Sample Preparation Methods for MS Based Proteomics

Lecture 2

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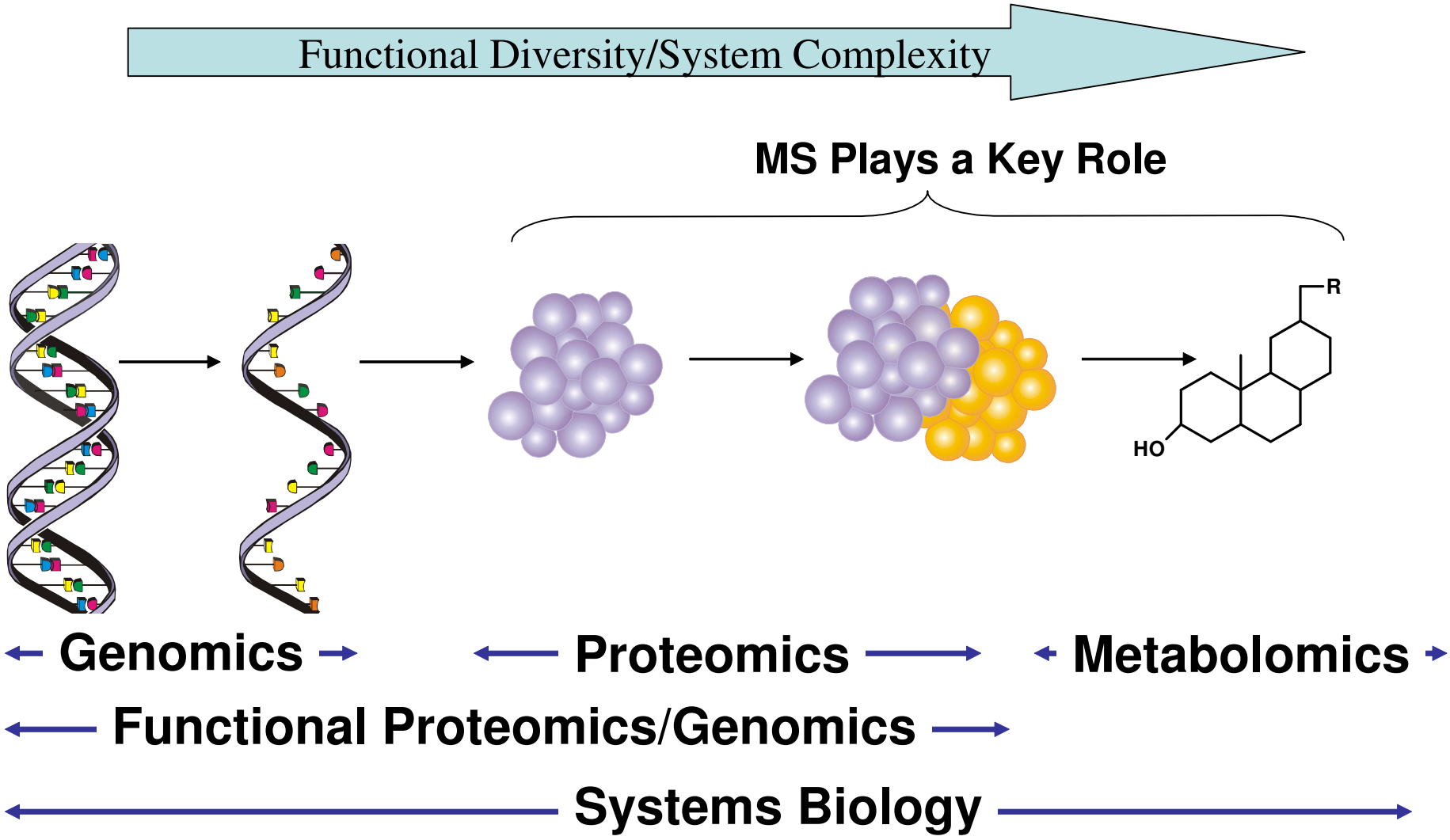
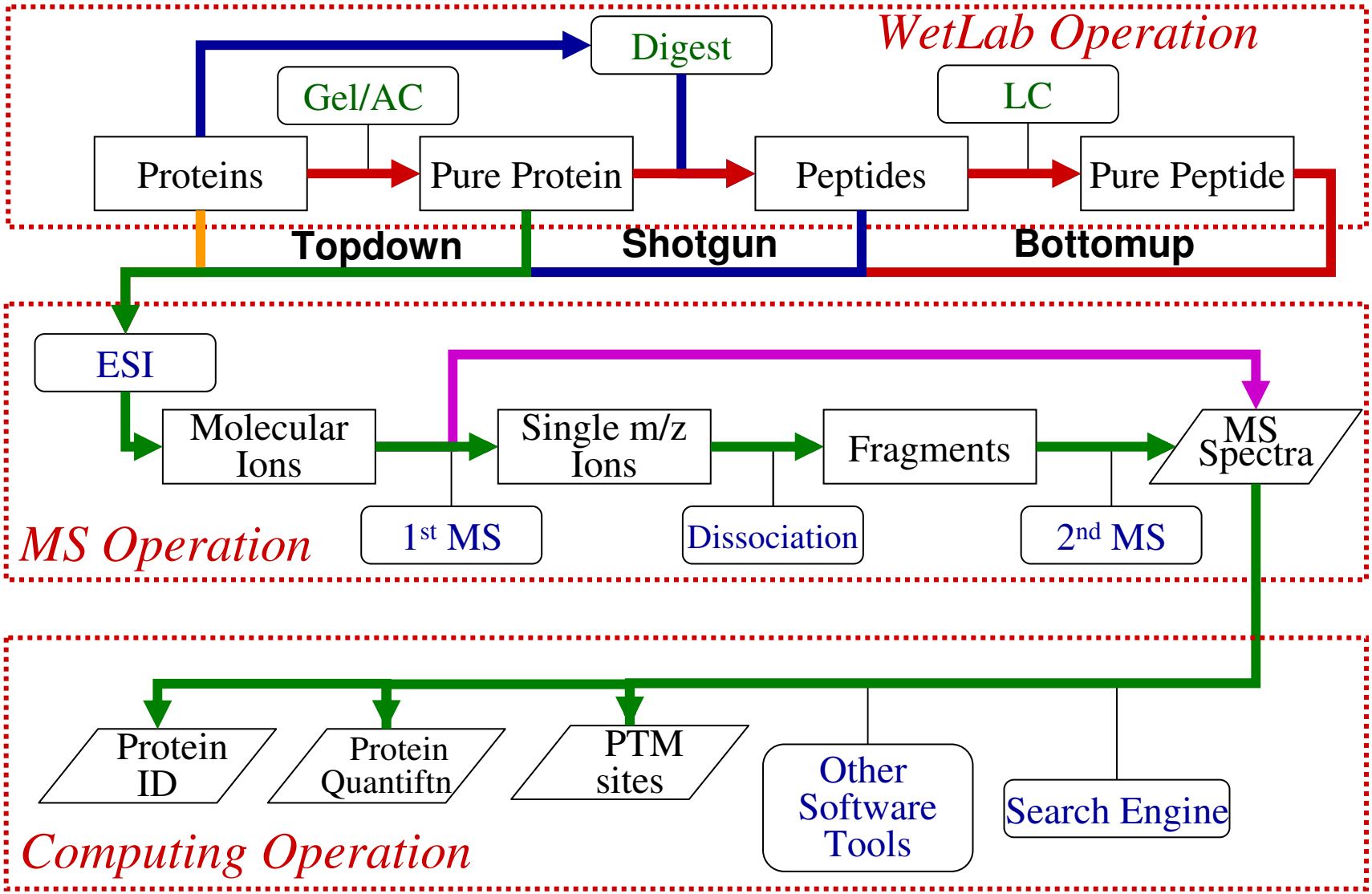
Lecture 2 Outline

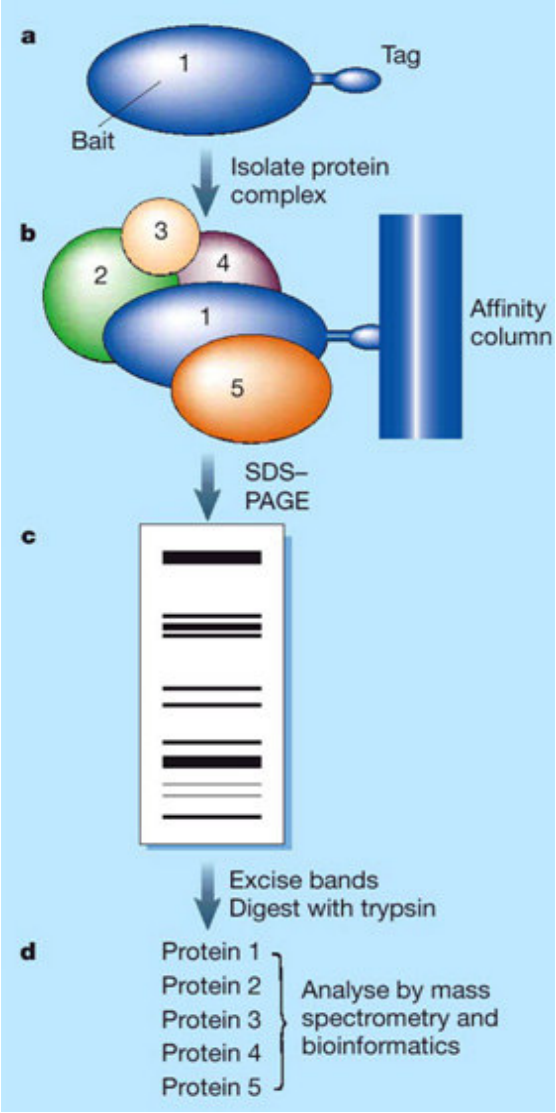
- Digestion Enzymes
- Protein and Peptide Separation Methods
- HPLC
- Multidimensional LC
- Microfluidics


Functional Diversity/System Complexity
MS Plays a Key Role
R
HO
Genomics
Proteomics
Metabolomics
Functional Proteomics/Genomics
Systems Biology

Proteomics Approaches



Protein complex analysis by mass spectrometry



Affinity purification/SDS gel separation/in-gel digest/LCMSMS mass spectrometry analysis/Protein sequence database search for protein identification is the commonly used bioanalytical methodology

Large scale experiments are possible
(Nature2006v440p637)

Nature2002v415p123

Isolating Interacting Proteins by Affinity chromatography

epitope tags

epitope tags	**composition**	**affinity matrix**
FLAG	DYKDDDDK	FLAG antibody
HA	YPYDVPDYA	HA antibody
C-MYC	EQKLISEEDL	c-MYC antibody
6XHIS	HHHHHH	Immobilized metal affinity (IMAC)
Biotinylation signal	78 amino acids	avidin/streptavidin
Strep binding	10-50 amino acids	avidin/streptavidin
Protein A	137 amino acids	IgG
Calmodulin binding peptide	26 amino acids	Calmodulin

Tandem Affinity Purification

Increased Specificity and Decreased Contaminations

The original yeast TAP tag

The optimised GS-TAP tag

Small tag for fast purification

The tag is used to purify cross-linked complexes in denaturing conditions

Preparation of Proteins for Digestion

Or for Topdown Analysis

Denature proteins

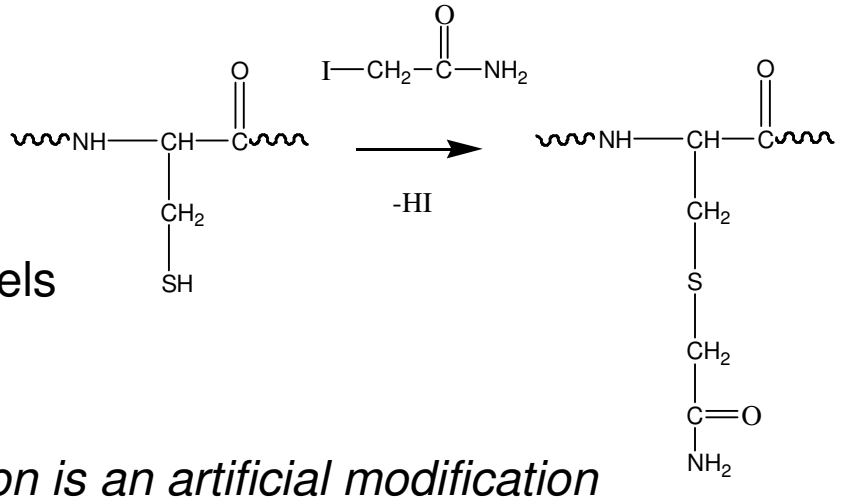
- •Make whole chain accessible
  - •Detergents: SDS, acid-cleavable (AnalChem2003v75p6642)
  - •8M urea – heated may cause carbamylation (*AnalBiochem*1999v267p57)
  - •6M Guanidine HCl; ACN or MeOH, etc

Reduce disulfide bonds

- •General in basic conditions
  - •Dithiothreitol (DTT, 2-SH), TCEP – wide pH range (NatBiotech2001v19p379)

Alkylate thiol groups (in dark)

- •Iodoacetic acid, +58 Da (historical)
- •Iodoacetamide, + 57 Da
- •May have opportunity to introducing labels (e.g., ICAT)



S-S bonds are special PTM and Alkylation is an artificial modification

Commonly Used Protein Digest Reagents

enzymes	cleavage	don't cut	side	type
Trypsin	KR	P	c	aspartic protease
Lys-C	K	P	c	serine protease
Glu-C	E	P	c	serine protease
Chymotrypsin	FYWL	P	c	serine protease
Arg-C	R	P	c	serine protease
PepsinA	FLE?		c?	aspartic protease
Asp-N	D		n	Metalloendopeptidase
Lys-N	K		n	Metalloendopeptidase
CNBr	M		c	chemical
Formic_acid	D		c	chemical

How to Choose A Suitable Enzyme

How specific the cleavage is

- Glu-C: Cuts after E, some D.
- Chymotrypsin: Cuts after F, W, Y, some L, occasionally V…

Generate suitable peptide size – 10-20 amino acid residues

- Conveniently analyzed by LCMSMS

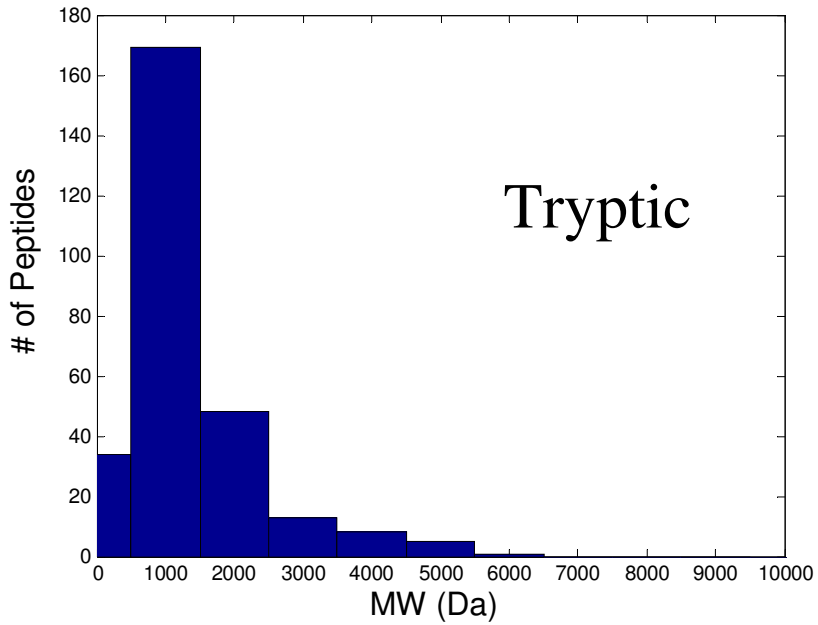
Digest condition is suitable to the sample

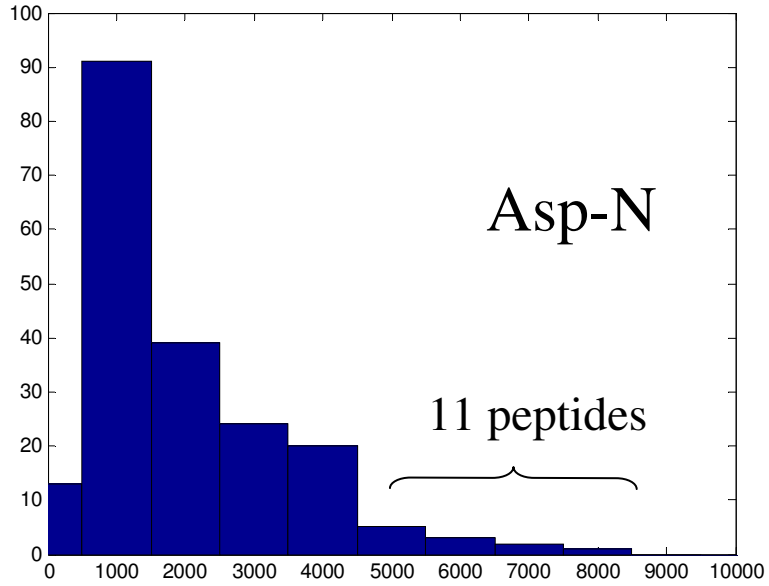
- Pepsin works at low pH and is good for H/D exchange

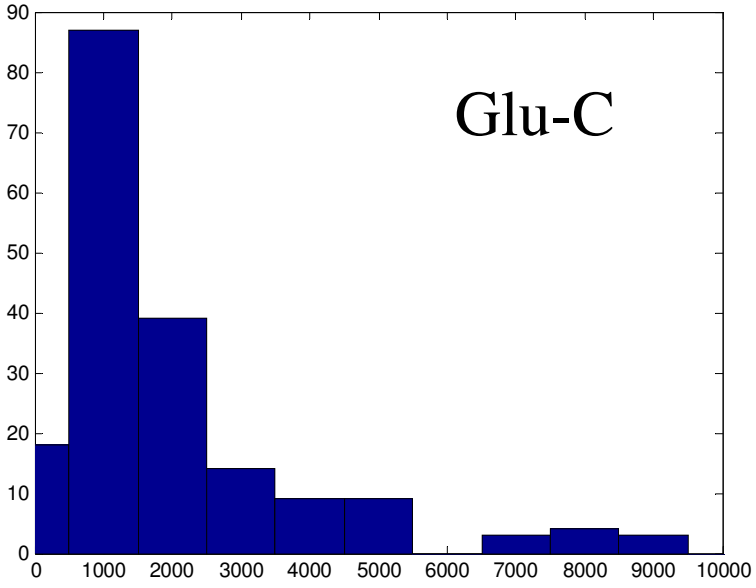
Autolysis and ligation (?)

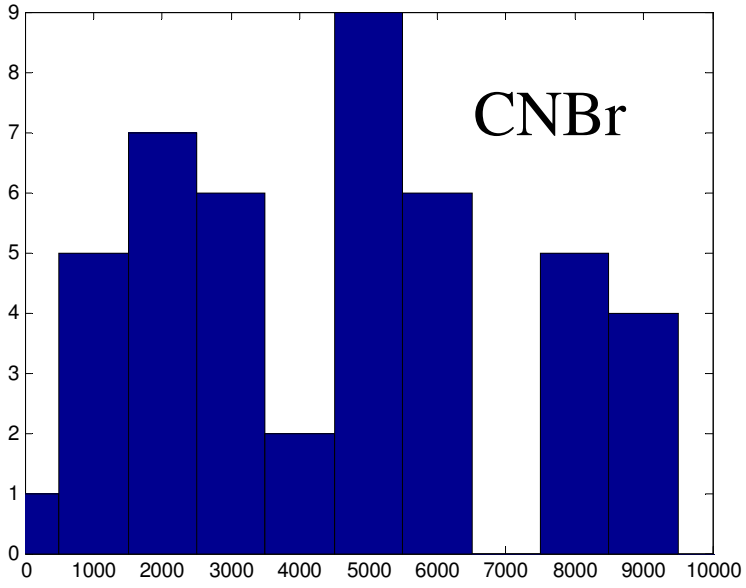
- Don’t want any peptides from enzyme itself

Yeast 20S Proteasome Peptides









Trypsin is the Most Popular Enzyme

- High Enzyme Specificity –cuts all Lys and Arg(to lesser extent followed by Pro).
- Produces peptides with basic C-terminus –give good (CID) fragmentation series
- Majority of peptides 7 -20 amino acids in length
- Relatively inexpensive compared to other proteases

New England BioLab's modified Trypsin is treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inactivate chymotryptic activity(Biochemistry1963v2p 252). It is modified by acetylation of the ε-amino groups of lysine residues to prevent autolysis.

Modified Trypsin Protein Sequence:

```
  1 IVGGYTCAENSVPYQVSLNAGYHFCGGSLINDQWVVSAAHCYQYHIQVRLGEYNID
 61 VLEGGEQFIDASKIIRHPKYSSWTLDNDILLIKLSTPAVINARVSTLLLPSACASA
121 GTECLISGWGNTLSSGVNYPDLLQCLVAPLLSHADCEASYPGQITNNMICAGFLEG
181 GKDSCQGDSGGPVACNGQLQGIVSWGYGCAQKGKPGVYTKVCNYVDWIQETIAANS
```

http://www.neb.com/nebecomm/products/productP8101.asp

Tryptic digest of hemoglobin b chain

VHLTPEEKSA VTALWGKVNV DEVGGEALGR LLVVYPWTQR FFESFGDLST
PDAVMGNPKV KAHGKKVLGA FSDGLAHLDN LKGTFATLSE LHCDKLHVDP
ENFRLLGNVL VCVLAHHFGK EFTPPVQAAY QKVVAGVANA LAHKYH

m/z	Start	End	Sequence
246.1812	60	61	(K)VK(A)
319.1401	145	146	(K)YH(-)
412.2303	62	65	(K)AHGK(K)
932.5200	9	17	(K)SAVTALWGK(V)
952.5098	1	8	(-)VHLTPEEK(S)
1126.5640	96	104	(K)LHVDPENFR(L)
1149.6739	133	144	(K)VVAGVANALAHK(Y)
1274.7256	31	40	(R)LLVVYPWTQR(F)
1314.6648	18	30	(K)VNVDEVGGEALGR(L)
1378.7001	121	132	(K)EFTPPVQAAYQK(V)
1421.6729	83	95	(K)GTFATLSELHCDK(L)
1669.8908	67	82	(K)VLGAFSDGLAHLDNLK(G)
1719.9727	105	120	(R)LLGNVLVCVLAHHFGK(E)
2058.9477	41	59	(R)FFESFGDLSTPDAVMGNPK(V)

Trypsin May Not Be The Best Choice

Rhodopsin (Membrane Protein)

MNGTEGPNFYVPFSNATGVVRSPFEYPQYYLAEPWQFSMLAAYMFLLIVLGFPINFLTLY
VTVQHKKLRTPLNYILLNLAVADLFMVLGGFTSTLYTSLHGYFVFGPTGCNLEGFFATLG
GEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLAGWSRYIP
EGLQCSCGIDYYTLKPEVNNESFVIYMFVVHFTIPMIIIFFCYGQLVFTVKEAAAQQQES
ATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTHQGSNFGPIFMTIPAFFAKSAAI
YNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASATVSKTETSQVAPA

Mass	Residues	Sequence
903.4424	340 – 348	(K)TETSQVAPA (-)
1357.574	315 - 325	(R)NCMLTTICCGK (N)
1403.665	326 - 339	(K)NPLGDDEASATVSK (T)
1490.709	232 - 245	(K)EAAAQQQESATTQK (A)
1499.75	136 - 147	(R)YVVVCKPMSNFR (F)
1727.887	297 - 311	(K)SAAIYNPVIYIMMNK (Q)
2257.071	1 - 21	(-)MNGTEGPNFYVPFSNATGVVR (S)
3231.569	148 - 177	(R)FGENHAIMGVAFTWVMALACAAPPLAGWSR (Y)
5058.603	253 - 296	(R)MVIIMVIAFLICWVPYASVAFYIFTHQGSNFGPIFMTIPAFFAK (S)
5388.76	22 - 66	(R)SPFEYPQYYLAEPWQFSMLAAYMFLLIVLGFPINFLTLYVTVQHK (K)
6503.206	178 - 231	(R)YIPEGLQCSCGIDYYTLKPEVNNESFVIYMFVVHFTIPMIIIFFCYGQLVFTVK (E)
7182.745	70 - 135	(R)TPLNYILLNLAVADLFMVLGGFTSTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLAIER(Y)

- Also, protein solubility may be an issue for membrane proteins

In-gel Digestion

If possible, alkylate proteins before running gel

1. Cut gel
2. **Wash** with 25mM ABC in 70% ACN
3. Reduce with 10mM DTT in 25mM ABC Alkylate with 50mM IAA in 25mM ABC
4. **Wash** with 25mM ABC in 70% CAN (dehydrate)
5. Digest with trypsin in 25mM ABC over night
6. Extract peptides with 5% FA in 50% ACN
7. Remove ACN before LCMSMS analysis

ABC (ammonium bicarbonate) solution, pH=7.8

In-gel digest is very robust, but hydrophobic peptides may not be easily extracted

In-Solution Digestion

- Remove impurity and dissolve in 25 mM ammonium bicarbonate (ABC)
  - Make sure digest mixture is slightly basic (tryptic digest)
  - Remove any inhibitors

- Protein may need to be denatured in order to get efficient digestion
  - ACN: most enzymes can tolerate up to 20-30% ACN
  - Urea or guanidine HCl: as much as tolerated by the enzyme:
    - Trypsin and LysC works in 2M urea or 1M guanidine HCl
  - Add a detergent only if necessary

- 1-10% enzyme (w/w), ~4 h, @37^{o}C

Other Useful Info on Digestion

1. Enzymes isolated from different sources may display very different activity(Roche vs WAKO Lys-C) and have different contaminants

2. Asp-N:
   - •has not been sequenced, so you will not identify Asp-N peptides in database searching.
   - •is ametallo-protease, thus chelating agents will inactivate it.

3. Covalent modifications on the target protein may slow down or prevent cleavages:Lys(Me), Lys(Me2), Lys(Ac)

4. Glycosylationmay stericallyhinder proteolysis
   - •N-linked sugars can be removed by peptide-N-glycosidase F (PNGase F)

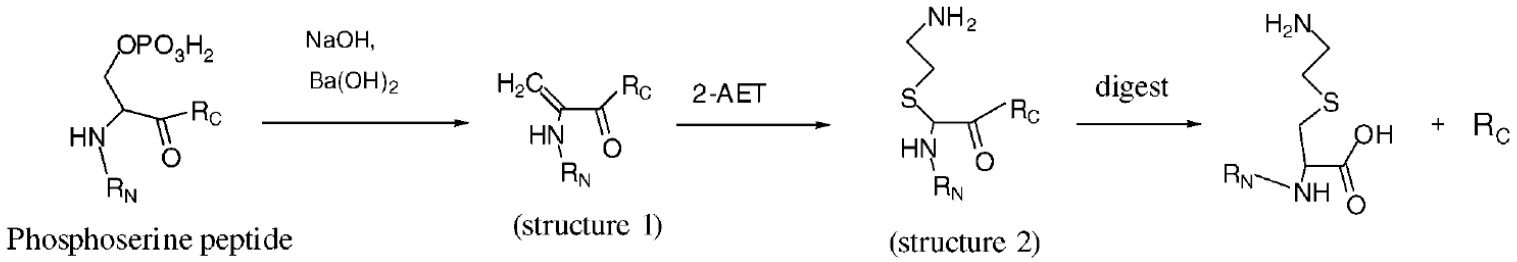
5. Endoproteases are poor exoproteases (cut at ends of peptides)

6. Enzymes may act as ligases – moving a few residues from one terminus to the other → transpeptidation

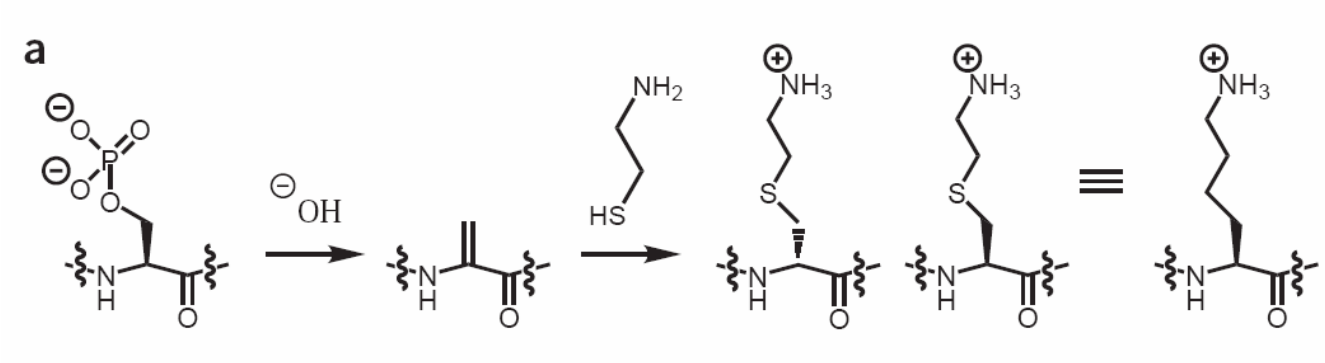
7. Proteases also tend to suffer from substrate inhibition

Other Useful Info on Digestion

Lys-C and trypsin cleave at lysine analogs. These can be generated by beta-elimination of phosphate, glycopeptides or sulfur groups, primarily on serines. The resulting dehydroalanine can be reacted with 2-aminoethanethiol or cysteamine HCl.



Rusnak, F. et al. (2002) *J. Biomol. Tech.* 13, 228-237



Knight, Z et al. (2003) Nat Biotech. 21, 1047-1054

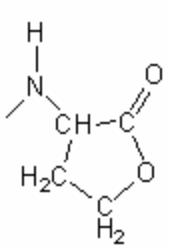
Digestion with Chemicals

- CNBr in 0.1M HCl, neat formic acid, or 70%TFA for 2days
  - Cleave at C-terminal after Met
  - Works with precipitated proteins
  - Can alkylate after digest
  - Methionine becomes homoserine AND homoserine lactone

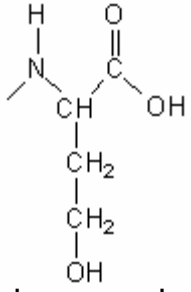
- Asn↓Gly -2M hydroxylamine,2M Guanidine HCl (pH 9), 45^{o}C, 4h

- Asp↓Xxx: 10 mM HCl, 108^{o}C, 2 h.

- Effective for membrane proteins.
  - Will work in-gel; on the blot; in a slurry
  - May access sites when protease cannot.



homoserine lactone



homoserine

Inhibitors

Your sample can continue to change after you isolate it.

•Proteases may still be active.

In the MS analysis of peptides you are assuming peptides are formed by the protease you add.

•Identifying ‘non-specific’ cleaved peptides is more difficult.

•If you are interested in phosphorylation, you should add phosphatase inhibitors.

Salt and Mass Spectrometry

•Proteins and Peptides in mass spectrometry are typically analyzed in a protonated state; i.e. [M+H]+

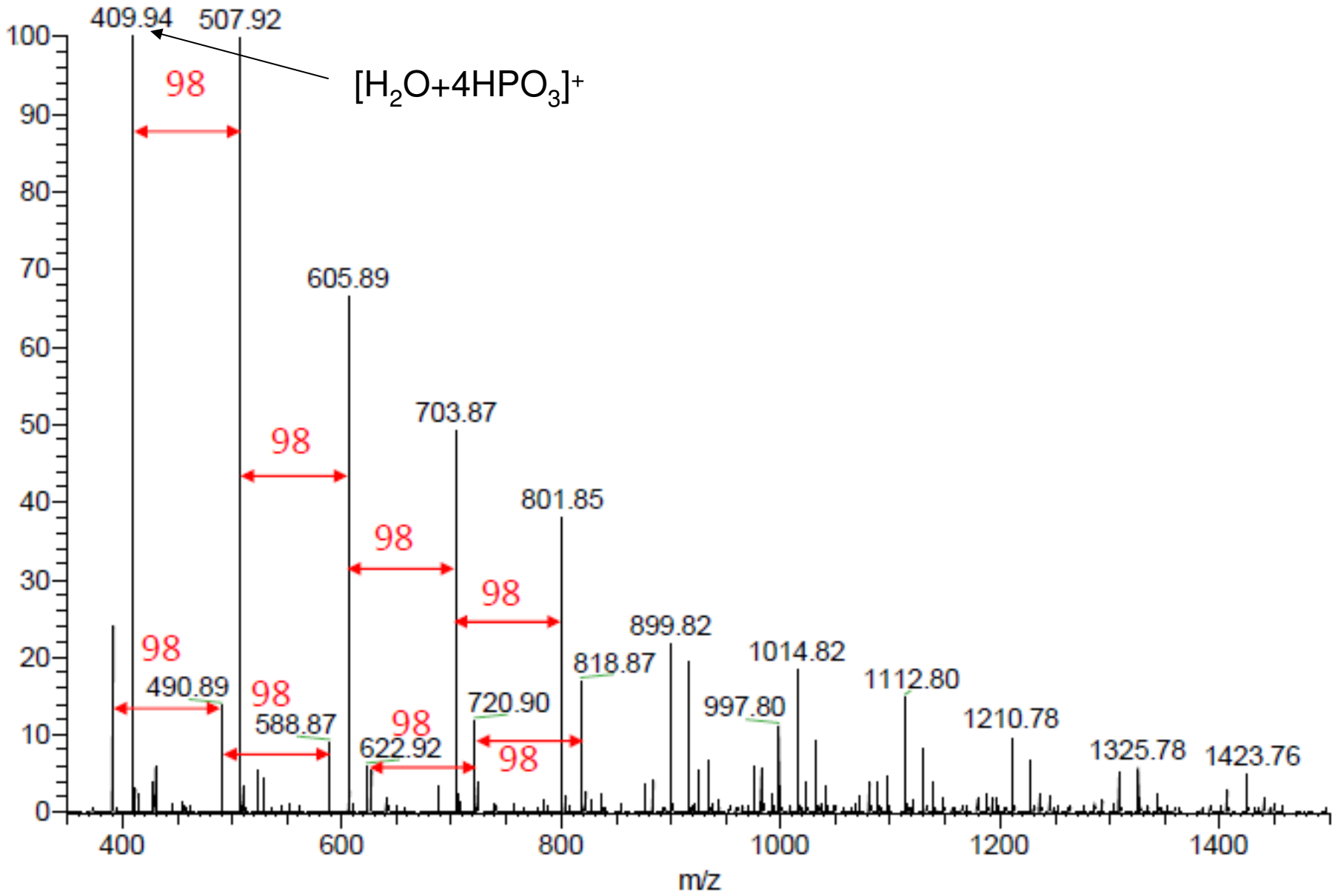
•If metal salts are present, then metal adducts can be formed; e.g. [M+Na]+or [M+K]+.

- •Having protonated and metal adducts makes the spectrum more complicated to interpret.
- •Metal adducted peptides do not fragment as readily as protonated, making identification by fragmentation analysis difficult.

•If a salt crystallizes in the electrospray capillary it can block flow, meaning it has to be replaced and sample is lost. It can also block the orifice into the mass spectrometer.

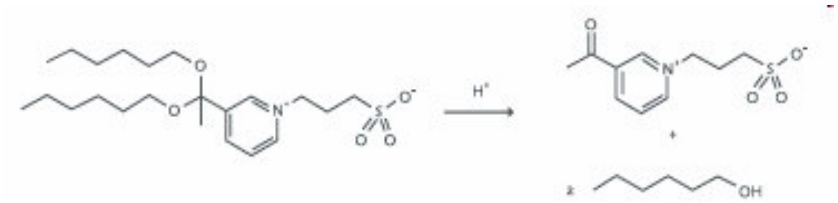
•Some salts form clusters (most notably phosphate), and these can drown out the signal from all other components in the sample.

Phosphate Clusters



Other Common Contaminants

- Detergents
  - Co-elute with peptides
  - Suppress ionization (surface tension)
  - Acid cleavable (PPS Silent Surfactant)

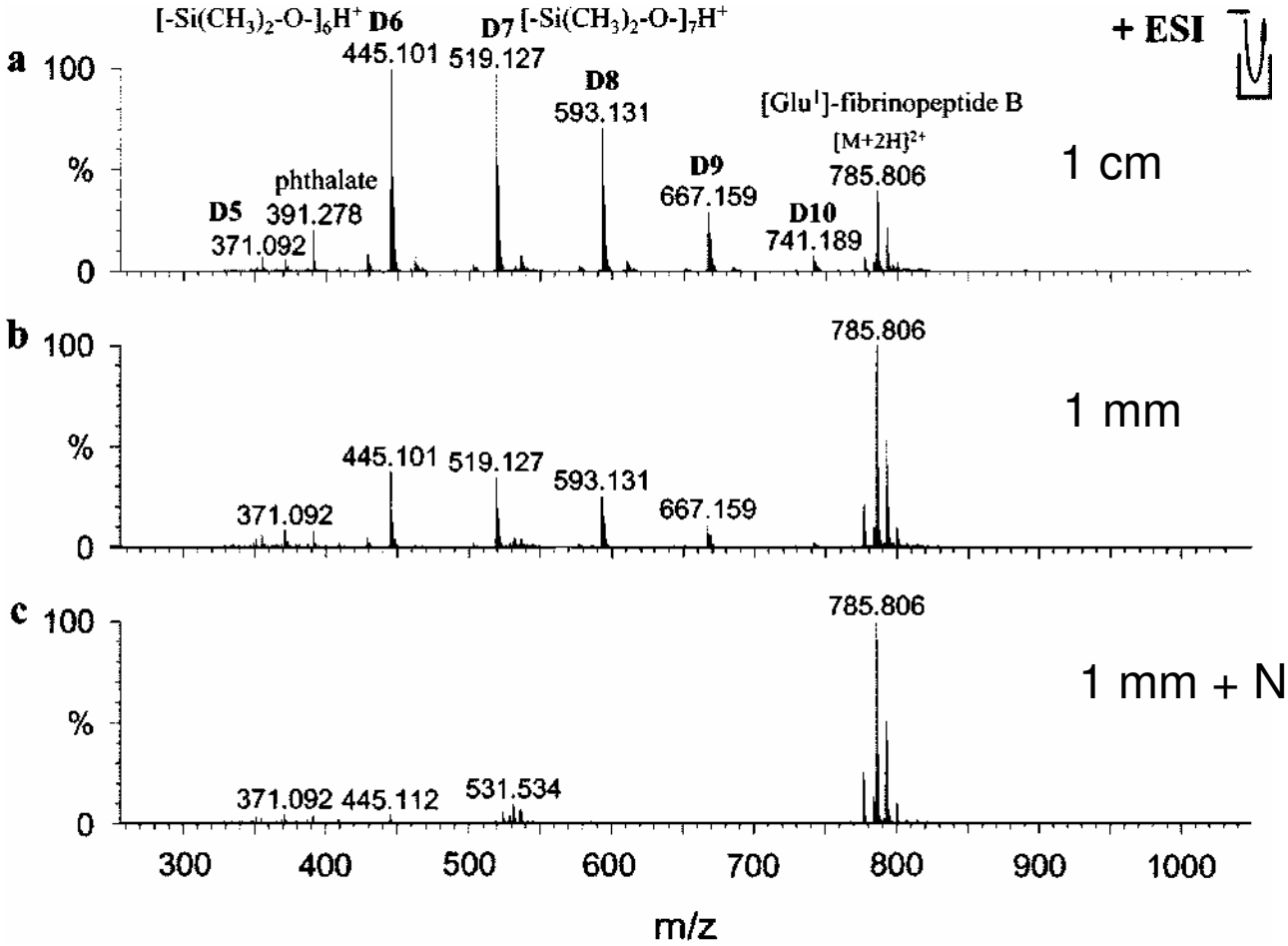


http://www.proteindiscovery.com/pages/products/pps.html

- Glycerol
  - Stabilizing agent for protein storage. Viscous, involatile.
  - As with detergents, it changes ESI behavior
    - and can prevent spray by forming a large droplet

- Trifluoroacetic acid (TFA)
  - Reduce charges of peptide ions
  - Forms adducts with peptide, especially with proteins

Contaminants in the Air

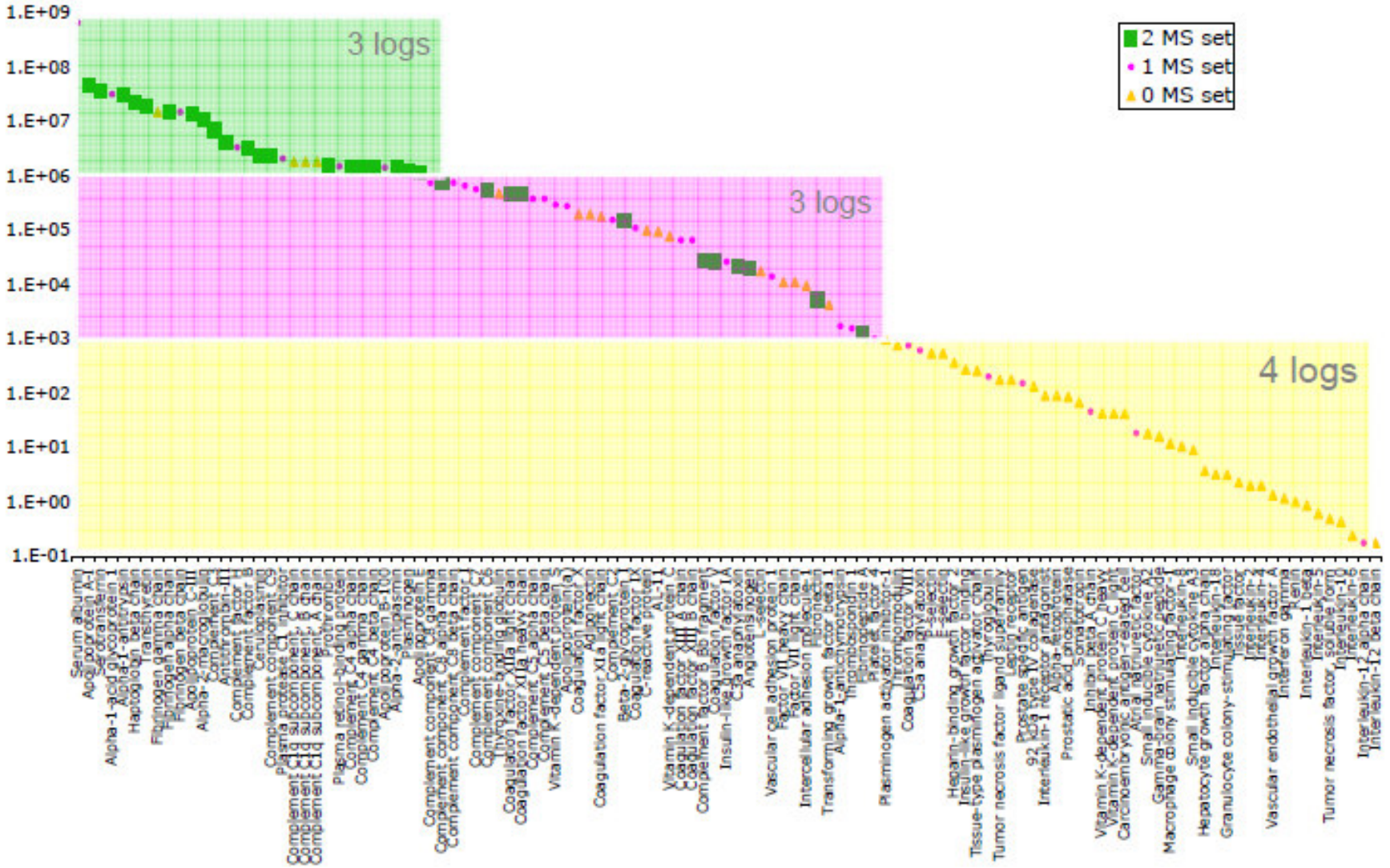


Polydimethylcyclosiloxane ions can be used as mass calibritants
(MCP2005v4p2010)

JMassSpec2003v38p523

The Needs for Protein/Peptide Fractionation

Protein Concentrations in Human Plasma



Immunodepletion of 12 Human Serum Highly Abundant Proteins

α_1-Acid Glycoprotein	Fibrinogen
α_1-Antitrypsin	Haptoglobin
α_2-Macroglobulin	IgA
Albumin	IgG
Apolipoprotein A-I	IgM
Apolipoprotein A-II	Transferrin

JProteomeRes2007v7p828

Protein Separation Methods

Electrophoresis

SDS PAGE; Blue native gel, complexes (MCP2004v8p176)

IEF

Capillary electrophoresis (CE)

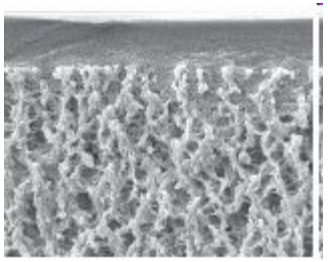
Affinity Separation

Beads: avidin-coated beads

Nanoparticles, goldNP (Cheng,MCP_Oct4, 2009)

Physical Separation

MW cutoff filter, Dialysis membrane



10-400 Ä

0.1- 1.5 µm

Millipore's Ultrafiltration membrane

Protein Separation in MS

Ion mobility – separate by conformation (AnalBioanalChem2008v391p905)

HPLC

HPLC Systems

Autosampler

- Automatically load samples into the system
- Keep samples at low temperature (~10^0C)

Binary pumps

Deliver solvent gradient

UPLC can deliver <100nl/min at ~10,000psi pressure

Detector

MS - online

UV is most common, 208nm for peptides

Conductivity is useful in ion exchange

Sample collector

Useful for manual 2D LC

Online LC

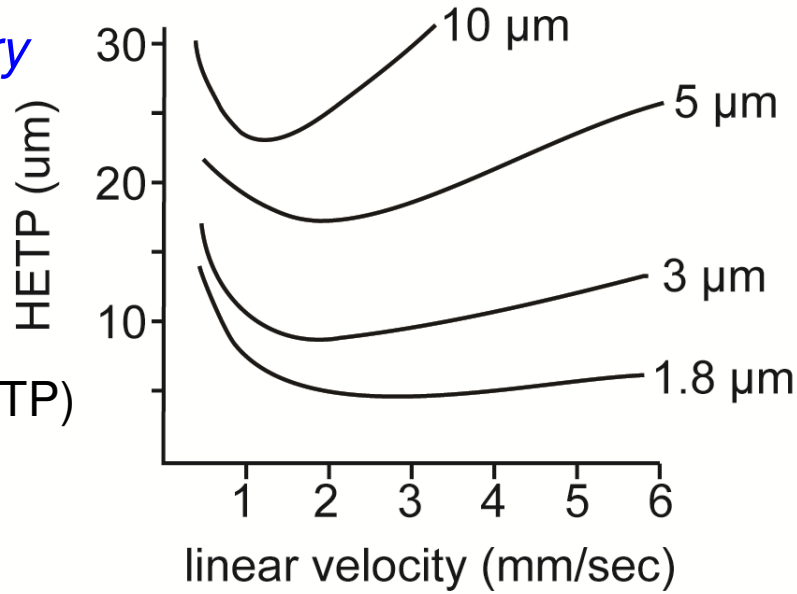
- Low flow rate: ~300nl/min with nanospray
- Reverse phase (RP) and MS compatible mobile phase

HPLC

Separation Theory

Van Deemter equation

$$H = A + \frac{B}{u} + C \cdot u$$



- H = Height equivalent of theoretical plates (HETP)
  (smaller, the better separation)
- A = Eddy-diffusion
- B = Longitudinal diffusion
- C = mass transfer kinetics of
  the analyte between mobile and stationary phase
- *u* = Linear Velocity.

Assumption: analyte is in equilibrium between stationary and mobile phases
(it never happens!)

HETP is a measurement of the column efficiency, determined by particle size flow rate etc. as shown in the figure

UPLC needs high back pressure to operate!
(Small particles and higher linear velocity)

HPLC
Separation Theory

retention factor, $k'_A = (t_R - t_M)/t_M$, is proportional to partition constant ($K = C_m/C_s$) between mobile and stationary phases

selectivity, α , for the separation of two species (A and B)

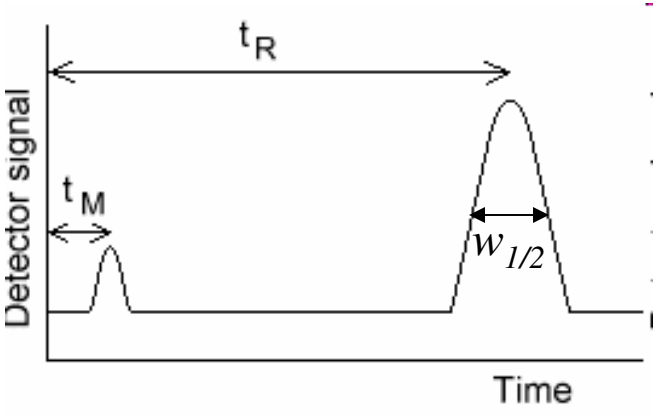
$$\alpha = k'_B / k'_A$$

number of theoretical plates is defined with length of column, *L*, as

$$N = L / HETP$$

and can be calculated from chromatogram

$$N = \frac{5.55 t_R^{\,2}}{w_{1/2}^{\,2}}$$



resolution R of two species, A and B, is defined as

$$R = \frac{(t_R)_B - (t_R)_A}{[(w_{1/2})_A + (w_{1/2})_B]/2}$$

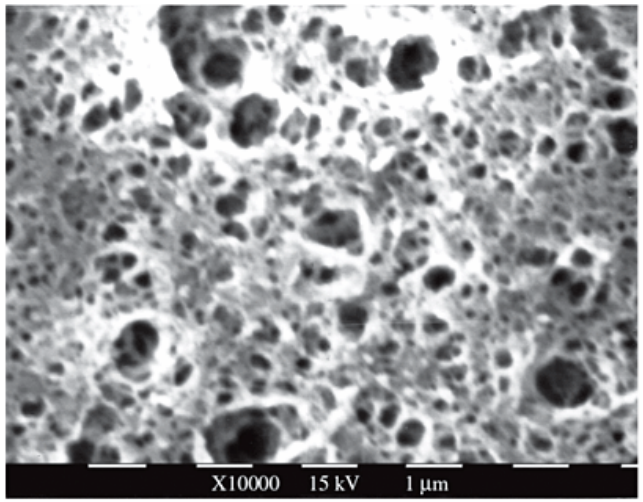
and is related to efficiency (N), selectivity (α), and retention factor (k'_B) as

$$R = \frac{\sqrt{N}}{4}\frac{(\alpha - 1)}{\alpha}\frac{k'_B}{(1 + k'_B)}$$

Master Resolution Equation

Liquid Chromatography
Types

	Proteins	**Peptides**
Reverse Phase (RP)	Low pH	Low pH and High pH
Ion Exchange	WCX and WAX	SCX
Size Exclusion (SEC)	Yes	-



Surface of a copolymer SEC Bead
MatRes2005v8pJan/Mar

Hydrophilic Interaction Chromatography (HILIC)
Is of weak cation exchange (WCX) type

HPLC
Column Formats

	Column Diameter (mm)	Typical Flow Rate	Sample Capacity	Maximum Practical Sample Load
Capillary 	0.075 0.15 0.30 0.50	0.25 µL/min 1 µL/min 5 µL/min 10 µL/min	0.05 µg 0.2 µg 1 µg 2 µg	
Microbore 	1.0	25–50 µL/min	0.05–10 µg	
Narrowbore 	2.1	100–300 µL/min	0.2–50 µg	
Analytical 	4.6	0.5–1.5 mL/min	1–200 µg	10 mg
Semi-preparative 	10	2.5–7.5 mL/min	1,000 µg	50 mg
Preparative 	22	10–30 mL/min	5 mg	200 mg
Process 	50 100	50–100 mL/min 150–300 mL/min	25 mg 125 mg	1,000 mg 5,000 mg

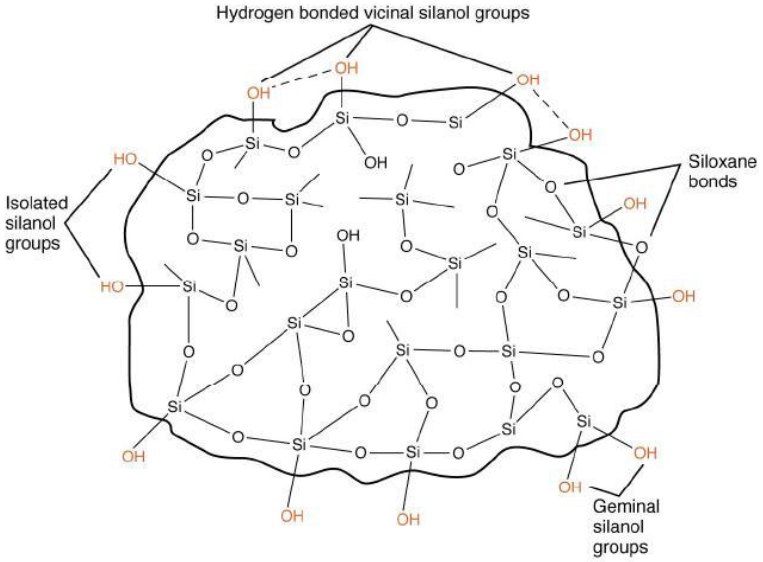
Online LCMSMS

General purpose separation

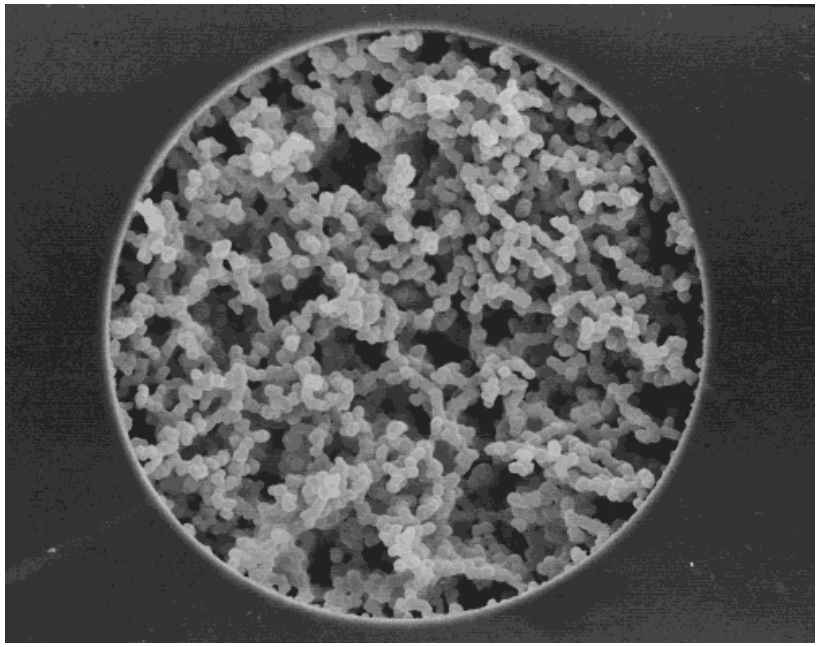
HPLC
Columns

Chromatographic Support

- Silica beads: 5µm diameter, 300A pore size
  Can be packed in lab
- ethyl-bridged hybrid support can stand high pH
  (AnalChem2003v75p6781, Water's Xbridge columns)
- Monolith: can be made with Sol-gel process (JSeparationSci2005v28p1628)
  Low back pressure



A

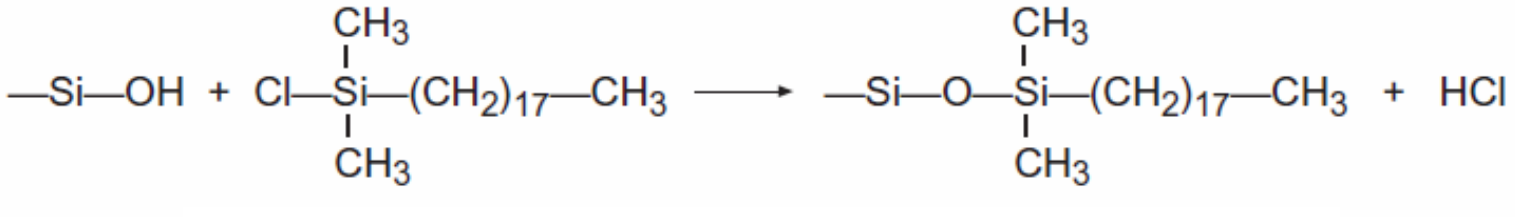


Cross section of a monolith column
AnalChem2000v72p1275

HPLC

Reverse Phase

- Organic solvent – Acetonitrile (CH3CN, ACN)
  - Low viscosity, UV cut off at 190nm
- Peptides online:
  - Stationary phase: C18
    - Solvent A: 0.1% formic acid (FA) in water
    - Solvent B: 0.1% formic acid in acetonitrile
    - Gradient: 0% B – 42% B in 40mins
- Proteins offline:
  - Stationary phase: C4
    - Solvent A: 0.1% trifluoroacetic acid (TFA) in water
    - Solvent B: 0.1% TFA in acetonitrile (ACN)
    - Gradient: 0?% B – 100?% B in 60?mins



Functionize silica support with C18 stationary phase

HPLC
Reverse Phase

Why the ion pairing reagent is needed?

- •Lower pH to 2
  - All but –COOH protonated
- •Reduce charge interaction
  - By masking +ionic groups with –ions
- •TFA is an excellent ion pairing reagent
  - Flurocompounds are hydrophobic
  - TFA is very acidic (pKa, 0.3)
  - But it forms adducts with peptide ions
- •If TFA is not an option, use formic acid or acetic acid
  - Formic acid (pKa, 3.75) works better for peptide separation than acetic acid (pKa, 4.75)

Group on AA	**pKa**
carboxylates	2 to 4
thiols	~ 8
amines	6 to 11
guanidines	12 to 13

It is historical reason to have an ion pairing reagent, because silica beads is not stable when pH>8.

LC Retention Time Prediction

Quantitative structure-retention relationships (QSRR) (ChemRev2007v107p3212)

t_r = (t_r for AAs) + (t_r from Van der Waals volume) + (t_r from partition)

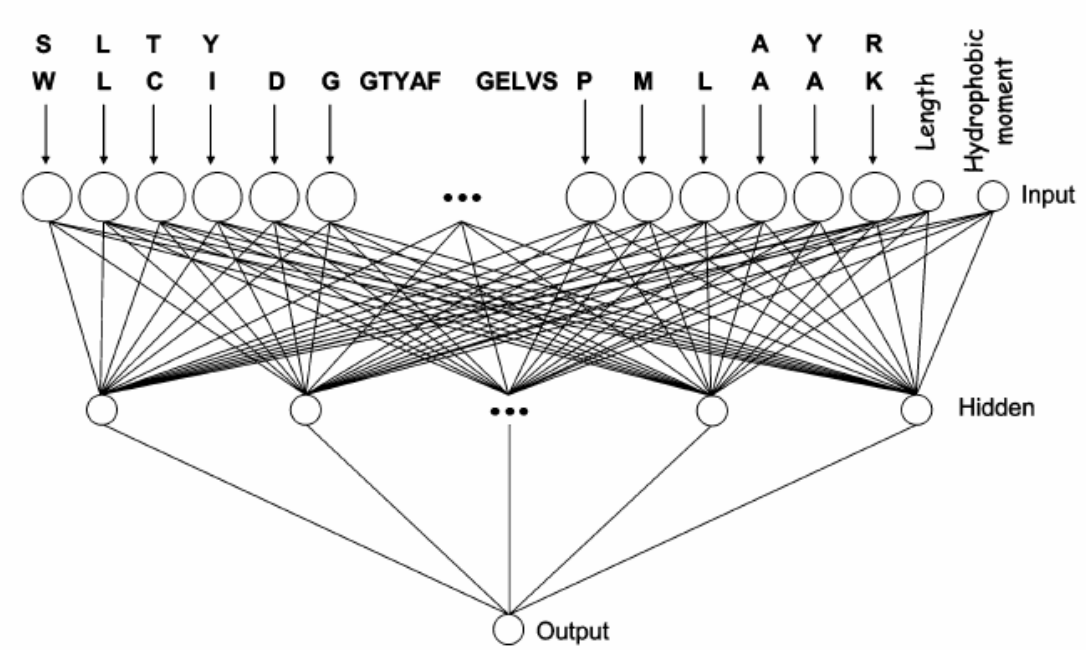
Relative hydrophobicity(MCP2004v3p908)

Retention coefficients of amino acid residues

http://hs2.proteome.ca/SSRCalc/SSRCalc.html

Artificial Neural Network (ANN) (AnalChem2006v78p5026, JProteomeRes2006v5p3312)

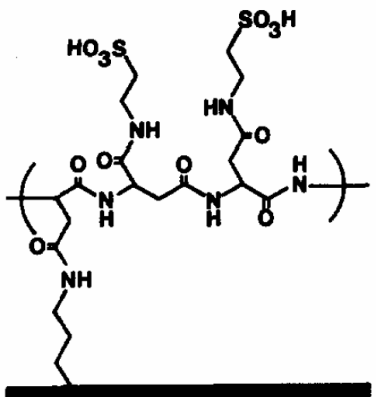
Use known data to train the network



HPLC
Columns

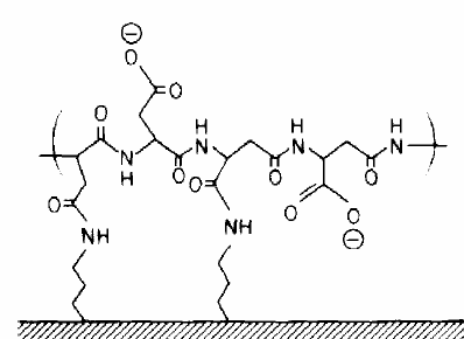
Stantionary phase

- *Strong Cation Exchange (SCX)*
  PolySULFOETHYL Aspartamide
  Salt gradient, pH=3
  Used as 1st dimension of 2DLC



SCX
JChromatog1988v443p85

- *Hydrophilic Interaction Chromatography (HILIC)*
  WCX (PolyCAT A): Poly Aspartic Acid
  Salt or pH gradient (histone PTM variants, MCP2009v8p2266)



HILIC (WCX)
JChromatog1983v266p23

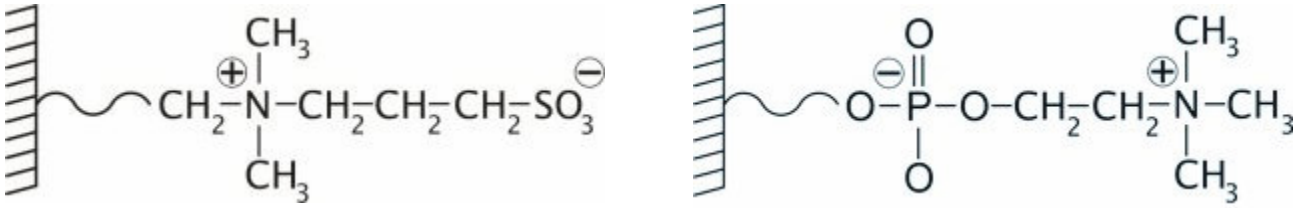
HPLC

Columns

Electrostatic Repulsion Hydrophilic Interaction Chromatography (ERLIC)

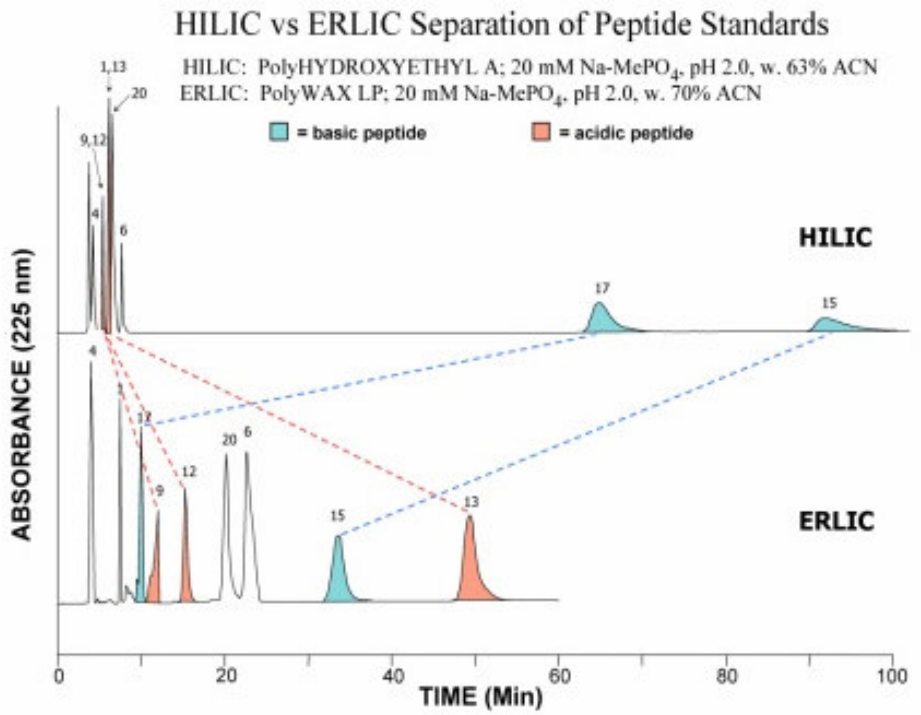
(AnalChem2008v80p62)

- Zwitterionic stationary phase (http://www.nestgrp.com/protocols/polylc/erlic/erlic.shtml)



- Excellent separation behavior according to peptide charge states over a wide pH range

- "Ideal" phosphopeptide separation (JProteomeRes2008v7p4869)



HPLC

Packing your own nanoLC columns

•Use a blank column with frit at the end

or frit at the spray tip

(http://www.newobjective.com/products/cols_index.html)

•Choose desired resin (packing material, buck media,…)

Type: RP, SCX, HILIC, etc

Particle size: 3µm, 5µm

Pore size: 300Å, 1000Å

Can buy hilic resin from:

(http://www.nestgrp.com/prices/PolyLC.shtml#polycatb)

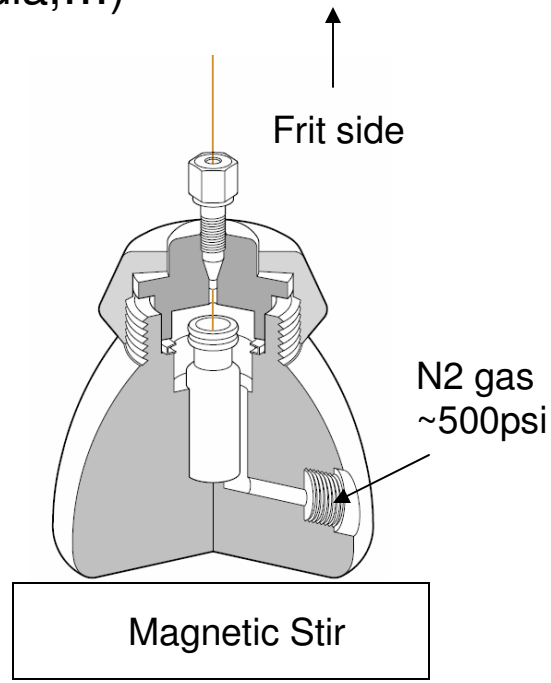
•Pack with a "bomb"

10% resin + 90% MeOH in a glass vial

Add a Teflon coated stir bar

Increase N2 pressure while stirring

http://www.celtaingenieros.com/uploads/pdf/nanobaume.pdf



A 100µm X 10cm column only uses ~3 mg of resin!

Multi-Dimensional Liquid Chromatography

Case Studies

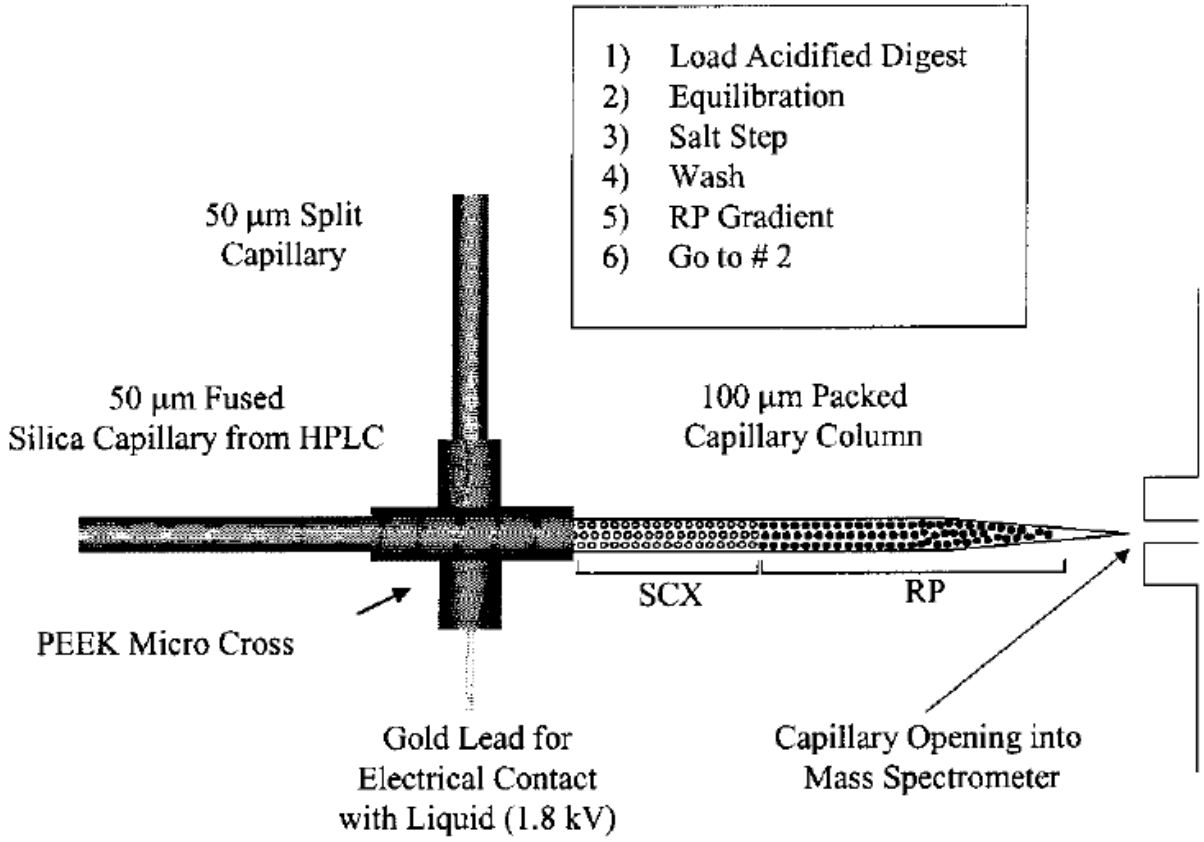
- LCs at different dimensions should be orthogonal
  - Separation based on different properties

- SCX-RP
  - General proteomics strategy with tryptic digest
  - Lys-N digest may be better

- High pH RP - low pH RP

- RP – HILIC
  - Histone analysis

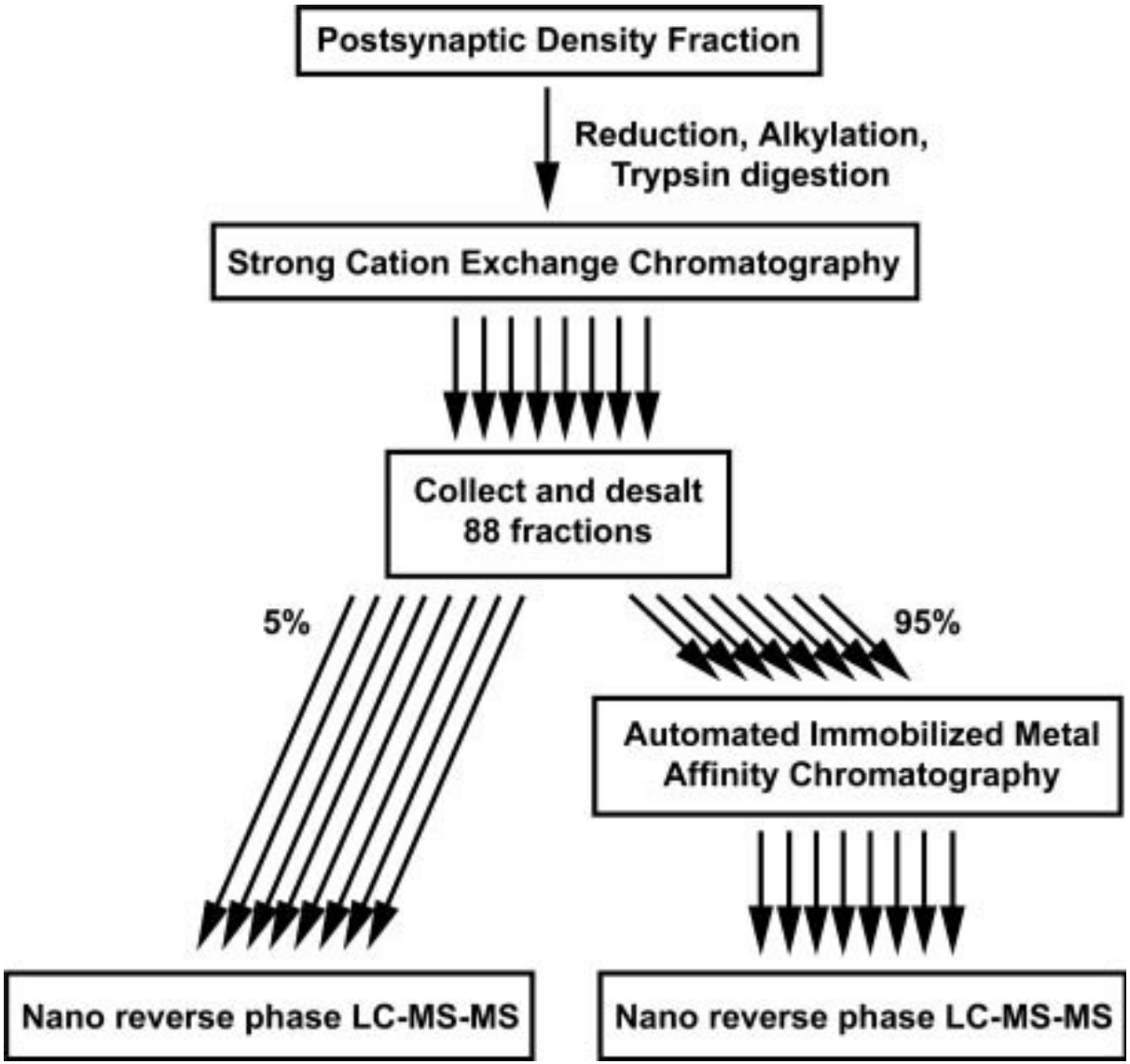
MudPIT (Multidimensional Protein Identification Technology)



SCX: Partisphere

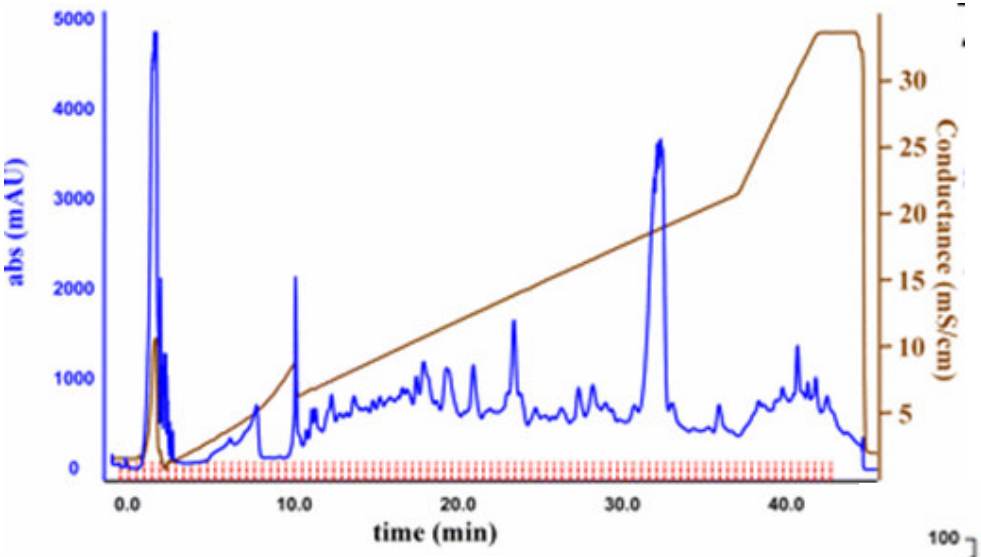
AnalChem2001v73p5683

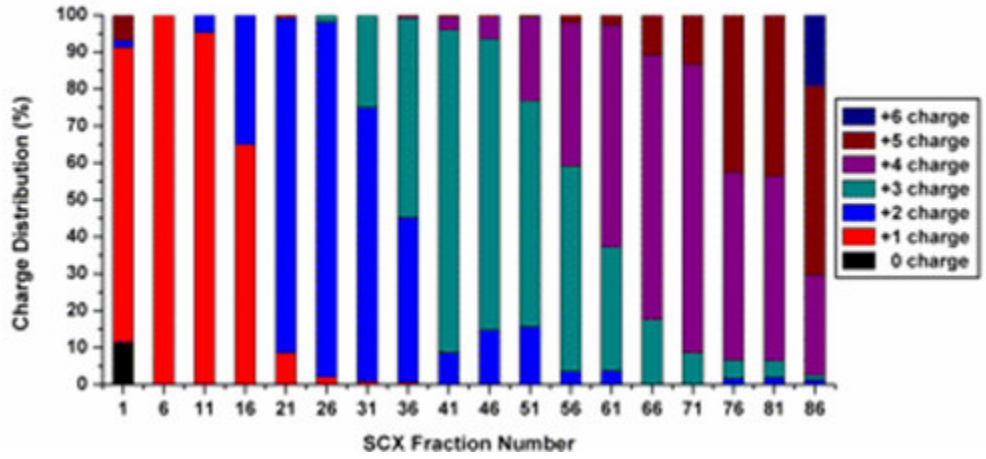
2D LC Identification of Phosphopeptides



SCX Separation of Tryptic Peptides

Column: 5X115-mm polysulfoethyl A
Gradient: 0 to 350 mM KCl in 30% acetonitrile,
5 mM KH2PO4, pH 2.7 in 90mins

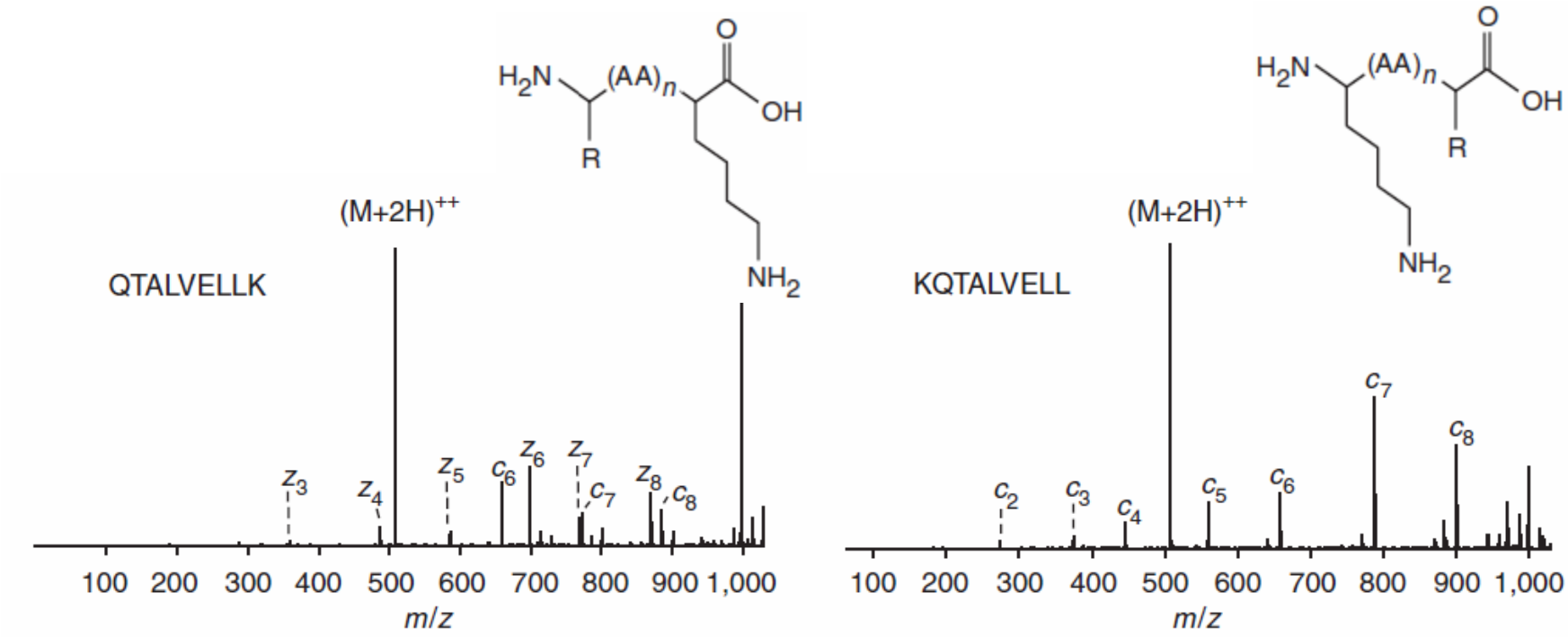




MCP2006v5p914

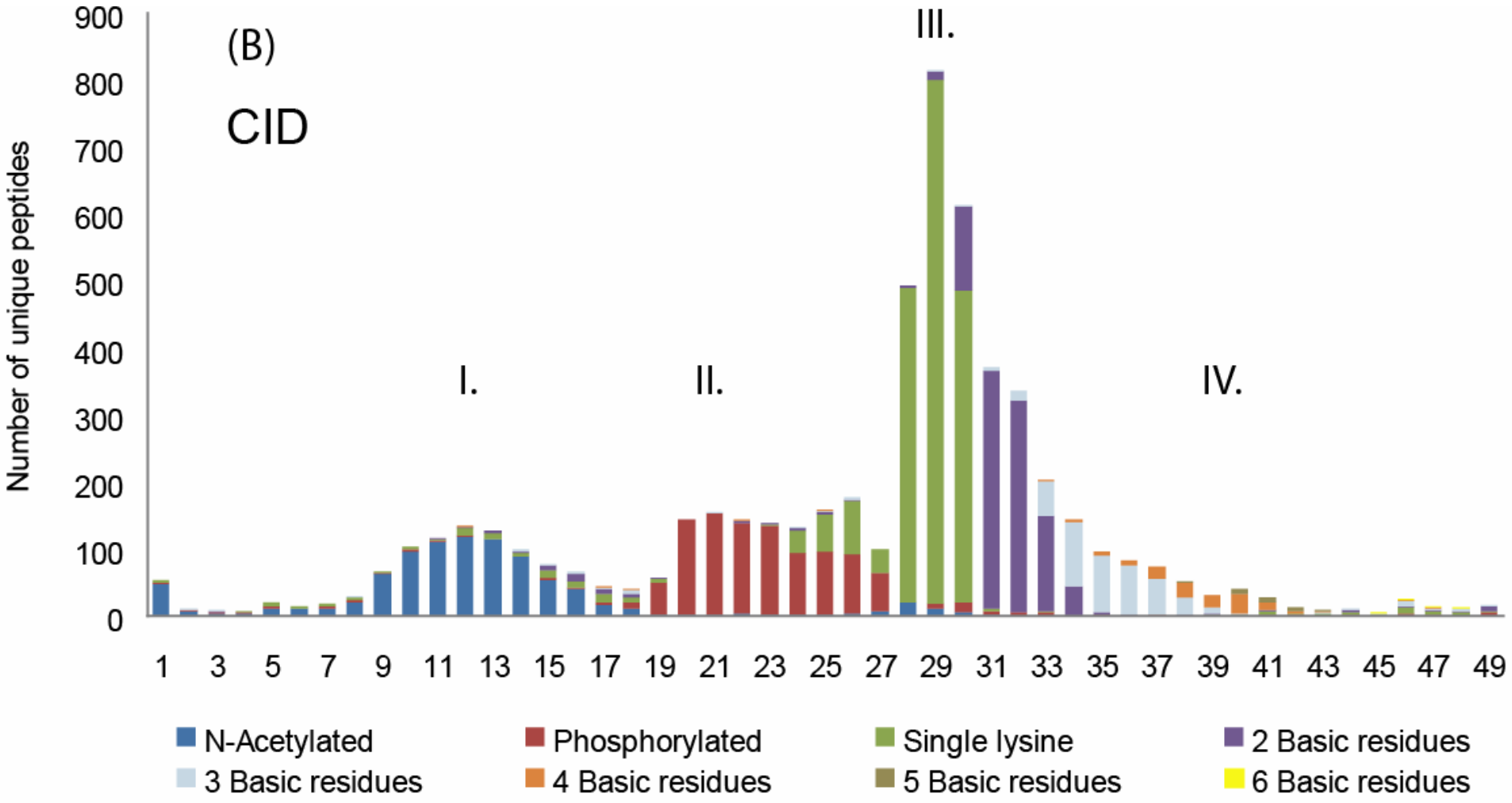
Lys-N vs Trypsin Digestion

1. Lys-N only incorporates a single O into peptide
   Good for 18O labeling (MCP2005v4p1550)
   Trypsin produces a mixture of <u>two</u> oxygen atoms
   (Electrophoresis1996v17p945)
2. Charge distributions are different
   MSMS fragmentation patterns
3. Lys-N cleaves –X↓KP- and trypsin does not cleave –KP-



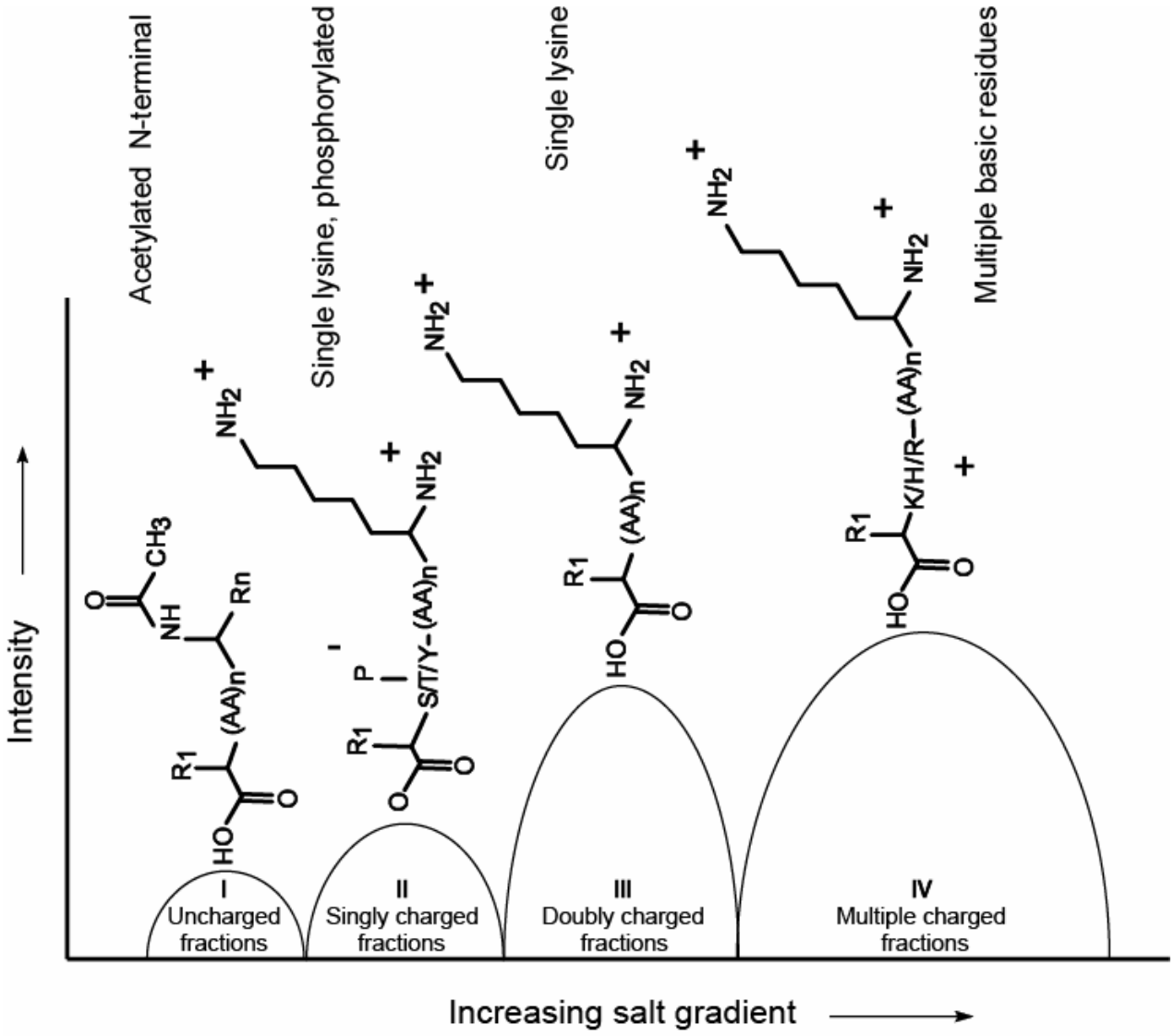
NatureMethos2008v5p405

SCX Separation of Lys-N Peptides



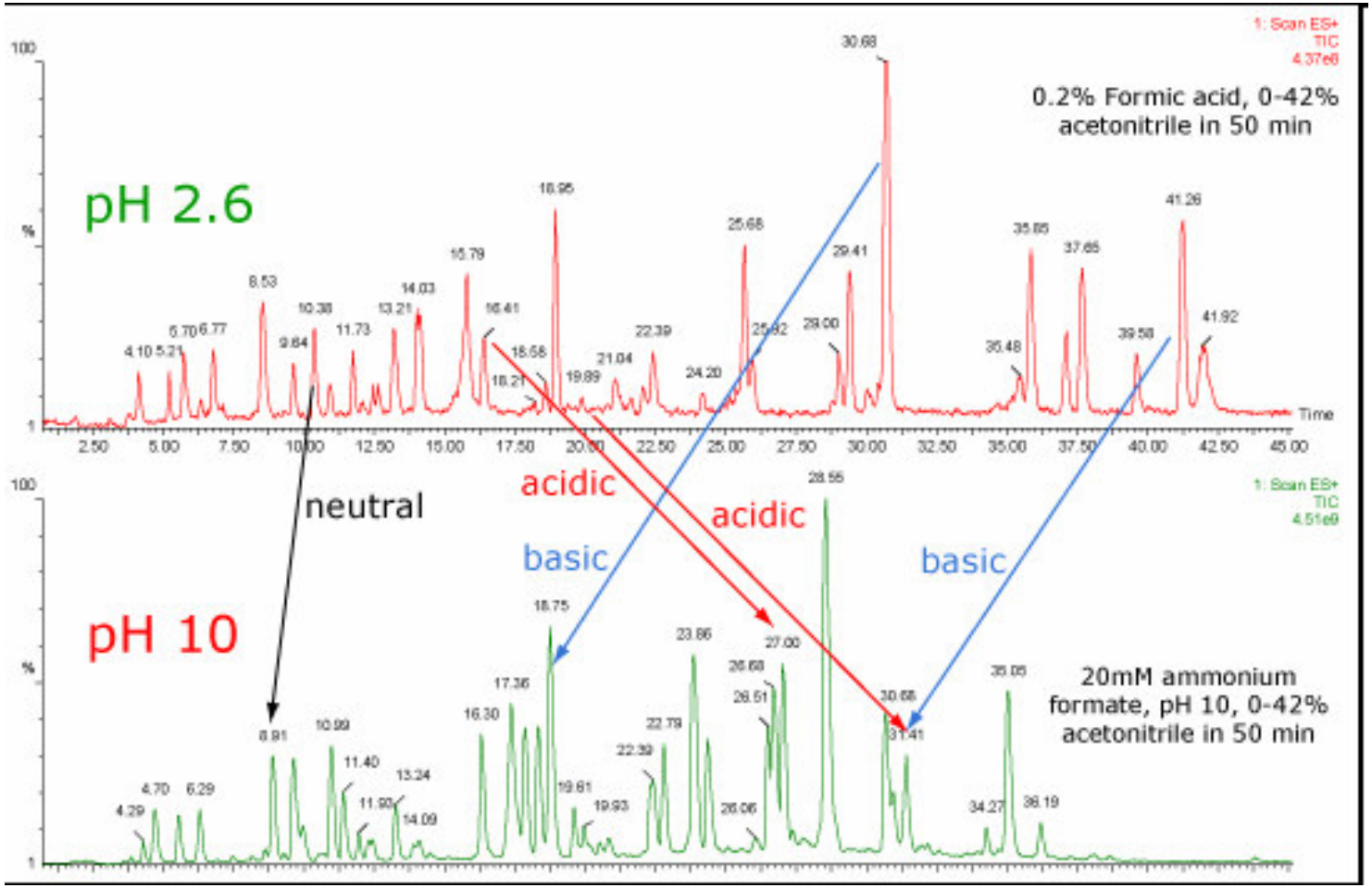
MolCelProteomics2009v8p190

SCX Separation of Lys-N Phosphopeptides



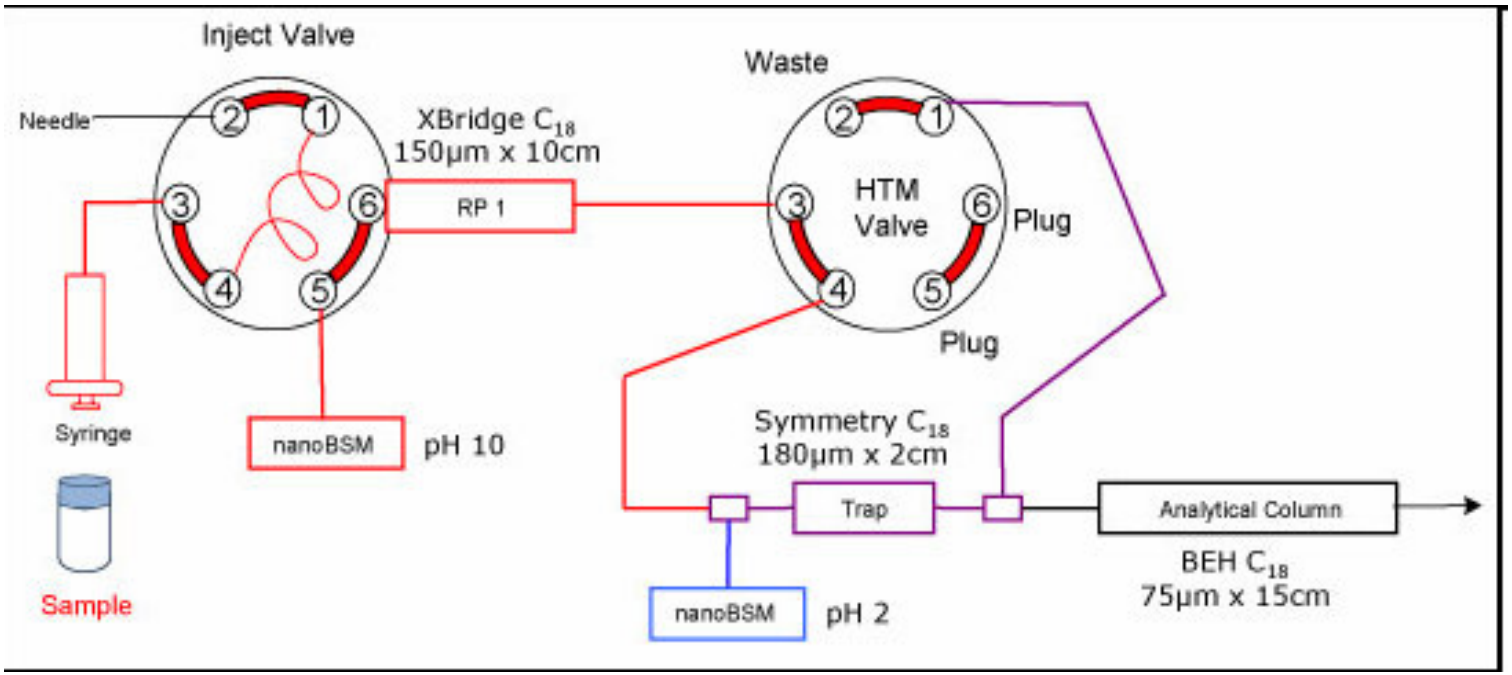
MolCelProteomics2009v8p190

Low pH RPLC vs High pH RPLC



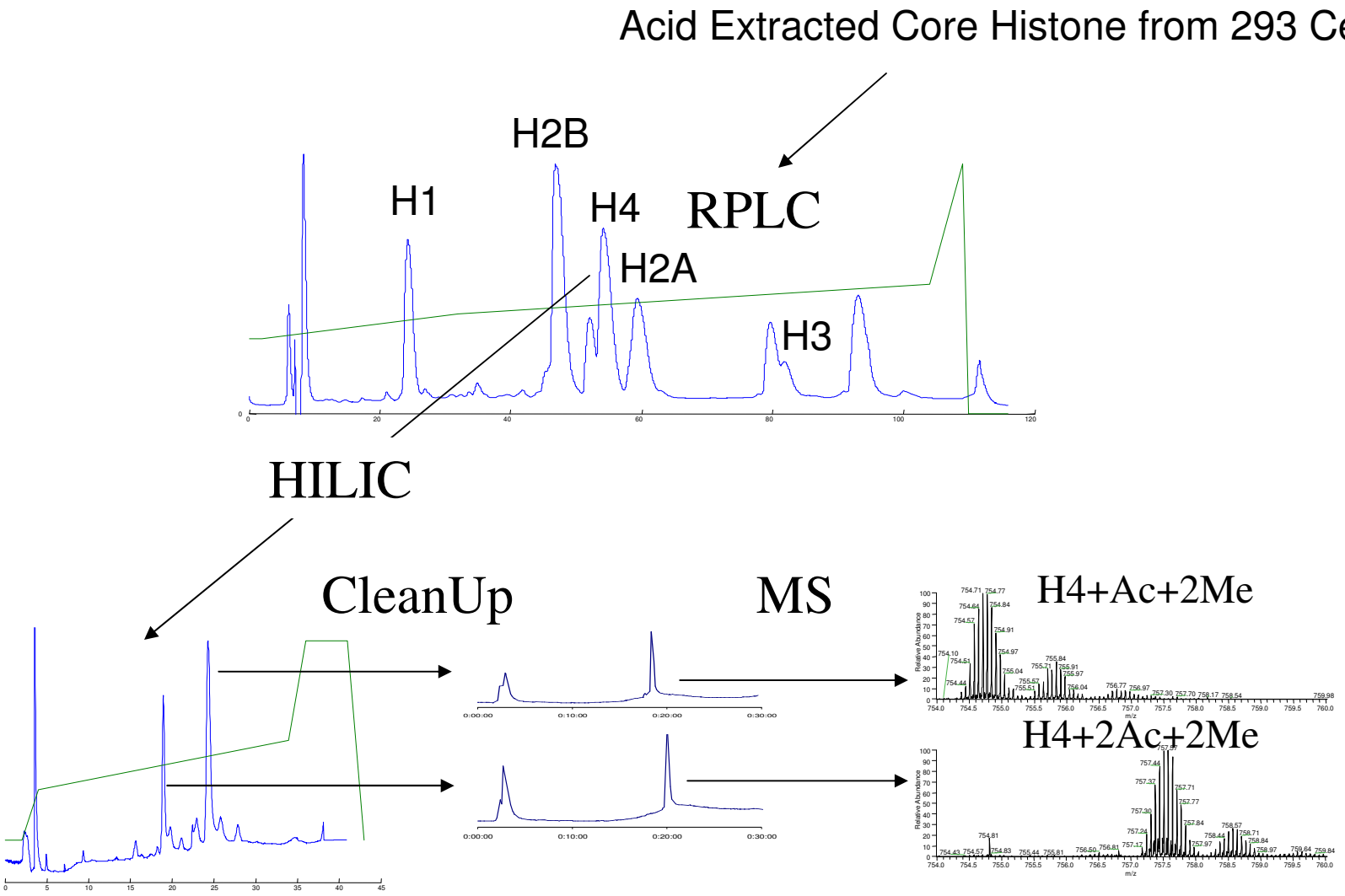
http://www.waters.com/waters/nav.htm?locale=en_US&cid=10070785

2DLC based on High pH RPLC and Low pH RPLC



http://www.waters.com/waters/nav.htm?locale=en_US&cid=10070785#

LC Separations for Histone Analysis



For online HILIC with volatile salt and pH gradient, see (MCP2009v8p2266)

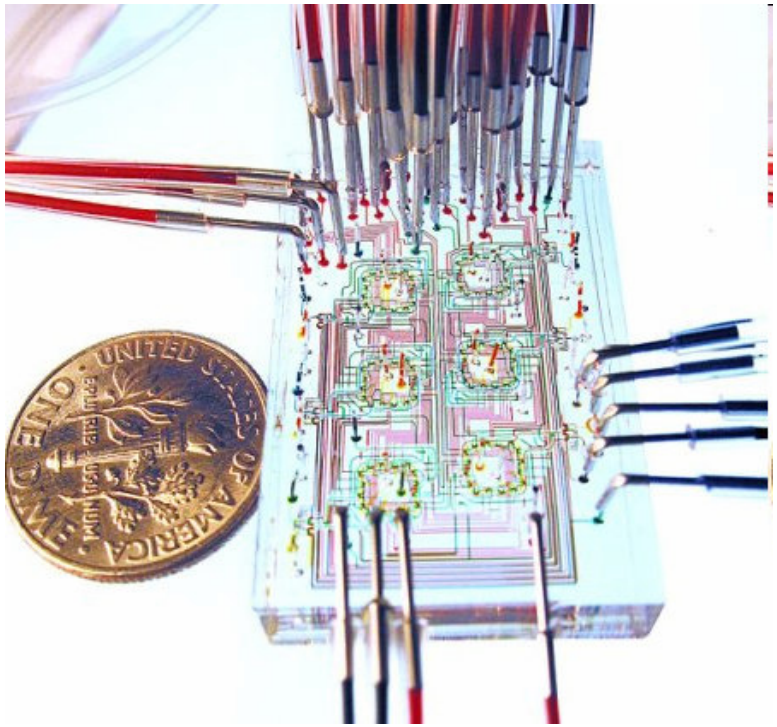
Microfluidics

Small channel dimensions

>1mm, *Laminar flow*
High surface to volume ratio

Device integration

Separation channels
    Packed or CE
Reactors
Mixers
Valves



Base materials

Polydimethylsiloxane (PDMS)
Glass, quartz
Silicon
Polycarbonate

Fabrication techniques

molding/bonding
NaOH etching
Plasma etching
Injection molding

Integrated circuits →Microelectromechanical systems (MEMS)
→Microfluidics → Biological Applications

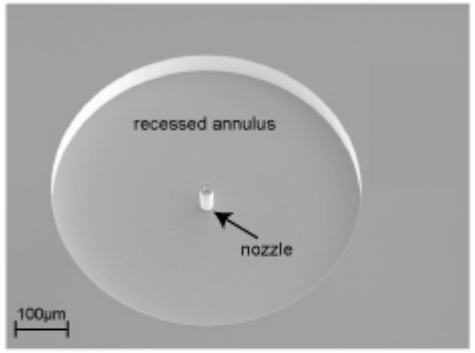
Nature2006v442p374, p394

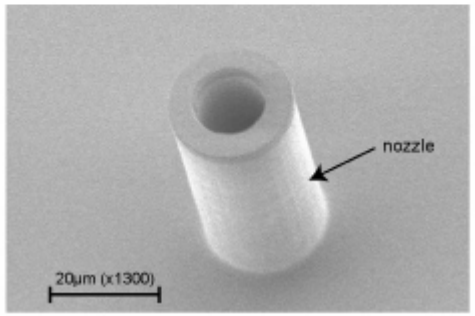
Microfluidics

Interface with MS

Nanomate

ESI spary tip array
Si deep etching





www.advion.com

- Automated sample loading for infusion
- LC sample collection and reanalysis
- Top-down
- System optimization

LabChip2007v7p1394

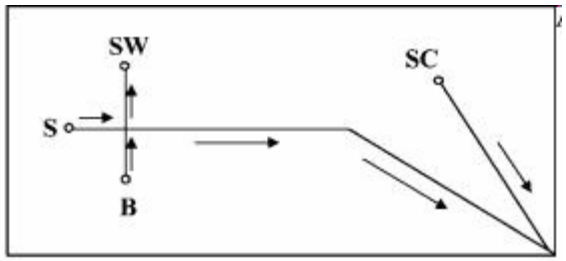
Microfluidics in Proteomics

Examples

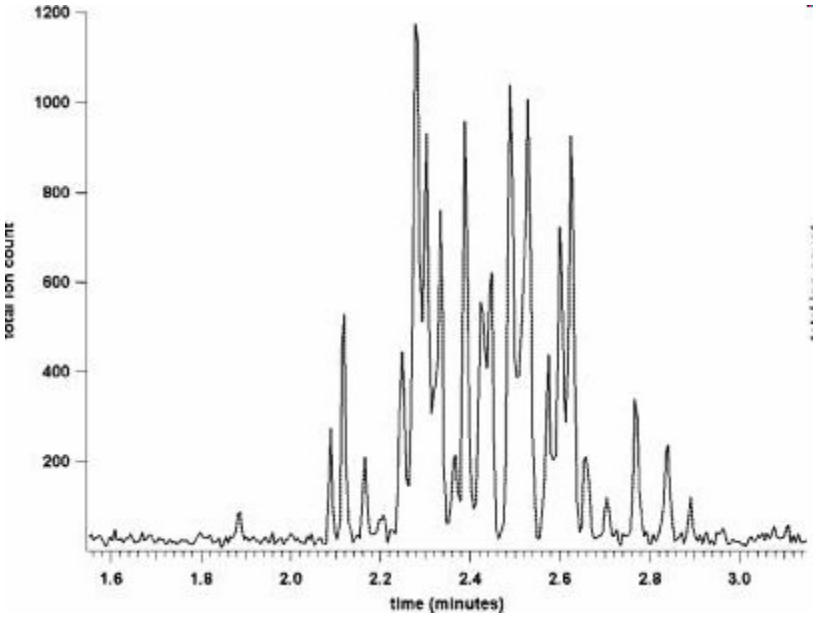
Immobilized microfluidic enzymatic reactors (IMERs)

- 10s digestion time
- Reduction of autolysis
- Easy separation interface
- Utilize monolithic material
  - High surface area
  - High mechanical strength
- Integrate nano-materials
  - High and homogeneous surface

Glass chip for electrophoretic separation and ionization of peptides
(AnalChem2008v80p6881)



BSA tryptic digest



Electrophoresis2004v25p3550
AnalChimActa2009v632p1

Microfluidics

Integrated Enrichment and NanoLC System

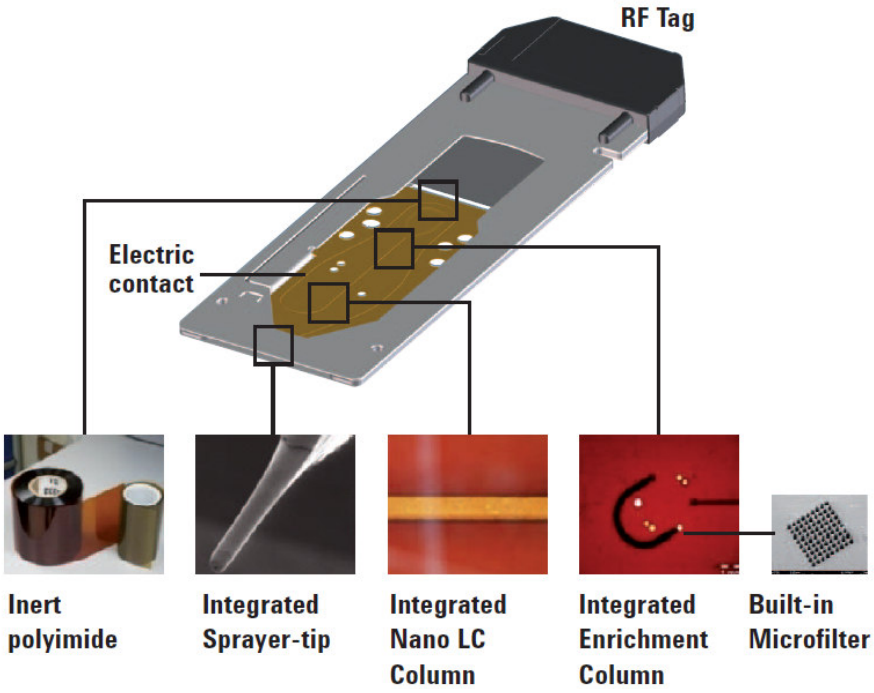
Agilent HPLC-chip

Functionility

- Graphitized carbon column for oligo-saccharide separation
- TiO2 column for phosphopeptide enrichment
- Trap column
- NanoRP analytical LC column
- Spray tip

Fabrication

- Polyimide
- Inkjet printhead processes

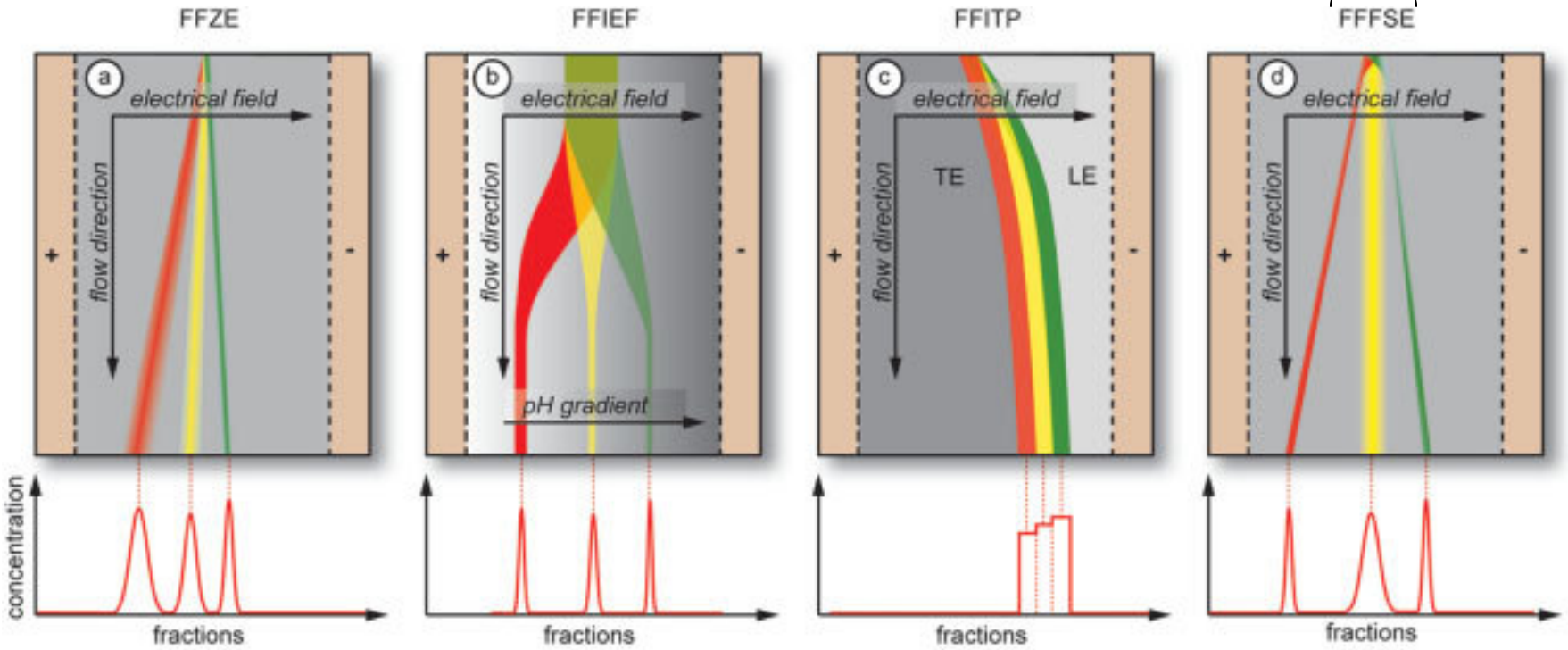


AnalChem2005v77p527
http://www.chem.agilent.com/en-us/products/instruments/lc/1200serieshplc-chipmssystem/pages/default.aspx

Microfluidics

Free-Flow Zone Electrophoresis

Different buffer



Zone
- Const. pH
- Const. E

IEF
- pH gradient
- Const. E

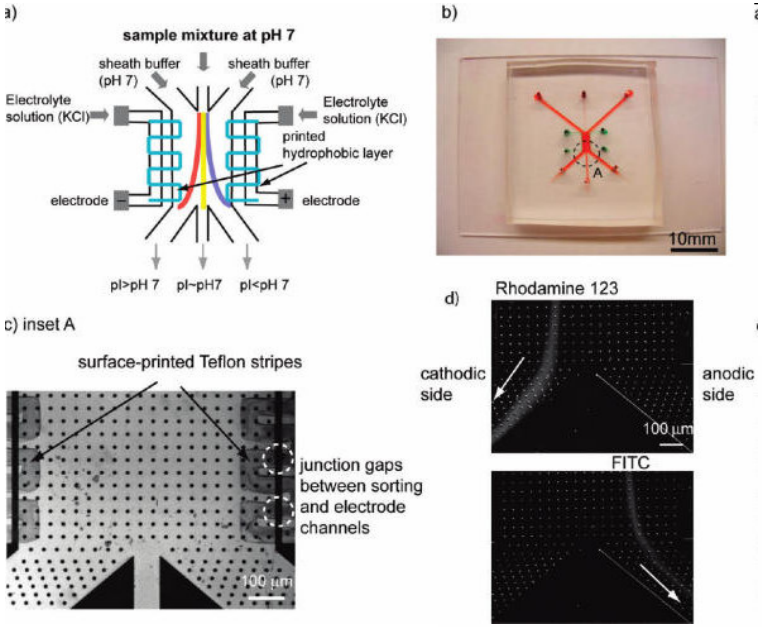
Isotachophoresis
- Two buffers
- Const. E

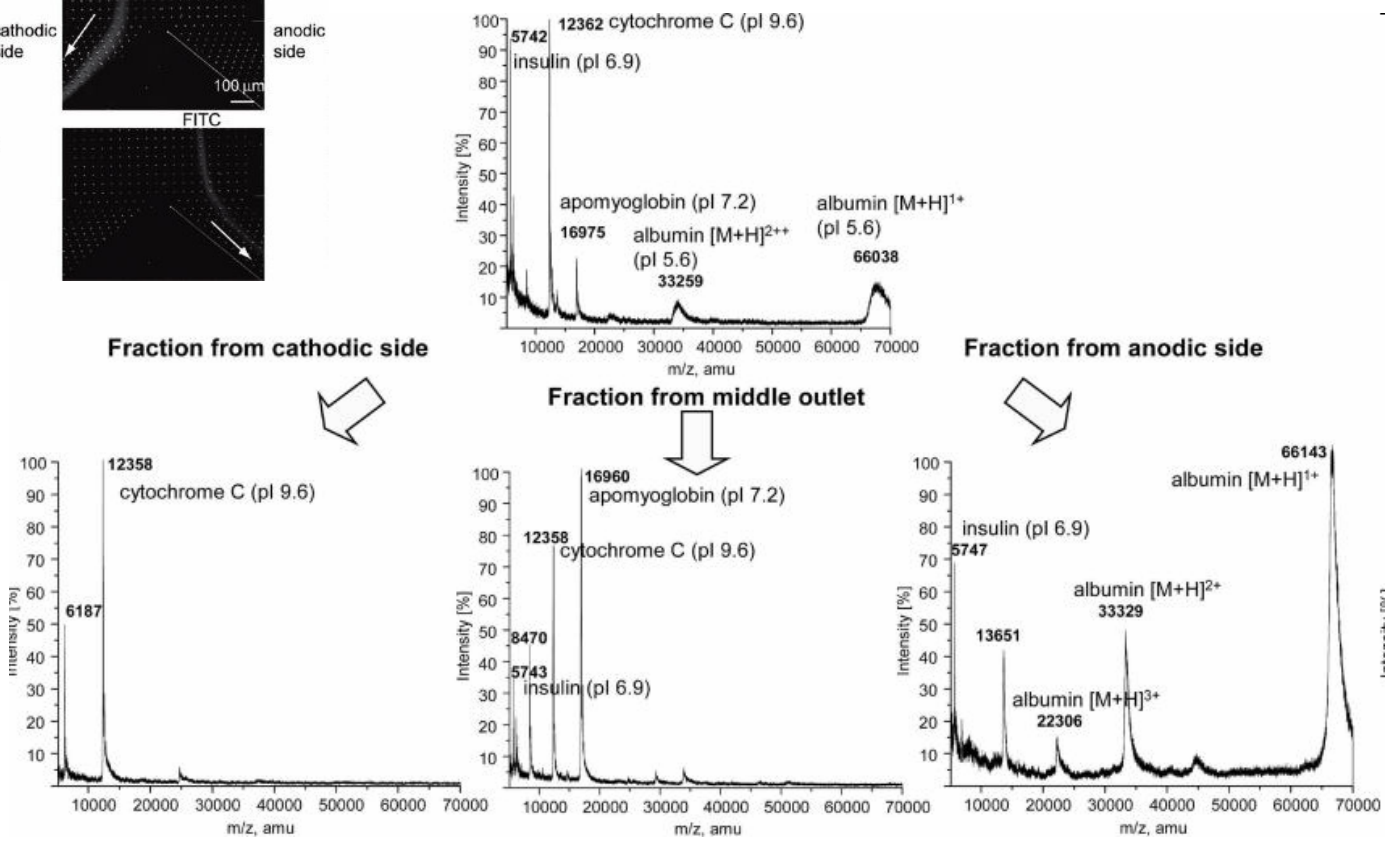
Field step
- Const. pH
- Field gradient

Electrophoresis2008v29p977

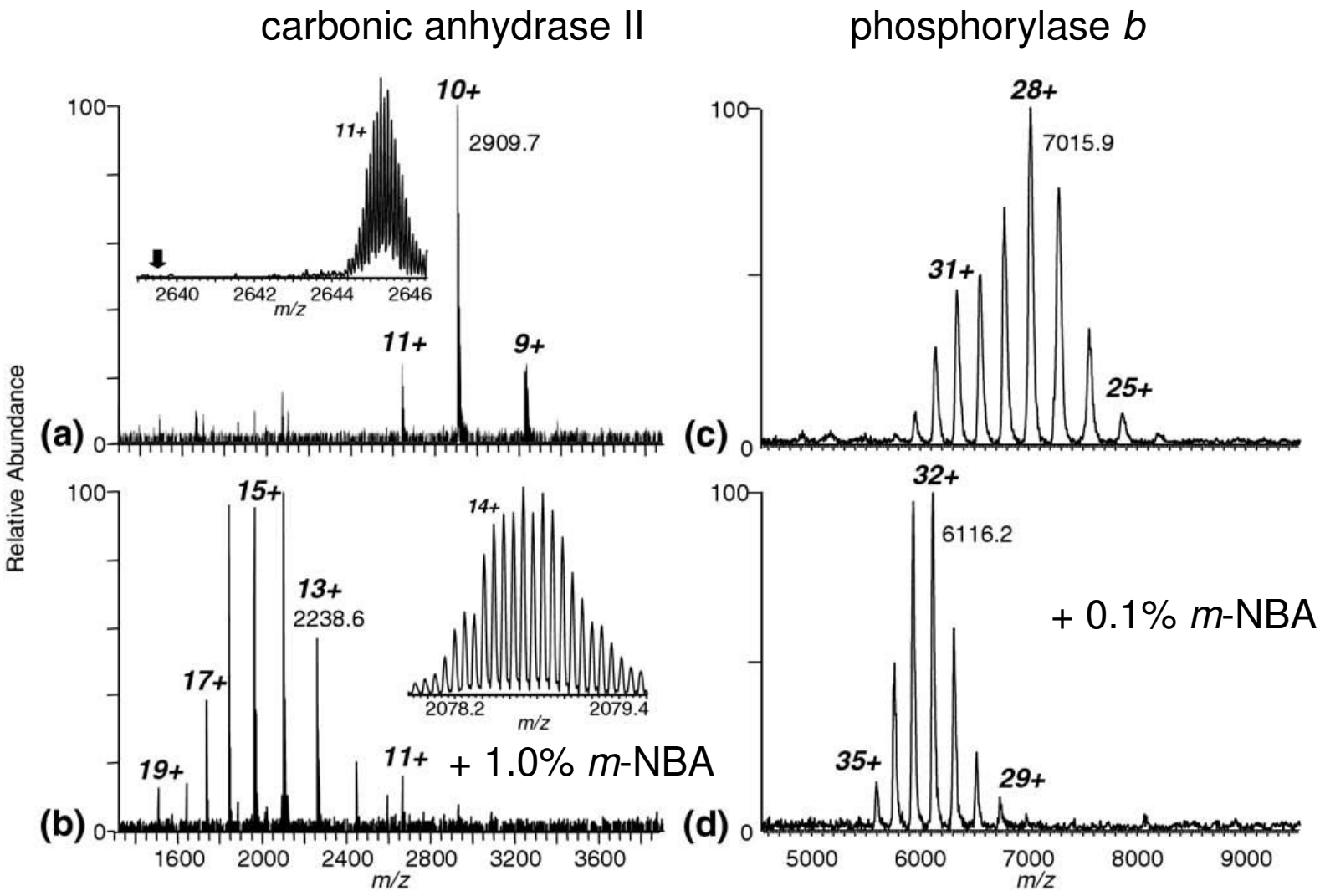
Microfluidics

Free-Flow Zone Electrophoresis



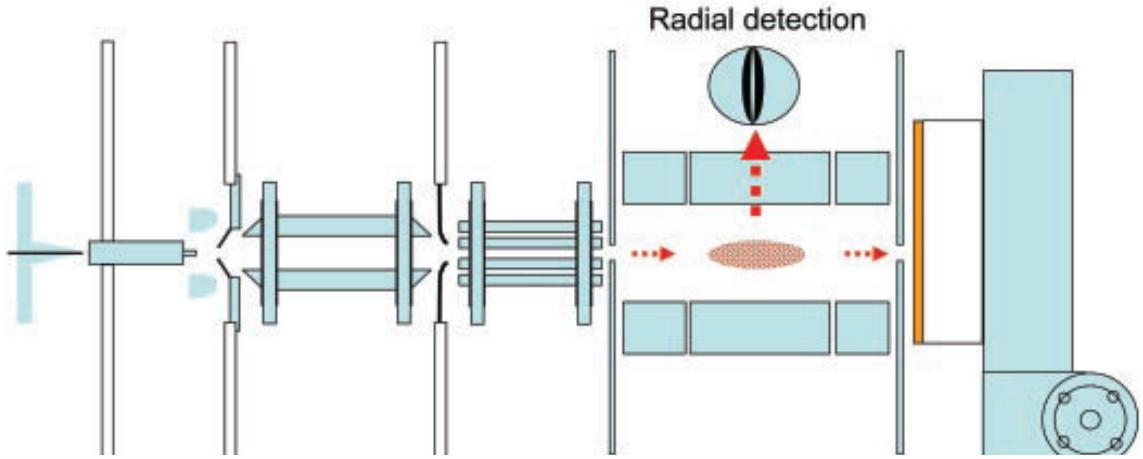


Increase Charge States in ESI



JASMS2009v20p593
AnalChem2007v79p9243 – for peptide ETD

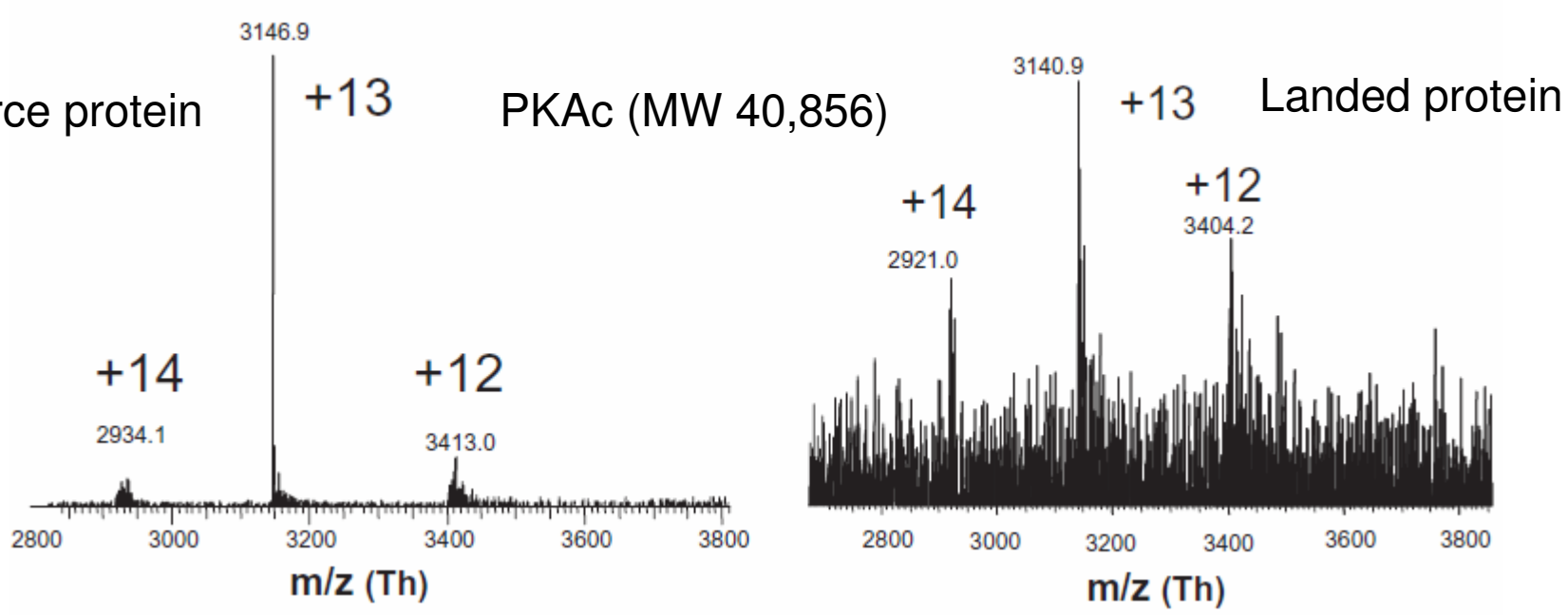
Soft-landing of Mass Selected Ions



Source protein

PKAc (MW 40,856)

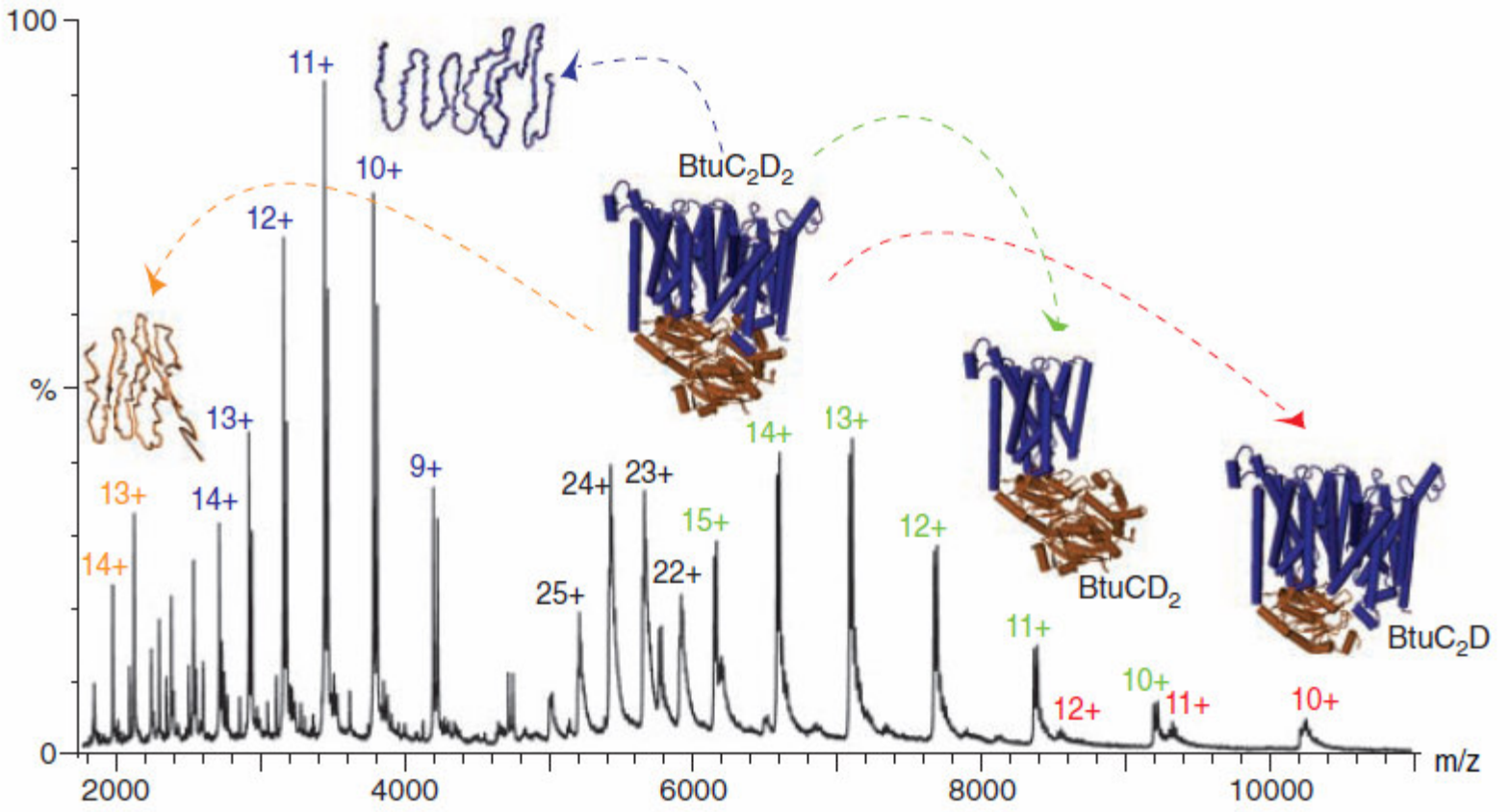
Landed protein



Science2003v301p1351

Direct MS Analysis of Intact Protein Complex

- adenosine 5´-triphosphate (ATP)–binding cassette transporter ($BtuC_2D_2$)
- nanoelectrospray of membrane protein complex in a micellar solution
- CID of protein complex into components
- Found α-N-Dgluconyl-His tag as a modification on BtC



Science2008v321p243

Conclusions

- 1D SDS gel – in gel digest is the most commonly used method
- 2D LC is widely used for shotgun proteomics
- Tryptic peptides are compatible with C18 RPLC and CID
- AspN and LysC are common alternatives; LysN becomes more popular
- Microfluidics allows for automation and fast analysis

Amino Acids

				C	H	N	O	S	Monoisotopic Mass (Da)	Occurrence(%)
Glycine	Gly	G		2	3	1	1	0	57.02146	6.89
Alanine	Ala	A		3	5	1	1	0	71.03711	7.36
Serine	Ser	S		3	5	1	2	0	87.03203	7.35
Proline	Pro	P		5	7	1	1	0	97.05276	5.01
Valine	Val	V		5	9	1	1	0	99.06841	6.46
Threonine	Thr	T		4	7	1	2	0	101.04768	5.92
Cysteine	Cys	C		3	5	1	1	1	103.00918	1.77
Isoleucine	Ile	I		6	11	1	1	0	113.08406	5.69
Leucine	Leu	L		6	11	1	1	0	113.08406	9.25
Asparagine	Asn	N		4	6	2	2	0	114.04293	4.59
Aspartic acid	Asp	D		4	5	1	3	0	115.02694	5.15
Glutamine	Gln	Q		5	8	2	2	0	128.05858	4.04
Lysine	Lys	K		6	12	2	1	0	128.09496	5.8
Glutamic acid	Glu	E		5	7	1	3	0	129.04259	6.18
Methionine	Met	M		5	9	1	1	1	131.04048	2.32
Histidine	His	H		6	7	3	1	0	137.05891	2.25
Phenylalanine	Phe	F		9	9	1	1	0	147.06841	4.08
Arginine	Arg	R		6	12	4	1	0	156.10111	5.18
Tyrosine	Tyr	Y		9	9	1	2	0	163.06333	3.24
Tryptophan	Trp	W		11	10	2	1	0	186.07931	1.34