Guest Lecture
Protein and Peptide Mass Spectrometry

CU- Boulder

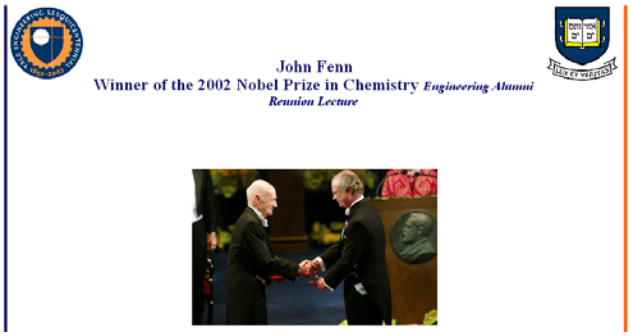
CHEM 5181

Mass Spectrometry & Chromatography

Prof. William Old

Fall 2011

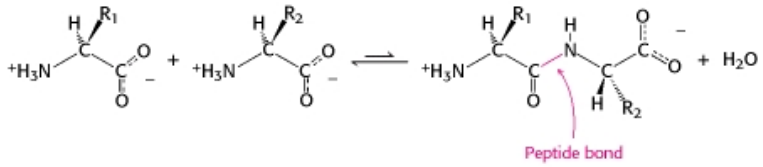
2002 Nobel Prize in Chemistry for developing electrospray ionization – John Fenn, Virginia Commonwealth University

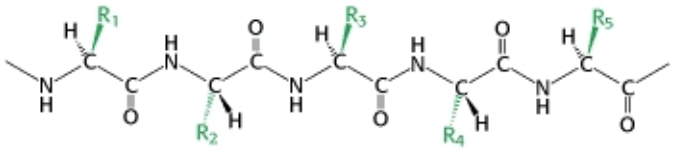


MS based proteomics is a discipline made possible by the availability of gene and genome sequence databases, and technical and conceptual advances in many areas, most notably the discovery and development of protein ionization methods, as recognized by the 2002 Nobel prize in Chemistry.

Electrospray Ionization for Mass Spectrometry of Large Biomolecules" J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, & C.M. Whitehouse Science 246, 64 (1989)

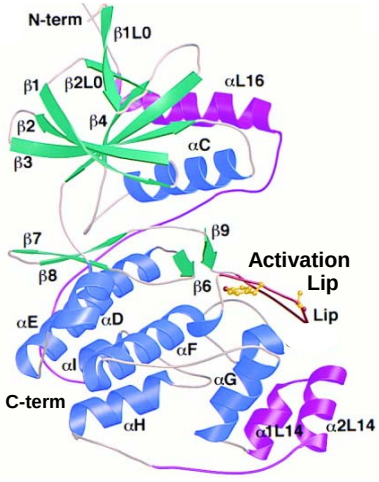
Peptide-Bond Formation

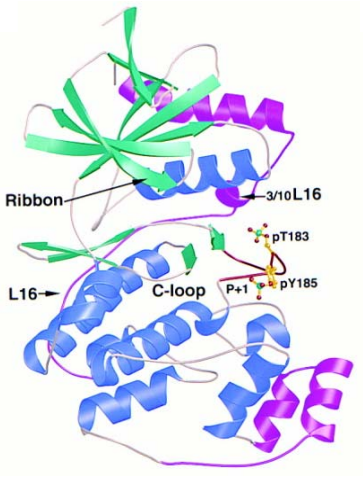




From Biochemistry. 5th edition. Berg JM, Tymoczko JL, Stryer L. New York: W H Freeman; 2002.

Conformational changes in ERK2 upon phosphorylation

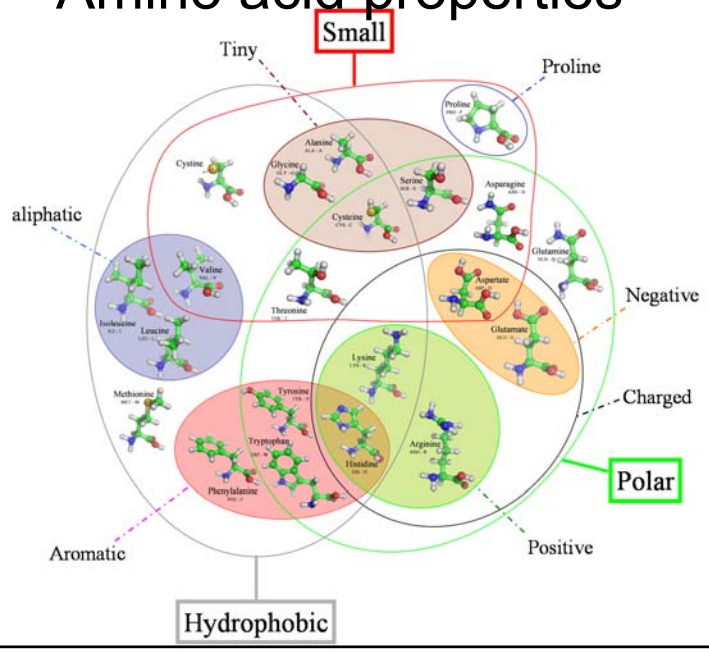


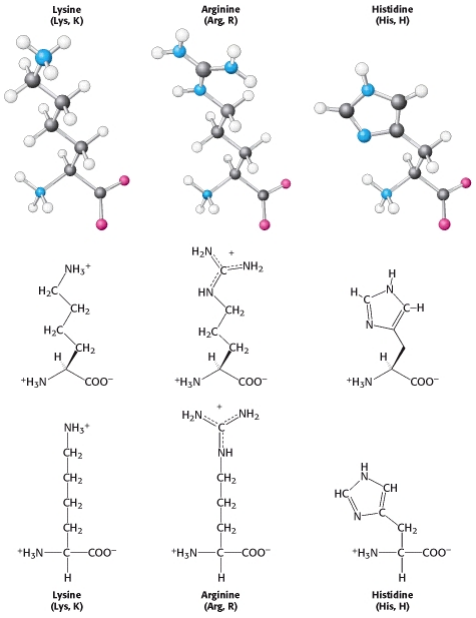


Unphosphorylated ERK2 **Phosphorylated ERK2**

(Canagarajah et al, 1997)

Amino acid properties



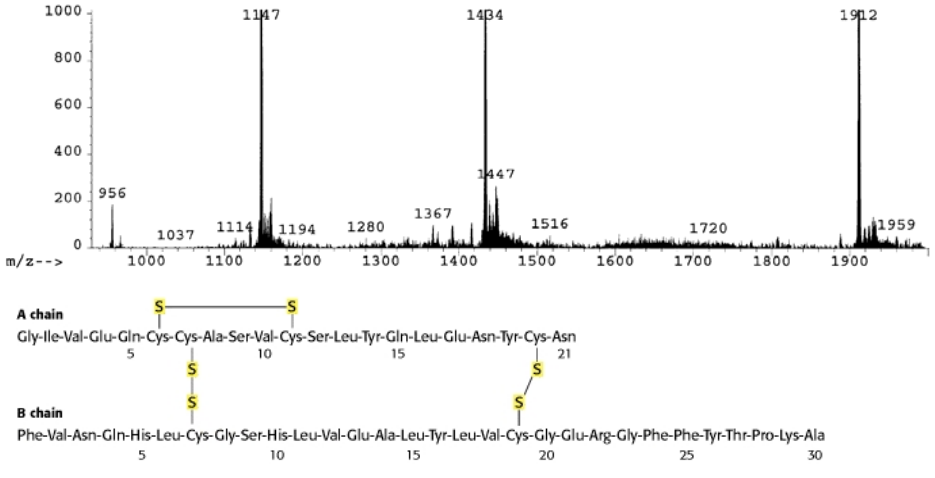


From Biochemistry. 5th edition. Berg JM, Tymoczko JL, Stryer L. New York: W H Freeman; 2002.

Protein Analysis by Mass Spectrometry

- Top Down
  - Intact protein mass measurement using high resolution MS (FTICR)
  - Good for identifying total modification level by mass shift, e.g. phosphorylation and a.a. variants
  - Cons: requires expensive equipment. Many proteins not soluble, and activation/fragmentation methods not efficient.
- Bottom up
  - Proteins are cut into smaller pieces with enzymes (proteases).
  - Advantage is that peptides have more uniform physico-chemical properties, i.e. solubility, hydrophobicity.
  - Ion traps, triple quadrupoles, and hybrid instruments are ideal
  - Cons: increased complexity requires chromatography.

ESI-MS of Insulin:



From Bob Barkley

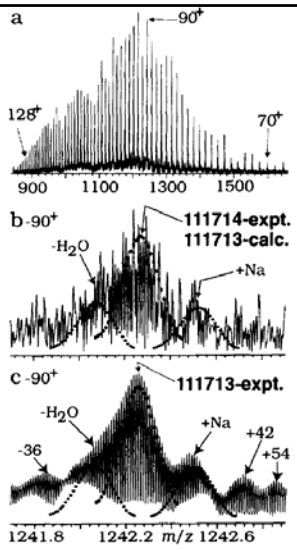


Figure 1. ESI/FTMS spectra of chondroitinase II. (a) Broadband, with external ion accumulation and data acquisition repeated 50 times. The small peaks between the large peaks correspond to dimer ions. (b) SWIFT isolation of m/z 1230–1255, data acquisition with heterodyne detection, repeated 10 times (RP > 170,000) [24]; dots, theoretical distribution of isotopic abundances [12b] for molecular, +22, and −18 Da ions. (c) Data from (b) with time domain data sampling prior to Fourier-transformation.

ESI-FTICR of chondronitase II (12 kDa)

$C_{5039}H_{7770}N_{1360}O_{1525}S_{22}$

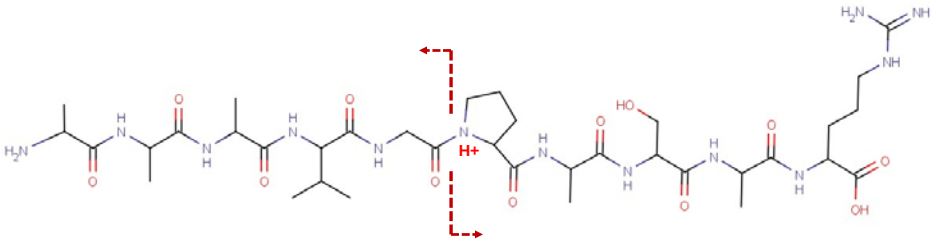
Table 1. M_r values of Chondroitinase measured by different techniques

	Chondroitinase I		Chondroitinase II	
Method	M_r value	Error (Da)	M_r value	Error (Da)
DNA	112,508-*69*	—	111,713-*68*	–
SDS-PAGE	110000	−2500	112000	+300
IR-MALDI/MS	112324	−183	111525	−188
ESI/Quad-MS	112553	+45	111750	+37
ESI/FTMS	112,509-*69*	+1	111,714-*68*	+1

From Kelleher NL, JASMS 1997

Peptides fragment in a predictable way

$[AAAVGPASAR + H]^{1+}$



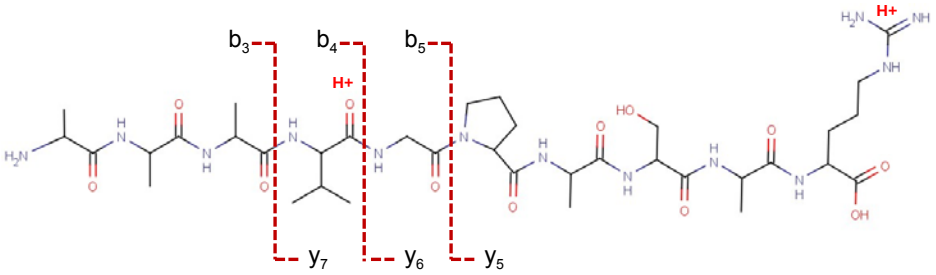
For a singly protonated peptide, either:

+ N-term ion and neutral C-term
or
+ N-term neutral + C-term ion

Peptides fragment in a predictable way

[AAAVGPASAR + 2H] $^{2+}$

For a doubly protonated peptide, both N- and C-terminal fragments can be generated from a single dissociation event

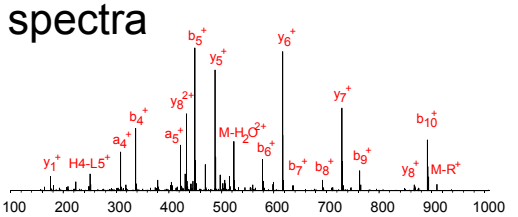


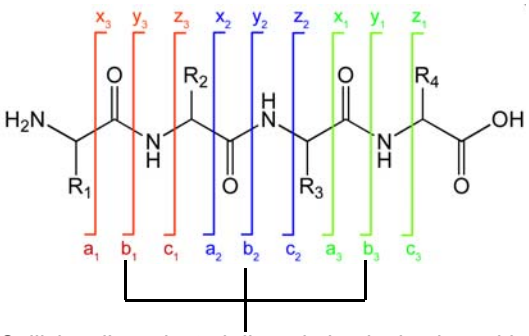
Biemann notation of peptide fragmentation spectra

Backbone fragmentations are denoted by a letter followed by a number:

Letter: Indicates the bond broken and the terminus contained in the fragment

Number: Indicates the number of alpha-carbons in the fragment





Collisionally activated dissociation is dominated by b- and y-ions which result from fragmentation at the amide

The proline effect – cleavage favored N-terminal to Pro

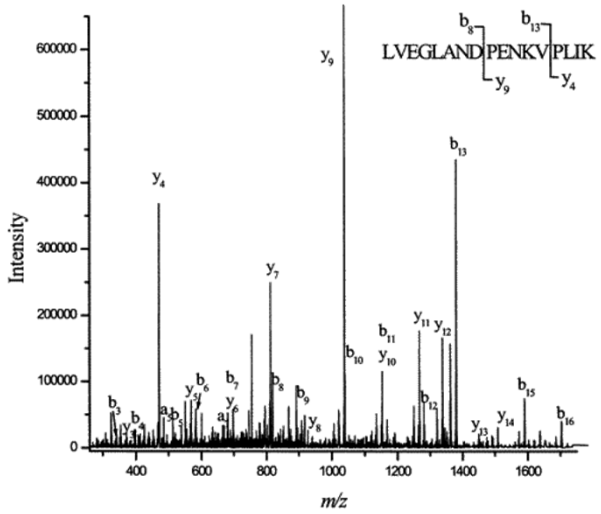


Figure 1 MS/MS spectrum of the peptide [LVEGLANDPENKVPLIK + 2H]2+ acquired by CID in an ion trap. Although many peaks are a-, b-, and y-sequence ions, many other peaks are unidentified.

Breci et al 2003 Anal. Chem., 75 (9), 1963 -1971

Mobile proton model: Wysocki, Gaskell and Harrison

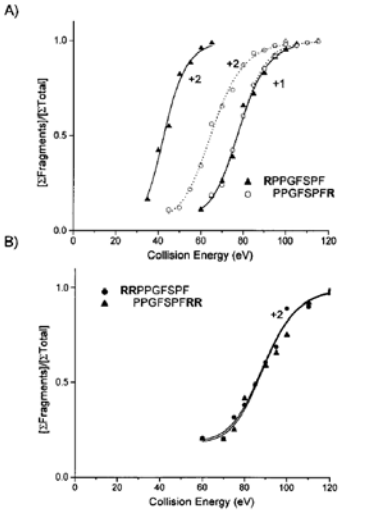


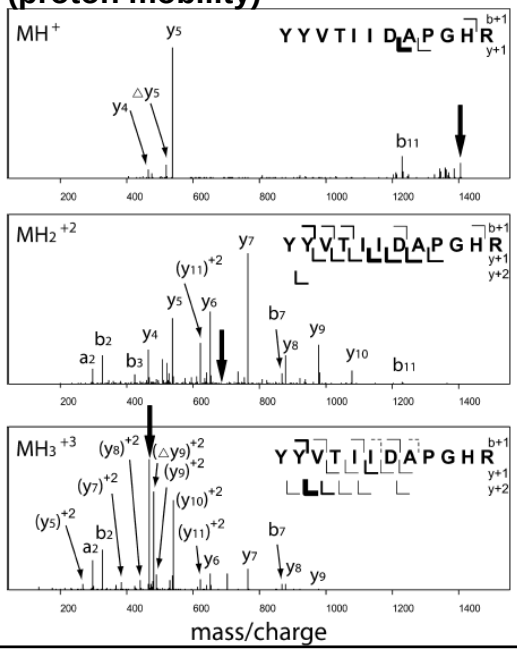
Figure 1. Fragmentation efficiency curves [(sum of fragment % relative abundance)/(total % relative abundance)] for singly and doubly protonated peptides that differ in the number and locations of arginines (**R**). Spectra were acquired by surface-induced dissociation on a tandem quadrupole mass spectrometer. From Ref. 14.

Correspondence between the basicity and the collision energy required to fragment the peptide

When protons are sequestered, greater energy is required to mobilize the protons to less basic sites, such as backbone amides, where protonation leads to fragmentation.

From Wysocki, *J. Mass Spectrom.* ***35, 1399–1406 (2000)***

Cleavage patterns depend on precursor ion charge state (proton mobility)



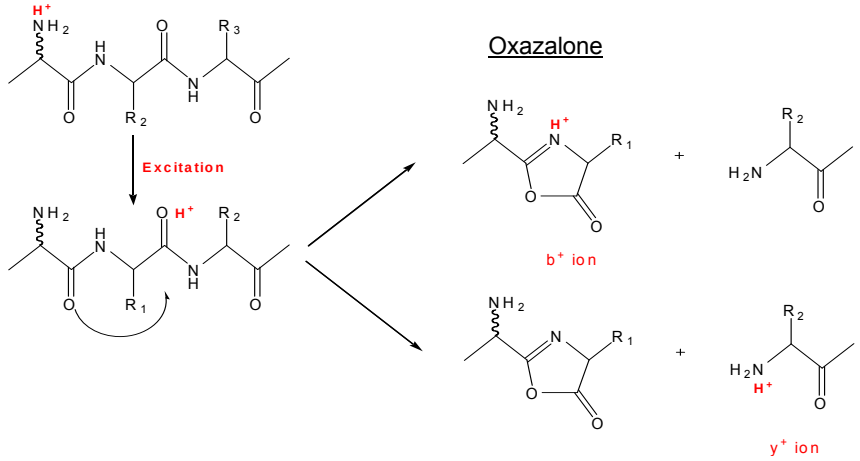
Localized proton, selective fragmentation

Free proton, non- or less selective fragmentation

Free proton, non-selective fragmentation and multiply charged fragments

A mechanism for low energy peptide fragmentation

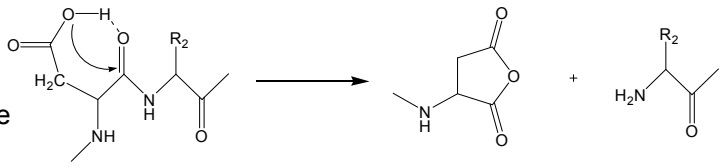
"Mobile proton" Charge directed mechanism



From Wysocki et al., *J. Mass Spectrom.* (2000) 35:1399

Charge remote fragmentation (non-mobile proton)

Charge remote mechanism



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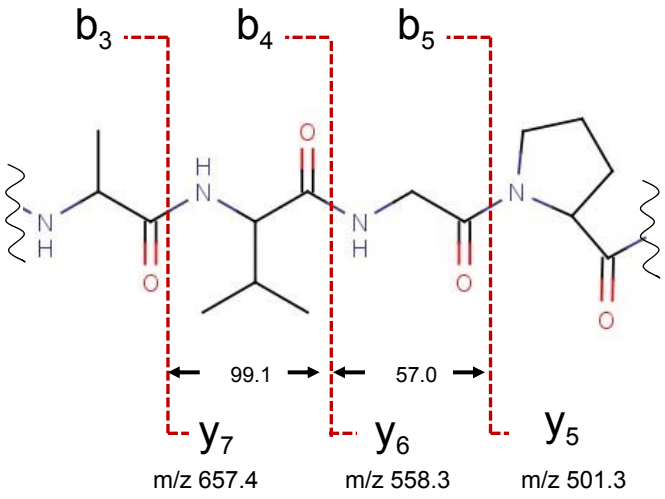
Peptide sequencing by MS/MS

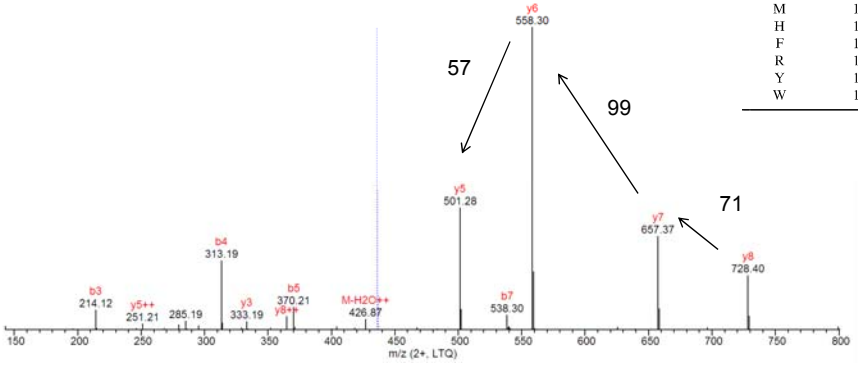
Amino acid sequence can be deduced by the Δmass between peaks



"residue mass"

Code (1 letter)	Monoisotopic mass
G	57.02147
A	71.03712
S	87.03203
P	97.05277
V	99.06842
T	101.04768
C	103.00919
I	113.08407
L	113.08407
N	114.04293
D	115.02695
Q	128.05858
K	128.09497
E	129.04260
M	131.04049
H	137.05891
F	147.06842
R	156.10112
Y	163.06333
W	186.07932

y-ions: AAAVGPASAR
9 8 7 6 5 4 3 2 1



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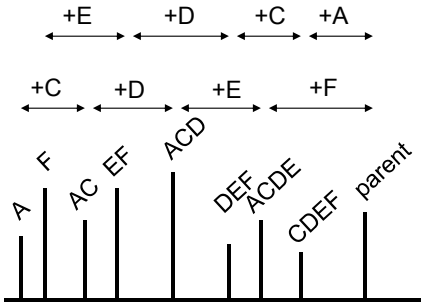
Generation of N-terminal and C-terminal fragments

$A\text{-}C\text{-}D\text{-}E\text{-}F^{+}$

A	C-D-E-F
A-C	D-E-F
A-C-D	E-F
A-C-D-E	F



Calculation of fragment ion masses

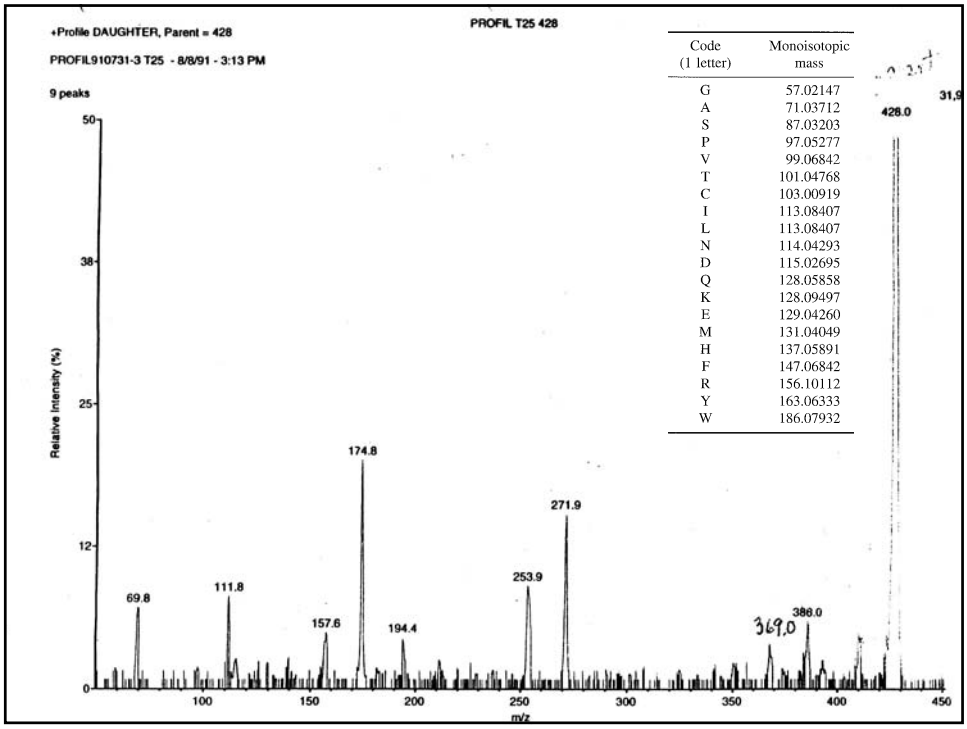
a_j = [N-term] + Σaa_i – [CO]
b_j = [N-term] + Σaa_i
c_j = [N-term] + Σaa_i + [NH_3]
$d_j = a_j$ - [R'_j] + [H]

x_j = [C-term] + Σaa_i + [CO]
y_j = [C-term] + Σaa_i + [H_{NH2}]+[H]
z_j = [C-term] + Σaa_i – [NH]
$w_j = z_j$ - [R'_j] + [H]
$v_j = y_j$ - [R_j] - [H]

b ion: M = 1 + Σ res. mass

y ion: M = 19 + Σ res. mass

a ion: M = M (b ion) – 28
= Σ res. mass - 27



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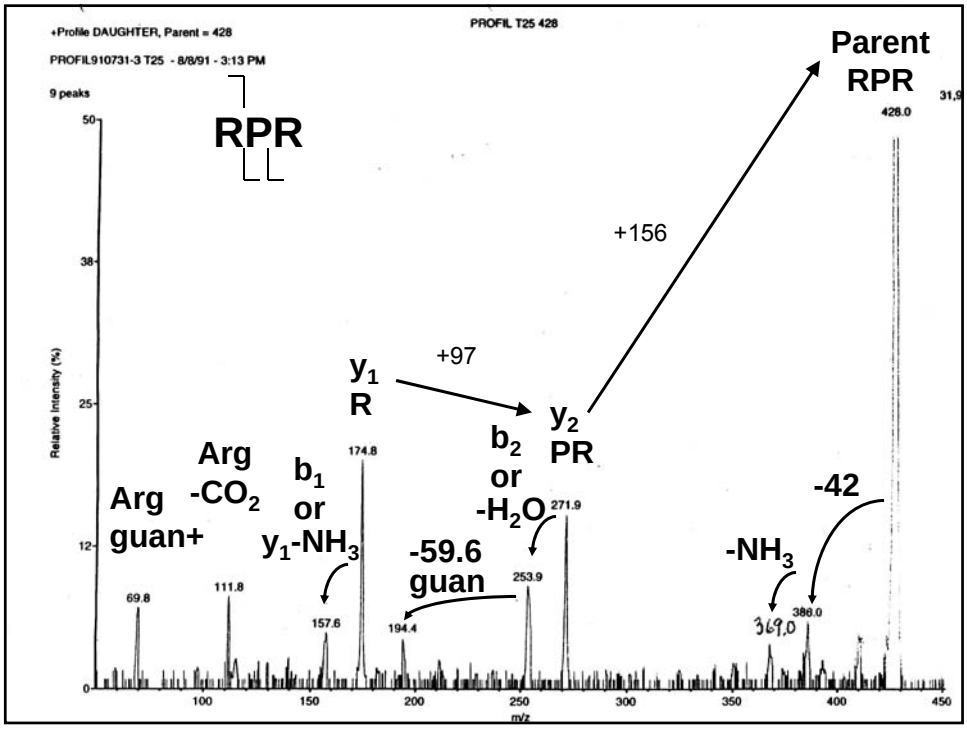


The proline effect – cleavage favored N-terminal to Pro

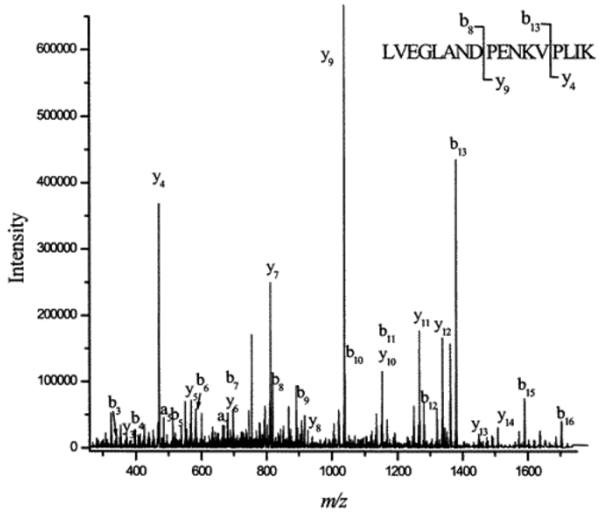


Figure 1 MS/MS spectrum of the peptide [LVEGLANDPENKVPLIK + 2H]2+ acquired by CID in an ion trap. Although many peaks are a-, b-, and y-sequence ions, many other peaks are unidentified.

Breci et al 2003 Anal. Chem., 75 (9), 1963 -1971

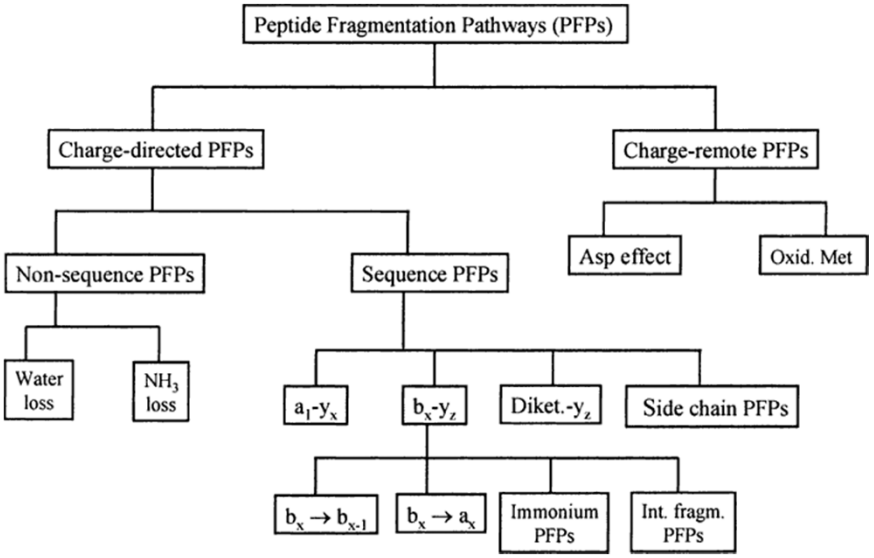
Besides sequence dependent fragments, you also obtain

1. Small ions that are breakdown products of amino acids (immonium ions) -- can give useful information about composition

2. Dehydration, deammoniation, and deamidation

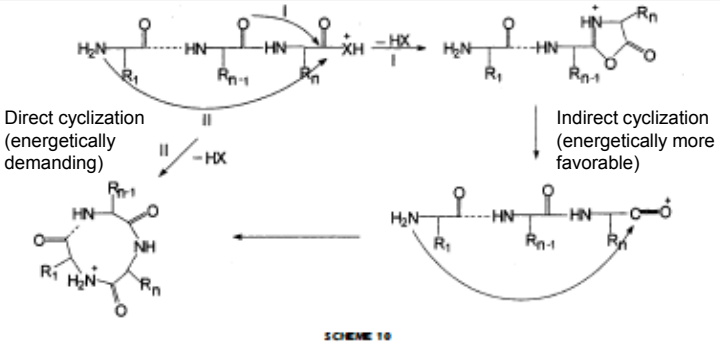
3. Internal fragment ions
   -- these are a or b type ions
   -- usually di- or tri- “peptides”

4. Cyclization products
   -- Arg and His very susceptible to cyclization
   -- can be confusing because “ring” reopens and begins sequence series from “new N terminus”

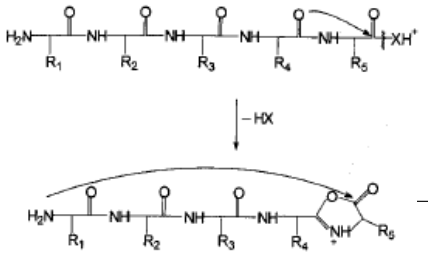


Paizs and Suhai 2005

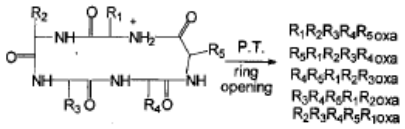
Macrocyclic structures for b ions



Cyclization probably via nucleophilic attack by N terminal amine to carbonyl of C-term oxazolone



Both oxazolone and macrocyclic structures validated by IRMPD depending on peptide chemistry



Infrared multiphoton dissociation (IR-MPD) spectroscopy – confirms oxazolone and macrocyclic structures

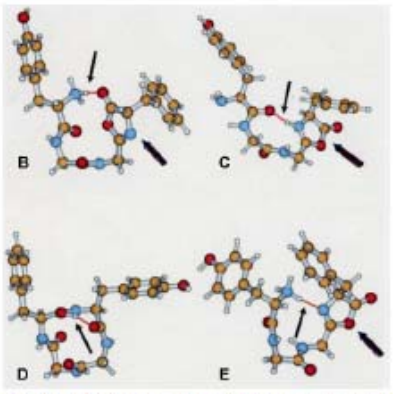


FIGURE 3. Structures corresponding to calculated spectra of Figure 2. Sites of proton solvation indicated by narrow arrows and oxazolone rings by broad arrows. Reprinted with permission from Polfer et al. (2007). Copyright 2007, American Chemical Society. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

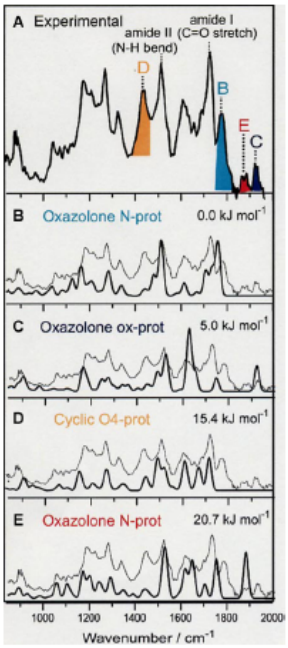


FIGURE 2. IRMPD spectrum of b_5 ion H-YGGFL.* (A) compared with calculated spectra for the four structures B to E shown in Figure 3 (relative energies given). Distinctive bands for each structure are color-coded. Reprinted with permission from Polfer et al. (2007). Copyright 2007, American Chemical Society. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Harrison (2009) Mass Spec Reviews "To b or not to b: the ongoing saga of peptide b ions."

Electron Capture Dissociation (ECD)
Electron Transfer Dissociation (ETD)

- Different mechanisms for fragmentation than CAD
- Free radical cleavage chemistries
- Non-overlapping coverage of peptides
- Favors high charge states (+3 and higher)
- Efficient sequencing of peptides with PTMs

ELECTRON TRANSFER DISSOCIATION (Donald Hunt, 2004)

$$[M+4H]^{4+} + C_{16}H_{10}{}^{-\bullet} \rightarrow [M+4H]^{3+\bullet} + C_{16}H_{10}$$

ETD fragments peptides by transferring en electron from radical anion to protonated peptide. This induces fragmentation along the backbone, generating c and z ions. The reaction allows cleavage without side chain chemistry, eg neutral loss.

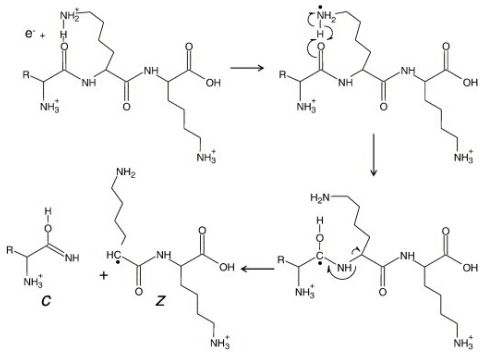


Fig. 2. ETD fragmentation scheme. Fragmentation scheme of a multiply protonated peptide after reaction with a low energy electron to produce c- and z-type ions [12]

Mikesh et al (2006) Biochem Biophys Acta 1764:1811-1822 – Review on ETD

Phosphate

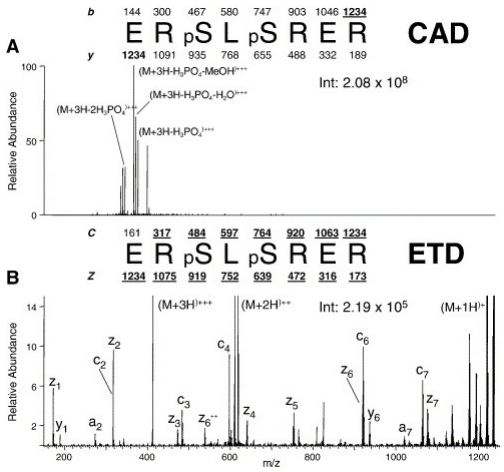
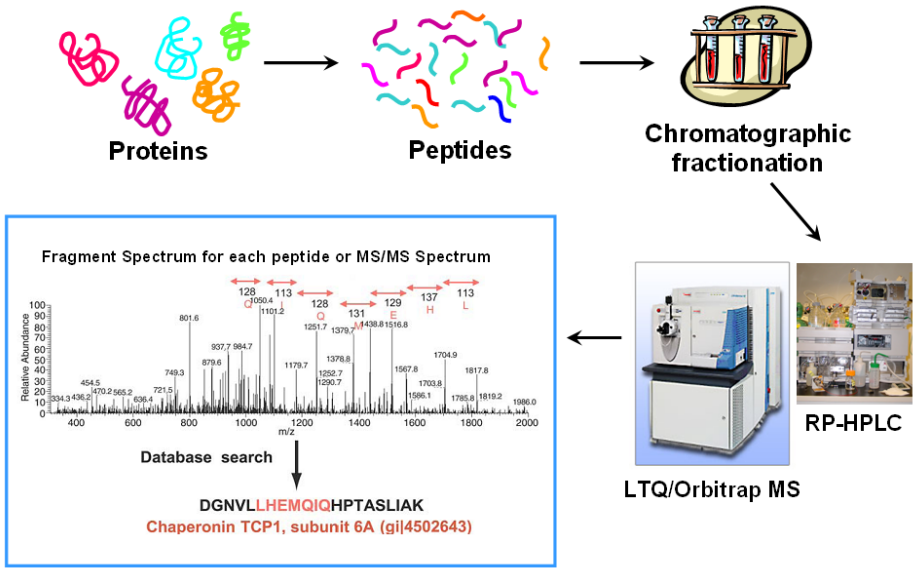


Fig. 3. Comparison of CAD vs. ETD spectrum of a phosphorylated peptide. Consecutive single-scan CAD vs. ETD mass spectrum comparison of phosphorylated peptides generated from a tryptic digest of human nuclear proteins recorded during a data-dependent analysis (nHPLC-μESI-MS/MS). All peptides were converted to methyl esters and enriched for phosphorylated peptides by immobilized metal affinity chromatography before analysis. (A) CAD spectrum dominated by fragment ions corresponding to the loss of phosphoric acid and either methanol or water. (B) ETD spectrum containing a near complete series of c- and z-type product ions. Note that the spectrum is devoid of fragment ions corresponding to the loss of phosphoric acid [12]

Mikesh et al (2006) Biochem Biophys Acta 1764:1811-1822 – Review on ETD

Bottom up “shotgun” proteomics:

Protein profiling by multidimensional LC/MS/MS



Bottom-up proteomics: the challenge of in-depth profiling by LC-MS/MS

- Up to 12,000 genes expressed in a given cell type
- This yields ~ 420,000 tryptic peptides
- Multiple charge states and redundancy in neighboring fractions yields ~3.4 M ions
- Splice variants and PTMs increase complexity further
- Current typical sampling depth: ~2000 proteins

LTQ-ORBITRAP -- HYBRID INSTRUMENT

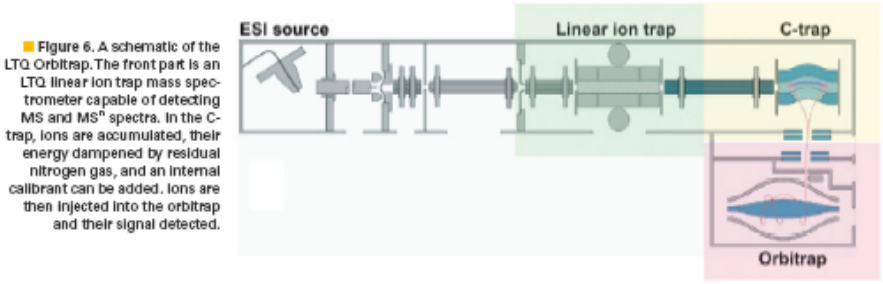
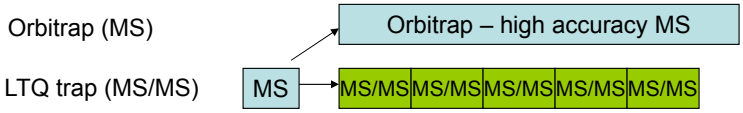
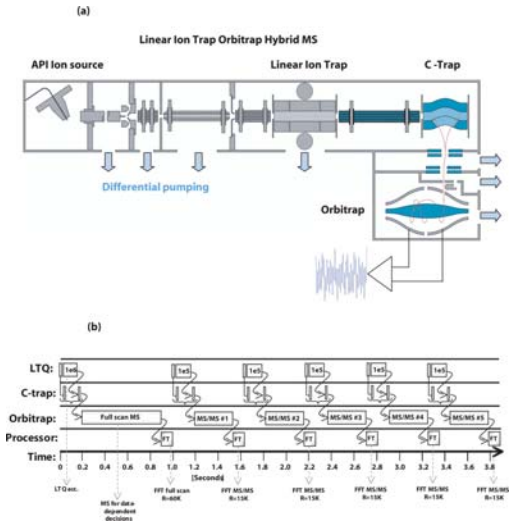


Figure 6. A schematic of the LTQ Orbitrap. The front part is an LTQ linear ion trap mass spectrometer capable of detecting MS and MS^n spectra. In the C-trap, ions are accumulated, their energy dampened by residual nitrogen gas, and an internal calibrant can be added. Ions are then injected into the orbitrap and their signal detected.

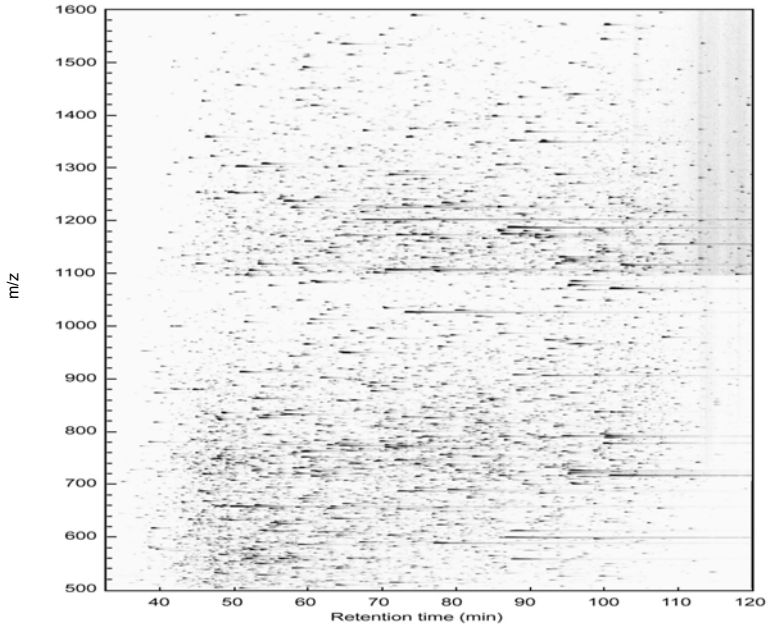


a, schematic of the LTQ Orbitrap.

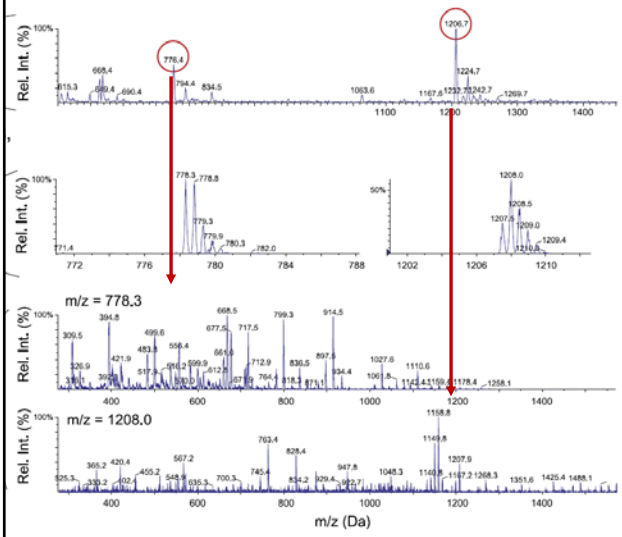


Olsen J V et al. Mol Cell Proteomics 2005;4:2010-2021

LC-MS/MS Contour map



Data dependent acquisition

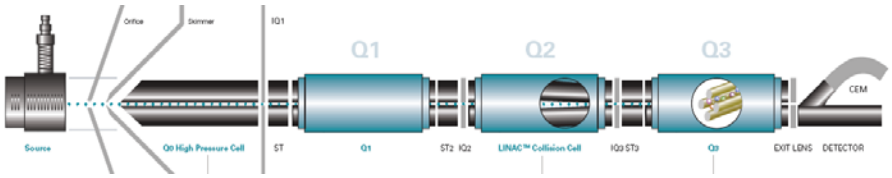


1) Survey scan (Orbitrap, QQQ precursor scan, etc)

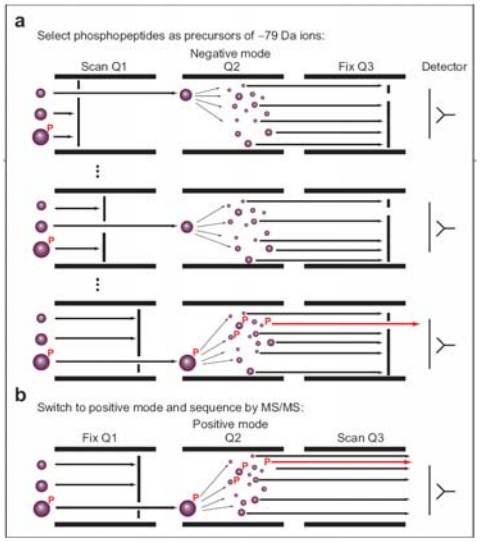
2) Target highest intensity ions detected in survey scan for MS/MS sequencing

From Old, WM Mol. Cell 2009

Hybrid triple quadrupole linear ion trap

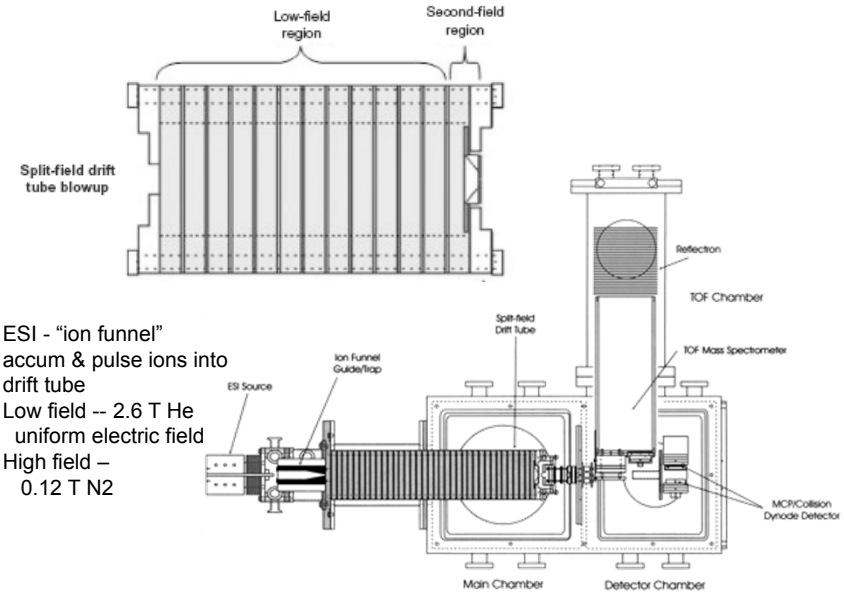


Negative ion precursor scanning to identify phosphopeptides in complex mixtures



Ahn et al (2007) *Chemical Biology* 2: *in press*

SPLIT-FIELD IMS-TOF INSTRUMENT -- David Clemmer, Richard Smith



ESI - "ion funnel"
accum & pulse ions into drift tube
Low field -- 2.6 T He
uniform electric field
High field –
0.12 T N2

Under low-field conditions, **the mobility (*K*) of an ion through the buffer gas is given as**

$$K = v_D \cdot E^{-1}$$

(v_D is the drift velocity of the ion and *E* is the electric field).64

Often, to permit comparison between different measurements, it is useful to convert values into reduced mobilities (*K*0) by using the relation

$$K_0 = \frac{L^2}{t_D \cdot V} \times \frac{273.2}{T} \times \frac{P}{760}.$$

In this expression, *t*D, *L*, *V*, *P*, and *T* correspond with the measured drift time, length of the drift region, the applied drift voltage, and the pressure and temperature of the buffer gas, respectively.

Ions that adopt compact conformations have higher mobilities than those that exist as extended conformers.

For ions of similar size, those that exist as higher charge states will have higher mobilities because they are influenced by a greater drift force.

Koeniger et al J Phys Chem 2006

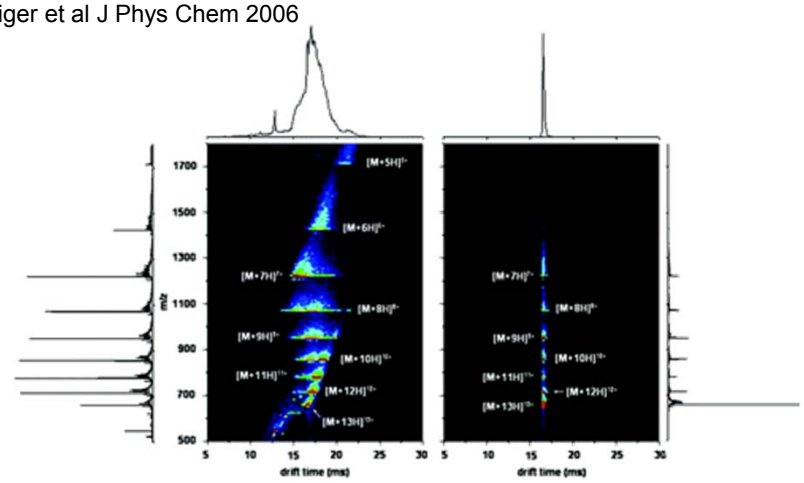


Figure 2 **Nested drift(flight) time distributions showing the total distribution of electrosprayed ubiquitin ions (left) and a narrow distribution of mobility-selected ions that were gated into D2 at 7.8 ms (right).** Also shown are the summed mass spectra (sides) and summed drift time distributions (top) obtained by integrating the two-dimensional data across all drift times and all *m*/*z* values, respectively. The drift time represents the total time required for the ions to travel through D1 to the TOF source.