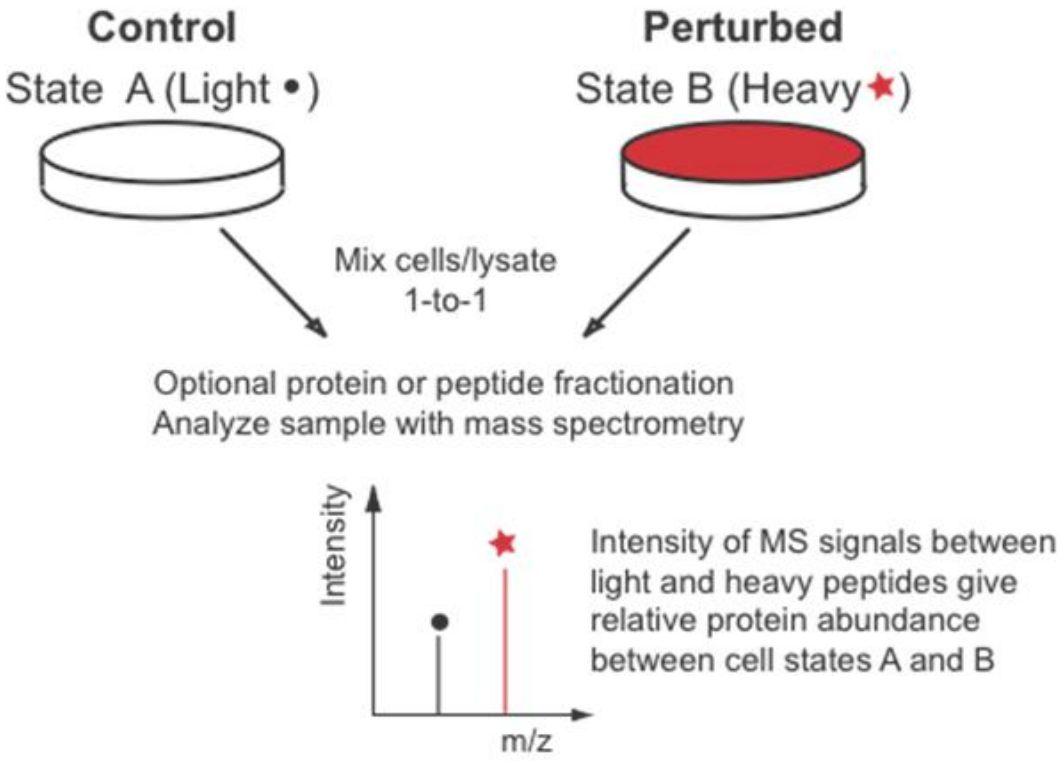
Change in Phenotype due to Mutations
(i.e. mutation in p53 as related to different types of cancers)

…….. etc. – leads into Clinical Proteomics

V. Protein Quantification

Experiments for Quantitation in Proteomics

Stable isotope labeling by amino acids in cell culture (SILAC)



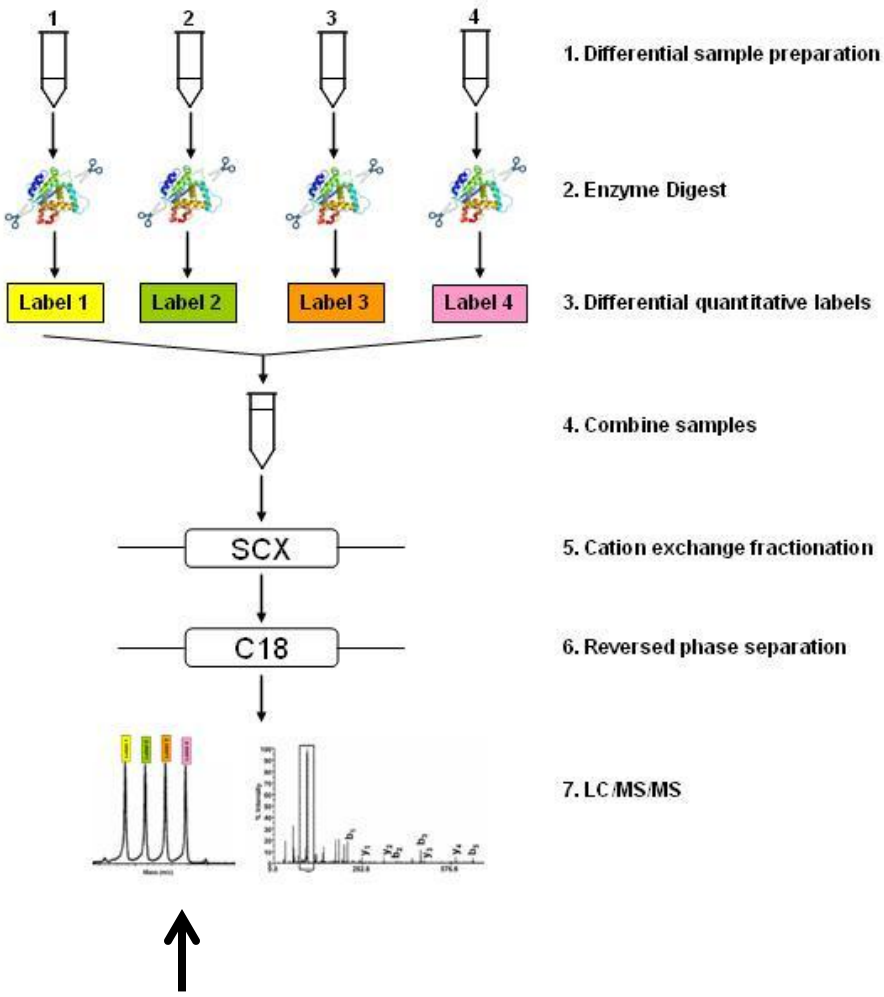
"**S**table **I**sotope **L**abeling by **A**mino acids in **C**ell culture (SILAC) is a simple, robust, yet powerful approach in mass spectrometry (MS)-based quantitative proteomics. SILAC labels cellular proteomes through normal metabolic processes, incorporating non-radioactive, stable isotope containing amino acids in newly synthesized proteins. Growth medium is prepared where natural ('light') amino acids are replaced by 'heavy' SILAC amino acids. Cells grown in this medium incorporate the heavy amino acids after five cell doublings and SILAC amino acids have no effect on cell morphology or growth rates. When light and heavy cell populations are mixed, they remain distinguishable by MS and protein abundances are determined from the relative MS signal intensities. A variant of the approach, Triple Encoding SILAC, allows three protein populations to be compared simultaneously. SILAC provides accurate relative quantification without any chemical derivatization or manipulation and enables steady-state proteome quantification or pulse-chase experiments."

http://www.broadinstitute.org/scientific-community/science/platforms/proteomics/silac

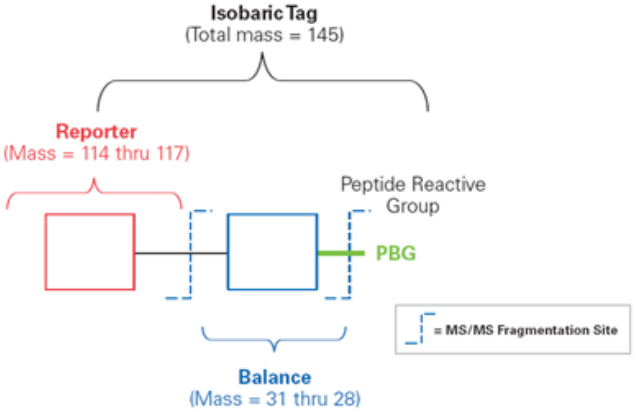
V. Protein Quantification

Experiments for Quantitation in Proteomics

Isobaric tags for relative and absolute quantitation (iTRAQ)



1. Differential sample preparation
2. Enzyme Digest
3. Differential quantitative labels
4. Combine samples
5. Cation exchange fractionation
6. Reversed phase separation
7. LC/MS/MS



The method is based on the covalent labeling of the N-terminus and side chain amines of peptides from protein digestions with tags of varying mass. There are currently two mainly used reagents: 4-plex and 8-plex, which can be used to label all peptides from different samples/treatments. These samples are then pooled and usually fractionated by SCX followed by nano liquid chromatography and analyzed by tandem mass spectrometry (MS/MS). A database search is then performed using the fragmentation data to identify the labeled peptides and hence the corresponding proteins. The fragmentation of the attached tag generates a low molecular mass reporter ion that can be used to relatively quantify the peptides and the proteins from which they originated.

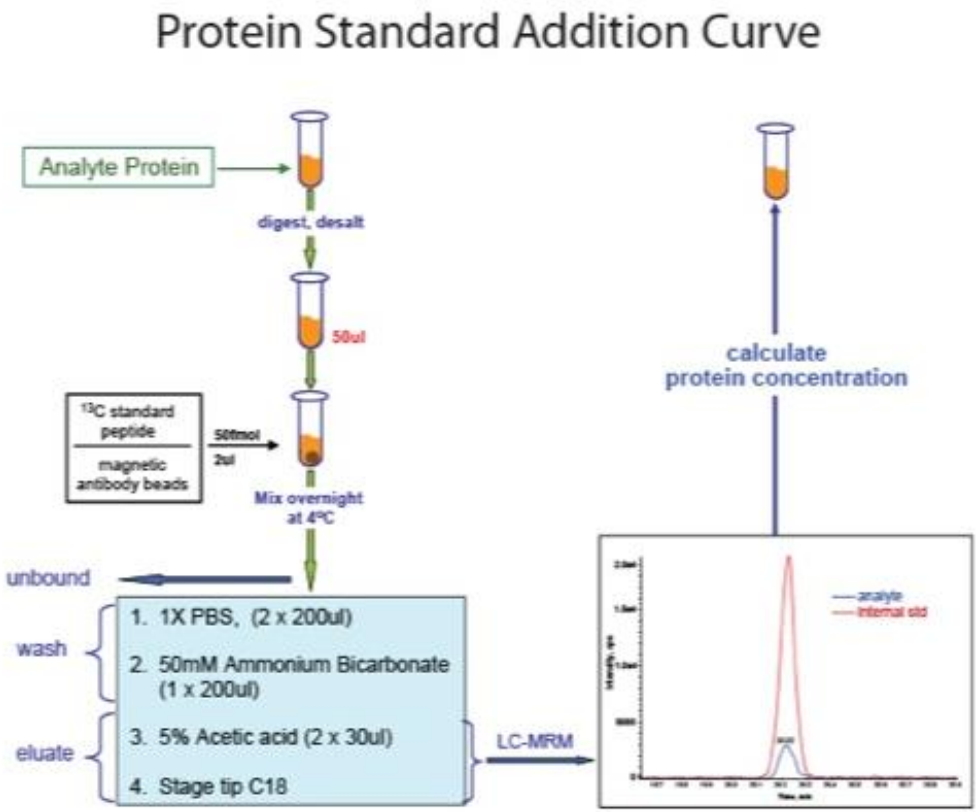
The signals of the reporter ions of each MS/MS spectrum allow for calculating the relative abundance (ratio) of the peptide(s) identified by this spectrum. The abundance of the reporter ions may consist of more than one single signal in the MS/MS data and the signals have to be integrated in some way from the histogram spectrum.

Reporter Ions For Relative Quantitation

V. Protein Quantification

Experiments for Quantitation in Proteomics

Isotopic Dilution – Addition of "heavy" Labeled Synthetic Peptide



A synthetic peptide with a "heavy" label is introduced to the mixture as an internal standard.

Absolute quantitation is achieved for single biomarker peptides

VI. Clinical Proteomics

Basic Science → Translational Science → Clinical Proteomics ('Omics') → New Treatments for Human Disease

"**Clinical Proteomics** is an emerging **interdisciplinary research field** that coalesces researchers from many different areas of biomedical research into one of the most likely disciplines to successfully foster the **translation of basic scientific knowledge into clinical applications for the benefit of the patient**. Major research interests that are expected to profit from the application of proteomic technologies to clinical issues include:

(i) early detection/diagnosis of disease

(ii) prediction of how a disease will behave over time and how a specific patient will respond to a given treatment

(iii) identification of novel targets for therapeutic intervention."

http://www.mcponline.org/content/7/10/1779.full

VI. Clinical Proteomics

"Today, clinical proteomics studies are increasingly shifting toward the analysis of tissues and bio-fluids and as a result, concerns such as tissue heterogeneity, abundance of the cell type of interest, marker quantitation, availability of samples with long-term clinical follow-up, as well as experimental design and optimal method selection have become some of the main challenges that researchers face. These and other inherent problems associated with using clinical samples make interdisciplinary joint efforts a necessity for any high-quality clinical proteomics study. Furthermore, the translation of basic proteomic discoveries to a clinical setting is a very long and expensive process that requires broad multidisciplinary collaborations between basic researchers, clinicians, surgeons, pathologists, and epidemiologists as well as infrastructural support and industry participation."

http://www.mcponline.org/content/7/10/1779.full

VI. Clinical Proteomics

Apply the basic methods described in previous slides to analyze real human samples.

Often need special certifications to work on clinical samples:
Institutional review board (IRB) a.k.a. independent ethics committee (IEC) or Ethical Review Board (ERB)

Clinical Proteomics is often combined with other 'Omics' (*slide 3)* results to describe the full 'systems biology' of a 'phenotype or organism'

With systems biology understanding of a human, we can start to prescribe "Personalized Medicine"