Qualitative and quantitative burrowing of the proteome

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Topics

- **Qualitative changes in the predicted proteome**
  - **Digging deeper - SELDI/MALDI**
  - **Posttranslational modifications**
    - **Isolation and characterization**
    - **Making using of the chemistry of the modification**
- **Quantitative aspects**
  - **Isotope labeling**
    - **ICAT, $^{18}O/^{16}O$, $^{15}N/^{14}N$**
    - **Chemical labeling**
  - **Absolute measures**

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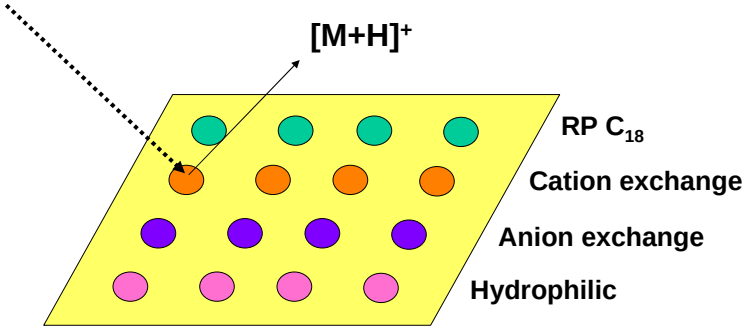
Surface enhanced laser desorption ionization (SELDI)



SELDI chip (plate) with local chemistry on surface causing selective binding of peptides

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MALDI-TOF analysis of peptides recovered by C_{18} extraction of sera from prostate patients, with and without metastases, and controls

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

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Identification of a significantly altered peptide in men with metastatic prostate cancer

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

But what is it?

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Purification of prostate peptide by SPE extraction and strong cation exchange

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TIFF (LZW) decompressor
are needed to see this picture.

Serum was first extracted with a C_{18} SPE cartridge

SPE fraction was passed over a [H+]-form cation exchange resin. Bound peptides were eluted with increasing step gradients of NaCl

Analyses of fractions were performed by MALDI-TOF MS

The most enriched fraction was contaminated with albumin

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Purification of prostate peptide needed 2D-electrophoresis

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Peptide mass fingerprint of purified prostate cancer peptide

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Non-mass spec verification of prostate peptide

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QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

Western blot analysis for PF4

ELISA analysis for PF4

Moral: proteomics is a serious business that requires multiple dimensions of separation - glib methods don't work

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General classes of modification

- **Biochemical events involving peptide processing**
- **Biochemical events stimulated by enzymes**
- **Chemical events driven by reactive species**
- **Chemical events determined by investigator**

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Examples of peptide processing of polypeptides

- Head groups of membrane proteins
- The family of proteins in HIV that are manufactured as one protein and then hydrolyzed by a protease
- Chromogranin - a brain protein consisting of several bioactive peptides
- Formation of β-amyloid

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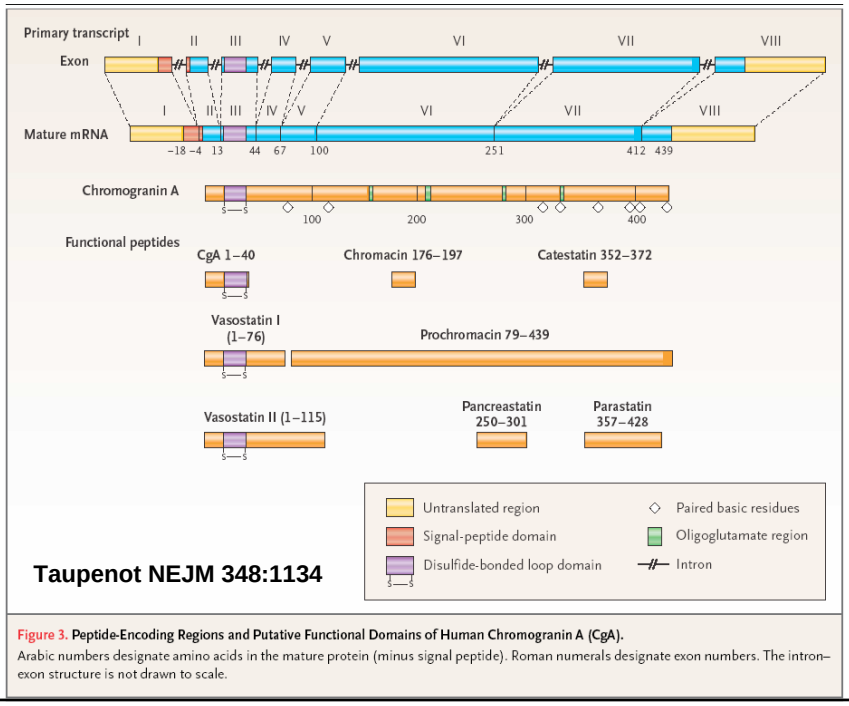

Taupenot NEJM 348:1134

Figure 3. Peptide-Encoding Regions and Putative Functional Domains of Human Chromogranin A (CgA).
Arabic numbers designate amino acids in the mature protein (minus signal peptide). Roman numerals designate exon numbers. The intron–exon structure is not drawn to scale.

Enzymatic modifications

- Phosphorylation/dephosphorylation
  - On serine, threonine, tyrosine
- Glycosylation
  - N-glycosylation (asparagine)
  - O-glycosylation (serine, threonine)
- N-Acetylation/deacetylation
  - On lysine
- N-Methylation/demethylation
  - On lysines - mono-, di- and trimethylation

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Phosphorylation of proteins

- in some cases, proteins are normally found in a stable, hyperphosphorylated state, e.g., casein
- In many cases, it is a transient event that causes 10-100 fold increase in enzyme activity. This is the way signals are propagated through a signal transduction pathway. However, the molar abundance of phosphorylation at an individual site may only be 1-2%.

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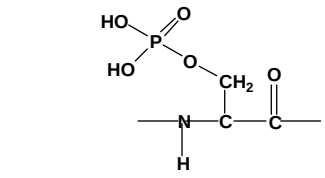
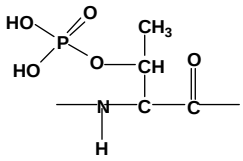
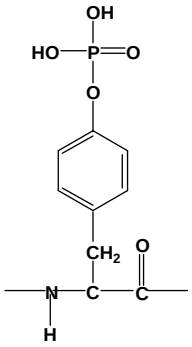
Chemistry of phosphorylation



Serine



Threonine



Tyrosine

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Mass spectrometry of proteins

- Adds H_3PO_4 (+98)
- Eliminates water (-18)
- Net change +80
- if the phosphate ion is released (i.e., in negative ion spectra), it is seen as *m/z* 79 and/or *m/z* 63
- Phosphate can be confused with sulfate and bromide (sulfate, 79.9568 Da; phosphate, 79.9663 Da; and ^{81}Br, 79.9083 Da)

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Mass spectrometry of proteins

Mass spectrometry has several advantages over other techniques

- **it is very accurate**
- **it can eliminate ambiguity by defining the site of phosphorylation**
- **it is very fast**
- **it does not require ^{32}P labeling**

But it is not nearly as sensitive as ^{32}P labeling

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Limitations of mass spectrometry

- **Although it can deliver sensitivity in the low fmol range (similar to immunological methods), because it is a universal detection method, finding the needle in the "haystack" of all the other peptides is a challenge**
- **Recovering the phosphopeptides from the matrix of the sample is more important than the mass spectrometry measurement**

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Finding a phosphate group

Several methods are in current use for detection of phosphopeptides

- **use of parent ion or neutral loss scanning**
- **phosphatase sensitivity**
- **affinity methods for enrichment of phosphopeptides**
  - **antiphospho-Ser/Thr/Tyr antibodies**
  - **metal ion affinity**
  - **chemical reaction/biotin affinity**

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Parent ion scanning to detect phosphopeptides

- **The procedure depends on the detection of the *m/z* 79 ion fragment (PO_3-) during collision-induced dissociation in a triple quadrupole instrument operating in the negative ion mode**
- **Parent ion scanning is a reversal of the more familiar daughter ion MS-MS where the parent ion is selected (in Q1) and a mass spectrum of the daughter ion fragments is obtained by scanning in Q3**
- **In parent ion scanning, the daughter ion fragment (in this case *m/z* 79) is held constant in Q3 and a mass spectrum of parent ions that give rise to the daughter ion obtained by scanning in Q1.**
- **having identified the phosphopeptides, the sample can be re-analyzed to obtain daughter ion MS-MS spectra on selected ions in the positive ion mode**

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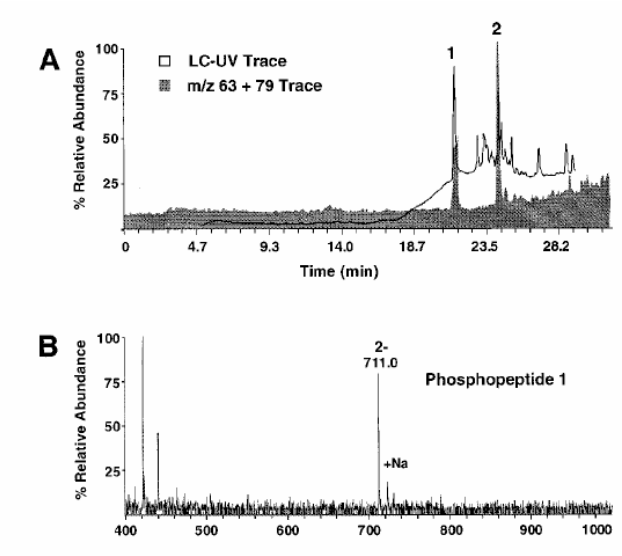
Parent ion scan to detect phosphopeptide



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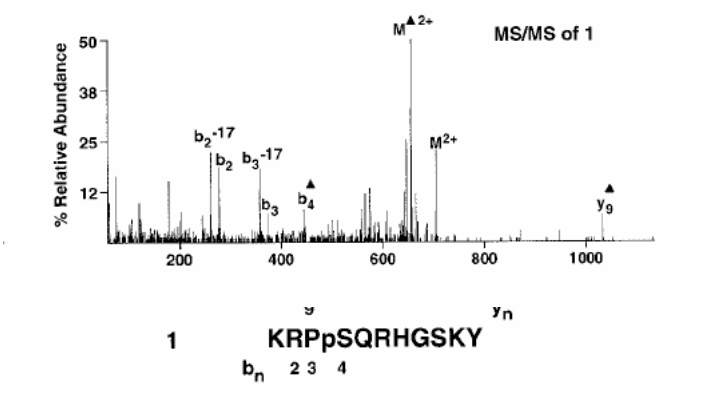
Tandem MS of phosphopeptide 1



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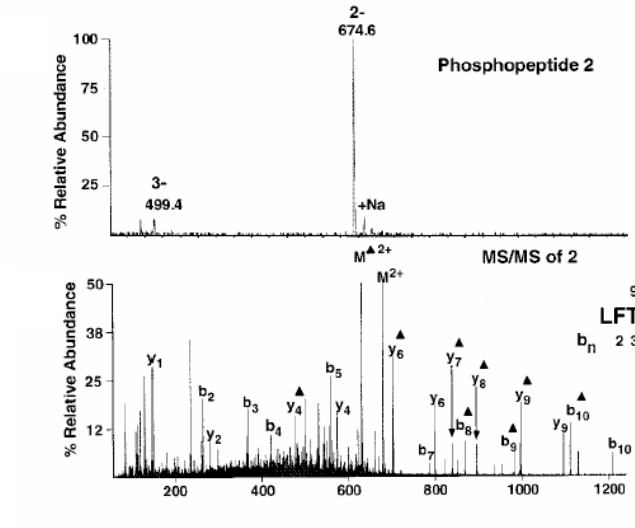
Phosphopeptide 2



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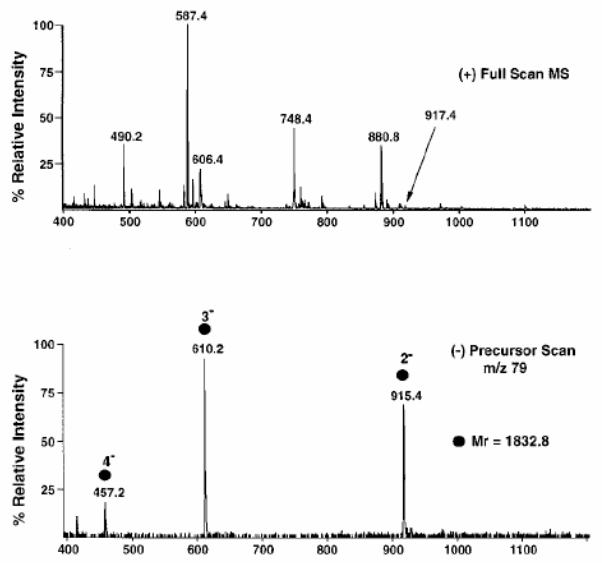
Unknown casein phosphopeptide



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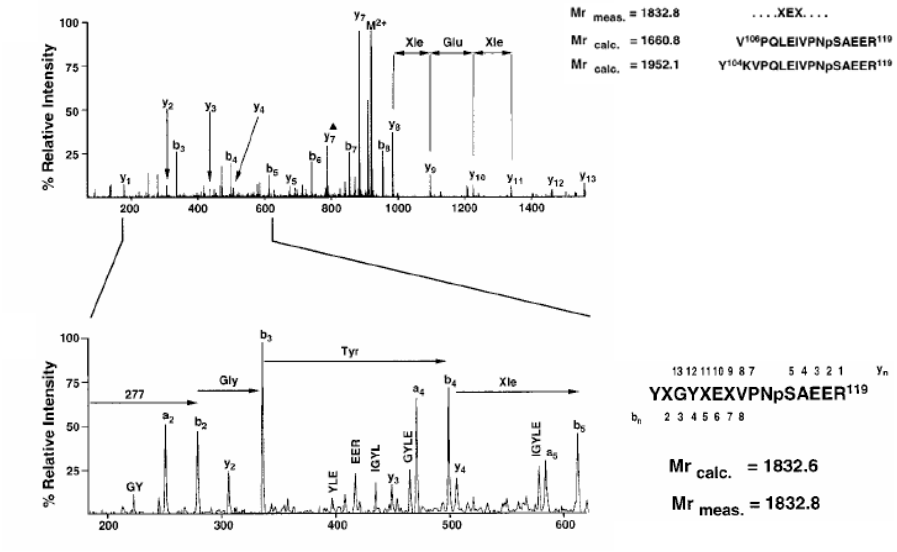
Deducing phosphopeptide



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Annan Anal Chem 73:393

Recovery and enhancement of phosphopeptides

The biggest problem in the detection of phospho-peptides is how to convert the initial sample matrix into a form suitable for mass spectrometry analysis.

- **how to handle minute samples with minimal losses**
- **how to recover and detect all the phosphopeptides**
- **how to recover and detect the non-phosphorylated proteins to determine the extent of phosphorylation at individual sites**

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Antibodies and phosphopeptides

- In this approach, both the phosphorylated and non-phosphorylated forms of a protein may be recovered from the sample matrix
- This can most easily achieved by immuno-precipitation of the protein with an antibody that recognizes epitope(s) that is(are) in common with both forms

NOTE *that as with all immunoprecipitation methods, the best results will be achieved if the antibody is coupled to agarose beads. This allows selective immuno-absorption of the antigen, washing free of contaminating proteins, AND following elution, minimization of the amount of antibody that is in the eluate and therefore would be analyzed by mass spectrometry*

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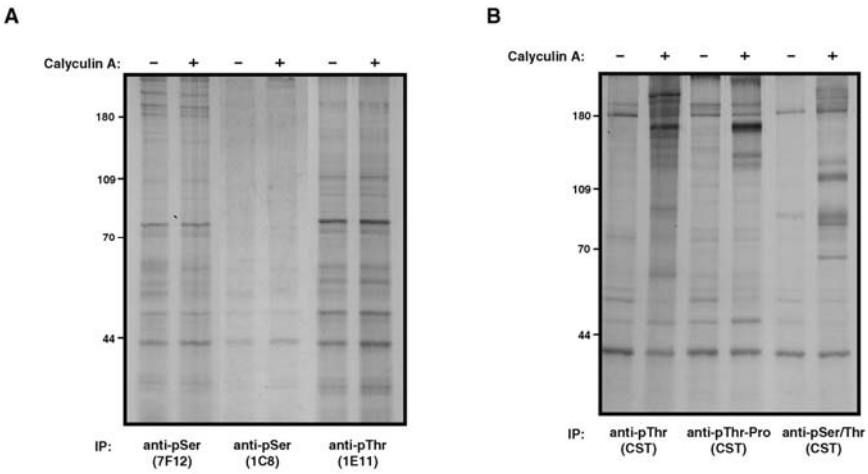
Variability of anti-phosphoserine and anti-phosphothreonine antibodies



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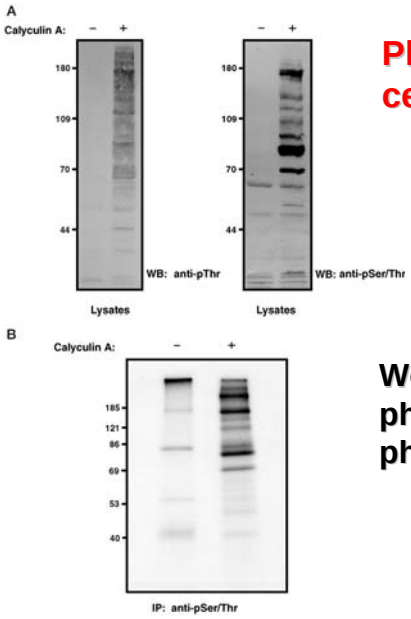

Phosphorylation in Hela cells stimulated by calyculin

Western blot analysis with anti-phosphoserine and anti-phosphotyrosine antibodies

Steve Barnes 2-10-06 Gronborg et al., 2002

Detection of phosphopeptides based on their sensitivity to phosphatase

- An alternative source of potentially phosphorylated proteins are individual spots on 2D-IEF/SDS gels. The protein preparation so isolated is either hydrolyzed by trypsin in solution (or in the gel piece) or using solid-phase trypsin
- One portion of the resulting tryptic peptides (in 50% acetonitrile:water) is analyzed by MALDI-TOF-MS. A second portion is diluted into 50 mM NH_4HCO_3 buffer and reacted with 0.5 U calf intestinal alkaline phosphatase at 37°C for 30 min. Sample is dried with a SpeedVac, redissolved in 50% acetonitrile:water, and re-analyzed by MALDI-TOF-MS

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Identifying phosphopeptides using alkaline phosphatase

- **Phosphopeptides shift down by *m/z* 80 (or units of 80 in the case of multiply phosphorylated peptides)**
- **The peaks identified as phosphopeptides can then be analyzed in a nanoelectrospray experiment where collision-induced dissociation is used to determine the identity of the peptide and the phosphorylation site in the sequence**

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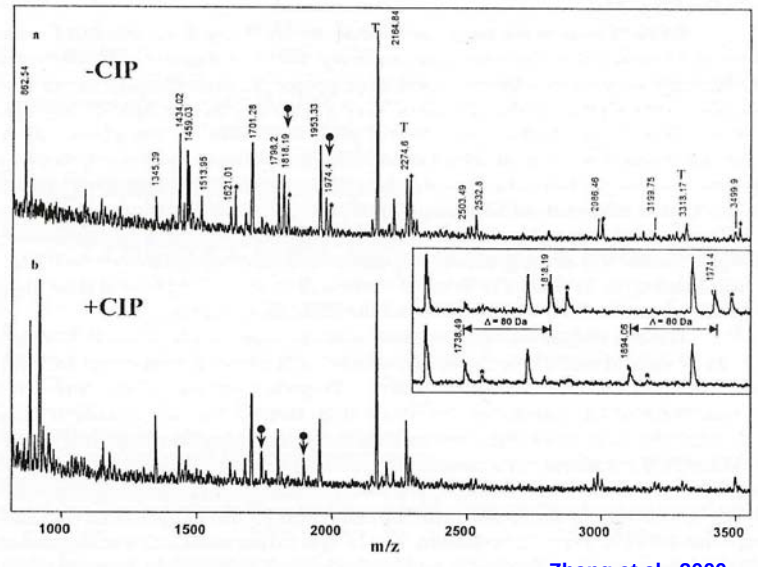
Detecting a phosphopeptide with alkaline phosphatase



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Zhang et al., 2000
MS in Biology & Medicine

Selective enhancement of phospho-peptides in tryptic digests

- Immobilized metal affinity chromatography (IMAC). Similar to Ni-affinity resins used in the purification of 6xHis-tagged proteins. The affinity phase can be charged with different metal ions (as their chlorides)
- Fe(III) and Ga(III), and to a lesser extent Zr(IV), were the most effective for the recovery of two synthetic phosphopeptides
- A tryptic digest containing both phosphorylated and non-phosphorylated peptides is passed over the IMAC column at acid pH (pH 2.5-3)
- The column is washed with 0.1 M acetic acid to remove unbound peptides
- Elute with sodium phosphate (have to desalt) or with NH_4OH
- Esterification may prevent Asp- or Glu-containing peptides from binding

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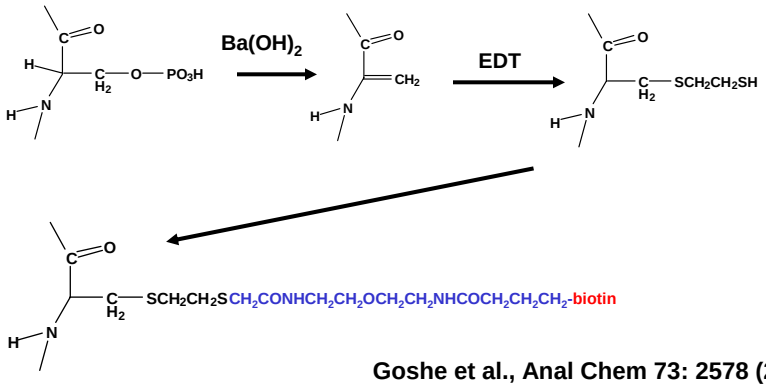
Selective biotinylation of phospho-groups



Goshe et al., Anal Chem 73: 2578 (2001)
Adamcyk et al., Rapid Commun Mass Spec 15; 1481 (2001)

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How to identify phosphorylated peaks by searching databases

- The databases you have already seen have some ability to predict the expected masses for a limited number of posttranslational modifications

  - MASCOT (http://www.matrixscience.com)
  - PROTEIN PROSPECTOR (http://prospector.ucsf.edu/)

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How to identify posttranslational modifications

FindMod at

http://www.expasy.ch/tools/findmod/

It examines mass fingerprinting data for mass differences between empirical and theoretical peptides. If the mass difference corresponds to a known modification, it also makes intelligent guesses as to the site of modification.

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FindMod

Modifications considered are:

acetylation	amidation	biotinylation
C-mannosylation	deamidation	flavinylation
farnesylation	formylation	geranyl-geranylation
γ-carboxyglutamic acid	hydroxylation	lipoylation
methylation	myristoylation	N-acyl diglyceride
O-GlcNac	palmitoylation	phosphorylation
pyridoxal phosphate	phospho-pantetheine	pyrrolidone-carboxylic acid
sulfation		

NOTE that none of the common chemical modifications (alkylation of sulfhydryl groups with iodoacetic acid, iodoacetamide, 4-vinylpyridine, and acrylamide) were included.

The list also omits nitration and the recently discovered halogenation of peptides.

See the article by Wilkins et al. (1999) in J. Mol Biol. for details on FindMod

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Site for compilation of PTMs

https://www.abrf.org/index.cfm/dm.home

This site was put together by Ken Mitchelhill, Len Packman and friends

Currently ranges from dephospho (-79) to (Hex)3-HexNAc-(dHex)HexNAc (+1,039)

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Nitration of proteins

- Peroxynitrite is a highly oxidizing and nitrating species produced by the reaction of nitric oxide and superoxide $NO^{\cdot} + O_2^{-\cdot} = ONO_2^-$
- UAB has an important place in the identification of nitrated proteins
  - 1996 Greis et al., Arch Biochem Biophys 335:396 (Surfactant protein A)
  - 1997 Crow et al., J Neurochem 69:1945 (neurofilament-L)
  - 2000 Cassina et al., J. Biol Chem 275:21409 (cytochrome C)
  - 2003 Aslan et al., J Biol Chem 278:4194 (actin)

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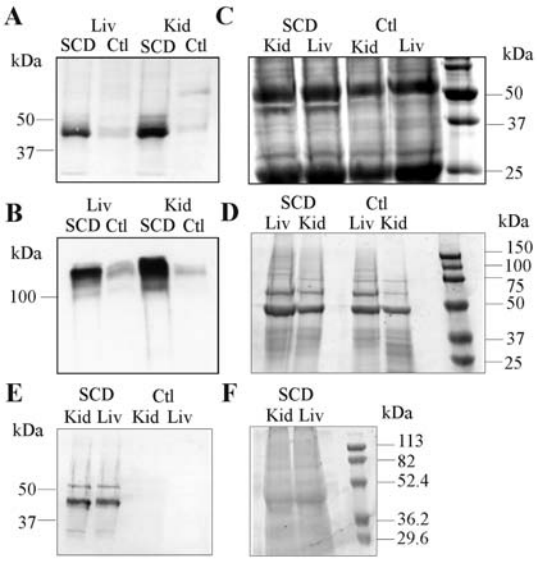

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SDS-PAGE and Western blot analysis

A = anti-nitrotyrosine and liver and kidney homogenates

B = anti-iNOS

C = immunoprecitated NO_2Tyr proteins run on SDS-PAGE and stained by Coomassie Blue

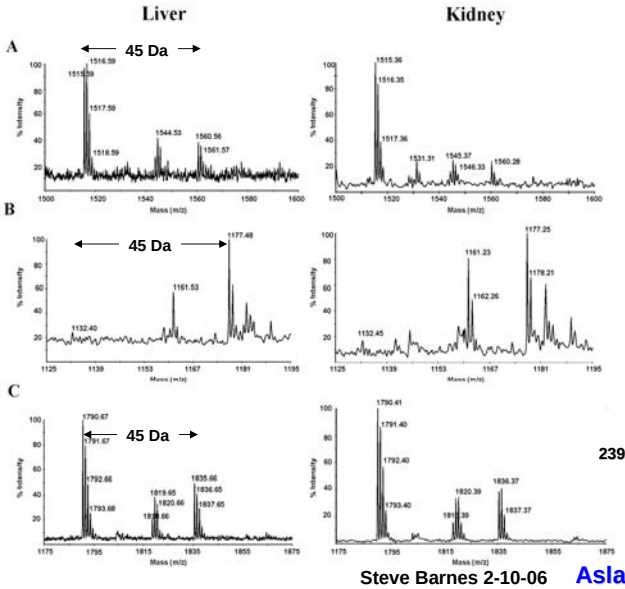
D = actin-enriched proteins run on SDS-PAGE and stained with Coomassie Blue

E = Western blot with anti-nitrotyrosine of actin-enriched proteins

F = Actin-enriched proteins bound to anti-nitrotyrosine affinity phase, eluted and run on SDS-PAGE and stained with Coomassie Blue

Aslan et al., JBC 278:4194

MALDI-TOF identification of NO_2Tyr peptides in actin - note the degradation pattern



^{85}IWHHTFYNELR^{95}

^{197}GYSFTTTAER^{206}

^{239}SYELPDGQVITIGNER^{254}

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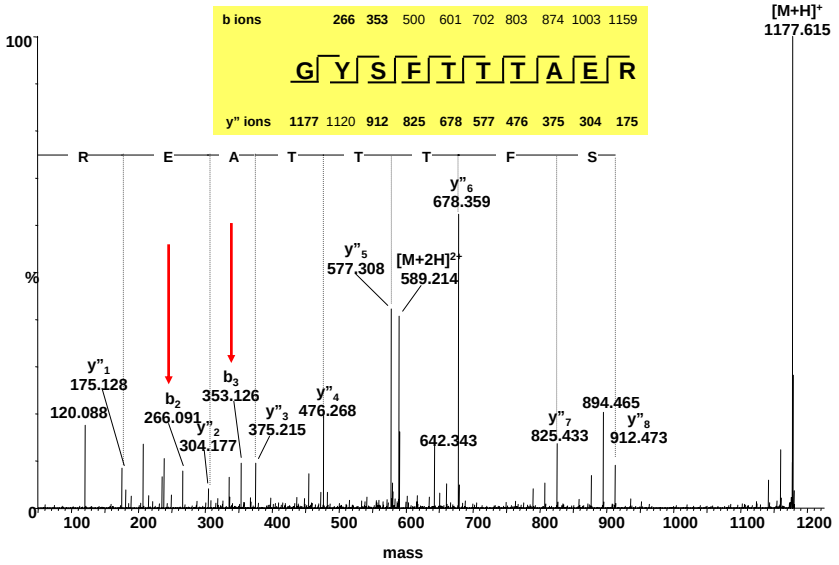
MSMS of actin tryptic peptide 197-206



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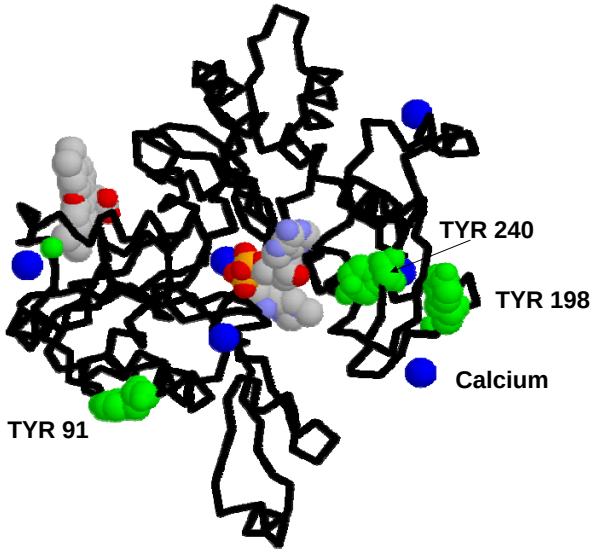
In Vivo Nitrated Actin



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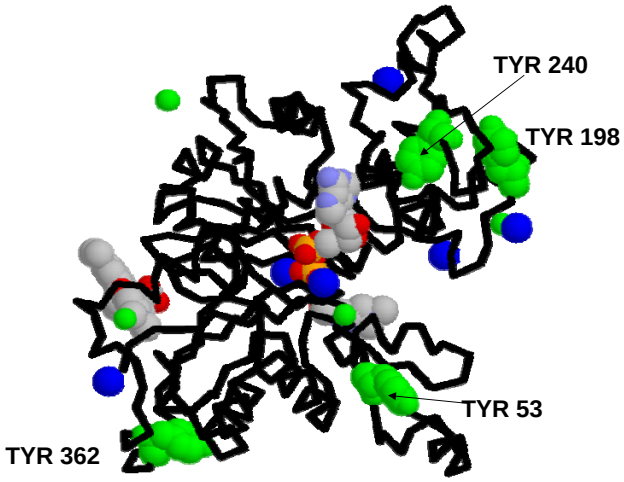
In Vitro Nitrated Actin



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Undetected actin peptides with tyrosine nitration

```
  1 MDDDIAALVV DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
 51 DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
101 HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
151 IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
201 TTTAEREIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
251 GNERFRCPEA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
301 GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS
351 TFQQMWISKQ EYDESGPSIV HRKCF
```

69	YPIEHGIVTNWDDMEK	= 1991.89
133/143	MTQIMFETFNTPAMYVAIQAVLSLYASGR	= 3298.60, 3343.59
166/169	TTGIVMDSGDGVTHTVPIYEGYALPHAILR	= 3230.64, 3275.63
188	DLTDYLMK	= 1043.48
218	LCYVALDFEQEMATAASSSSLEK	= 2539.81
294/306	DLYANTVLSGGTTMYPGIADR	= 2260.06, 2305.05
337	YSVWIGGSILASLSTFQQMWISK	= 2647.33

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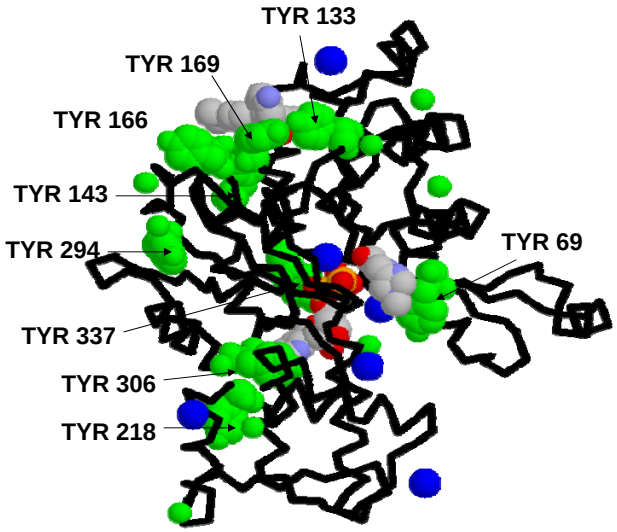
Tyrosine Residues Not Nitrated



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Amanda Isom

Alternative digestion with Glu-C

```
   1 MDDDIAALVV DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
  51 DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
 101 HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
 151 IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
 201 TTTAEREIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
 251 GNERFRCPEA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
 301 GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS
 351 TFQQMWISKQ EYDESGPSIV HRKCF
```

53	SYVGD	= 585.22	198	RGYSFTTTAE	= 1177.52
69	AQSKRGILTLKYPIE	= 1761.99	218	KLCYVALD	= 969.48
91	KIWHHTFYNE	= 1419.65	240	KSYE	= 571.24
133/143	TFNTPAMYVAIQAVLSLYASGRTTGIVMD	= 3090.56, 3135.54	294/306	LYANTVLSGGTTMYPGIAD	= 1988.93, 2033.92
166	GVTHTVPIYE	= 1160.56	337	RKYSVWIGGSILASLSTFQQMWISKQE	= 3188.63
169	GYALPHAILRLD	= 1384.74	362	YD	= 342.10
188	YLMKILTE	= 1055.55			

Use of Glu-C would reveal whether ^{69}Y, ^{166}Y, ^{169}Y, ^{188}Y, ^{218}Y and possibly $^{294/306}$Y are nitrated

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Key points to remember

- **Actin is a highly abundant protein in cells**
- **Proteins in the 40-44 kDa range are frequently heavily contaminated with actin**
- **2D-IEF/SDS-PAGE can help separate actin from other proteins**
- **Actin can be nitrated**
- **However, nitration is a low abundance event**
- **So, even detection of nitration of actin requires a preliminary immunopurification**
- **Nitration of β-actin is restricted to the protein surface; however, this may be an artifact of the distribution of trypsin cleavage sites in actin**

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Detecting PTMs

- If we don't have any idea about what the modification(s) is(are), how do we proceed?
- We won't have the modification on MASCOT or Protein Prospector
- No antibodies or convenient affinity phases

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Can we approach this globally?

- It's asking a lot
  - Too many degrees of freedom
  - Endless modifications
- Should we try?
  - John Yates' group has
  - MacCoss et al. PNAS 99:7900 (2002)

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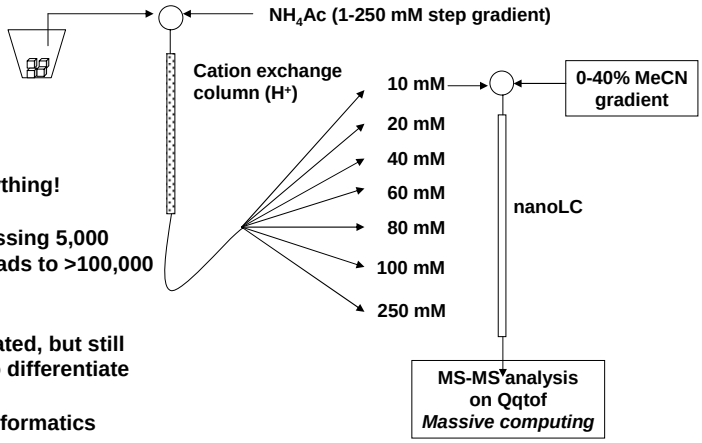
MudPIT - Multi-dimensional Protein Identification Technology



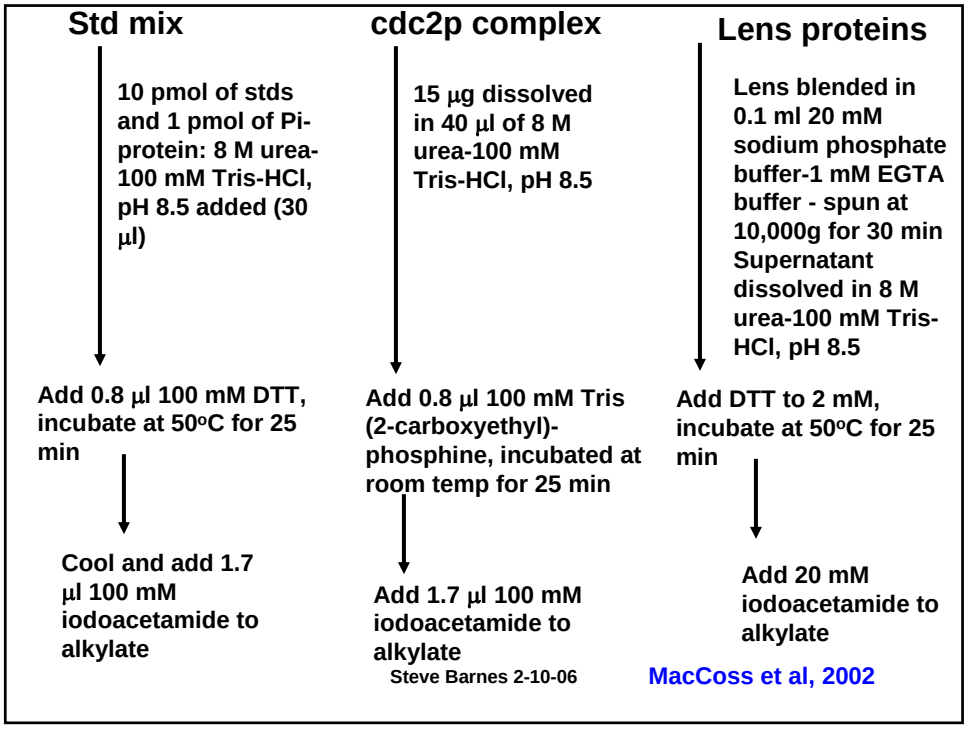
- Hydrolyze everything!
- For a cell expressing 5,000 proteins, this leads to >100,000 peptides
- Can be fractionated, but still 10,000-20,000 to differentiate
- Enormous bioinformatics problem

Steve Barnes 2-10-06 John Yates

The Yates approach - selection of "proteomes"

- BioRad mixed molecular weight standards (10 pmol each) plus 1 pmol of a phosphorylated glycogen phosphorylase
- Cdc2 protein complex isolated with TAP (15 μg)
- Lens proteins from 4-yr old

Steve Barnes 2-10-06 MacCoss et al, 2002


Std mix
10 pmol of stds and 1 pmol of Pi-protein: 8 M urea-100 mM Tris-HCl, pH 8.5 added (30 µl)
Add 0.8 µl 100 mM DTT, incubate at 50°C for 25 min
Cool and add 1.7 µl 100 mM iodoacetamide to alkylate
cdc2p complex
15 µg dissolved in 40 µl of 8 M urea-100 mM Tris-HCl, pH 8.5
Add 0.8 µl 100 mM Tris (2-carboxyethyl)-phosphine, incubated at room temp for 25 min
Add 1.7 µl 100 mM iodoacetamide to alkylate
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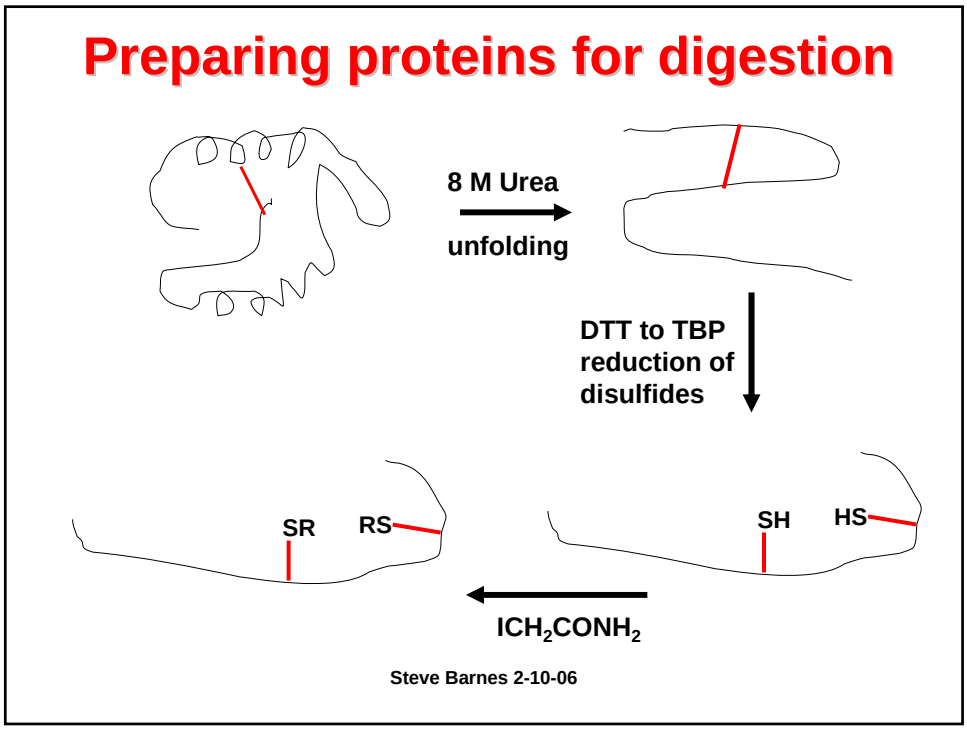
Lens proteins
Lens blended in 0.1 ml 20 mM sodium phosphate buffer-1 mM EGTA buffer - spun at 10,000g for 30 min Supernatant dissolved in 8 M urea-100 mM Tris-HCl, pH 8.5
Add DTT to 2 mM, incubate at 50°C for 25 min
Add 20 mM iodoacetamide to alkylate
MacCoss et al, 2002


Preparing proteins for digestion
8 M Urea
unfolding
DTT to TBP
reduction of
disulfides
SR
RS
SH
HS
ICH2CONH2
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Hydrolysis procedures

Reduced, carboxymethylated in 8 M urea

Diluted x 3 with 100 mM Tris-HCl, pH 8.5

↓

100 mM $CaCl_2$ added to 1 mM

↓

Roche trypsin 1:50, incubated 12-24 h at 37°C

↓

quenched with 90% formic acid to 4%

Diluted x 3 with 4.8 M Urea-100 mM Tris-HCl, pH 8.5

↓

subtilisin added 1:50, incubated 2-3 hr at 37°C

↓

quenched with 90% formic acid to 4%

Diluted x 3 with 100 mM Tris-HCl, pH 8.5

↓

incubated with elastase 1:50 12 h at 37°C

↓

quenched with 90% formic acid to 4%

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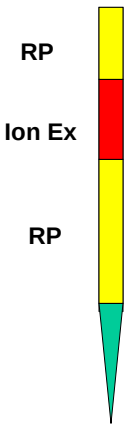
MacCoss et al, 2002

Elution from a triphasic column



sample

Wash with 5% MeCN-0.1% acetic acid - elute with 80% MeCN-0.1% formic acid

Wash with 5% MeCN-0.1% acetic acid - elute with 0-500 mM $(NH_4)_2CO_3$

Wash with 5% MeCN-0.1% acetic acid for 5 min - elute with 5-64% MeCN-0.02% HFBA

For lens digests, the sample was passed over a separate 3 cm RP column - after washing, the peptides were eluted with 80% aqueous acetonitrile-0.1% acetic acid

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MacCoss et al, 2002

Automated MS-MS analysis

- Limit analysis to 2^+ or 3^+-charged peptides
- Delete poor quality spectra
- Identify peptides with 98-Da neutral loss
- Analyze the remaining spectra with SEQUEST (operating on a 31-node beowulf computer cluster) to identify proteins
- Search sequences of identified proteins for PTMs by 80 (STY phosphorylation), 42 (K acetylation), 16 (MWY oxidation) and 14 (K methylation)

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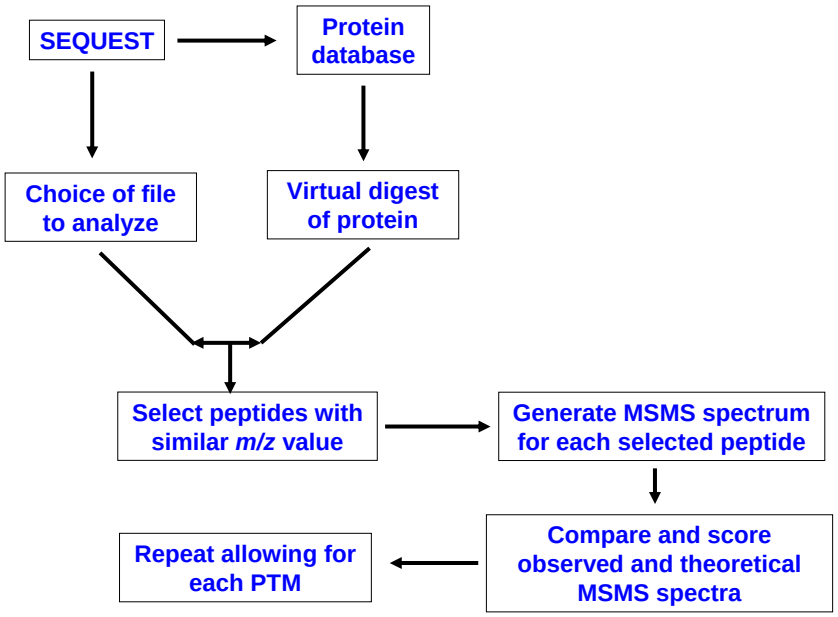

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How to best use SEQUEST

- SEQUEST is computer software to ease the burden of interpreting MS-MS data
- SEQUEST does not make an absolute judgement on the truth of an identification
  - For a given protein, there will be several peptides that should be identified - no one-hit wonders!
  - The overall score improves with greater sequence coverage
  - PTMs can cause confusion since they will imply a *m/z* value of a non-PTM peptide
  - The PTM can be accounted for if known

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Results

Protein standard mixture:

- The three digests were combined - 83.7% and 95.4% coverage for glycogen phosphorylase and BSA
- Identified the glycogen phosphorylase phosphorylation site as well as the two known sites (S69/S345) in ovalbumin
- N-acetylation found plus many sites of methionine oxidation (? due to work up or real)
- New sites found - phosphorylation at S237/S241 in ovalbumin and methylation at R652

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Results

Cdc2p complex:

- 200 proteins - 20 showed >40% sequence coverage
- Expected Y15 and T167 phosphorylation sites on Cdc2p found
- New phosphorylation sites found on cyclin partners Cdc13 and Cig1p
- Multiple methylation sites on Cdc2p

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Results

Lens proteins:

- Found 272 proteins - 52 had >40% sequence coverage
- 90% are crystallins
- PTMs accumulate over your lifetime
- Used 18-step MudPIT because of complexity
- 73 different PTMs found on the 11 crystallins
- Found the 13 of 18 PTMs previously described in all species
- Found 60 other new PTMs in phosphorylation, oxidation, acetylation and methylation

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PTMs in α-crystallin

	Known	New
αa-Crystallin	S45, S122	T13, T140
αb-Crystallin	S19, S45, S59	S53, S76
αa-Crystallin		Y18, Y34, M138
αb-Crystallin		Y48, W60, M68
αa-Crystallin		K70, K78, K88, K145
		R1, K88
αb-Crystallin		K92
		R22, R50

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What MudPIT missed

- MudPIT detected proteins with MWs from <10 kDa to >200 kDa
- But it missed all the protein dimers, trimers, tetramers and higher species of the α-crystallins
- These oligomers contain difunctional PTMs

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Quantitative proteomics

Use of isotopes

- **ICAT (d_0/d_8) and ICAT $^{13}C_0/^{13}C_8$**
- **latter needed because of deuterium isotope effects on LC-MS mobility**
- **d_0/d_{10} propionic anhydride (N-terminal labeling**
- **$^{15}N/^{14}N$ (whole cell labeling)**
- **$^{18}O/^{16}O$ (trypsin)**

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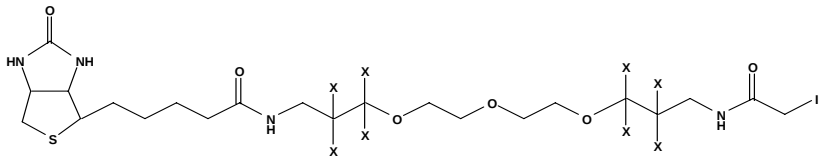
Isotope-coded affinity technology (ICAT)



biotin **Linker region (heavy or light)** **Thiol-specific reactive group**

This reagent reacts with cysteine-containing proteins (80-85% of proteome)

Labeling can be replacement of hydrogens (X) with deuterium, or better to exchange ^{12}C with ^{13}C in the linker region (this avoids chromatography issues)

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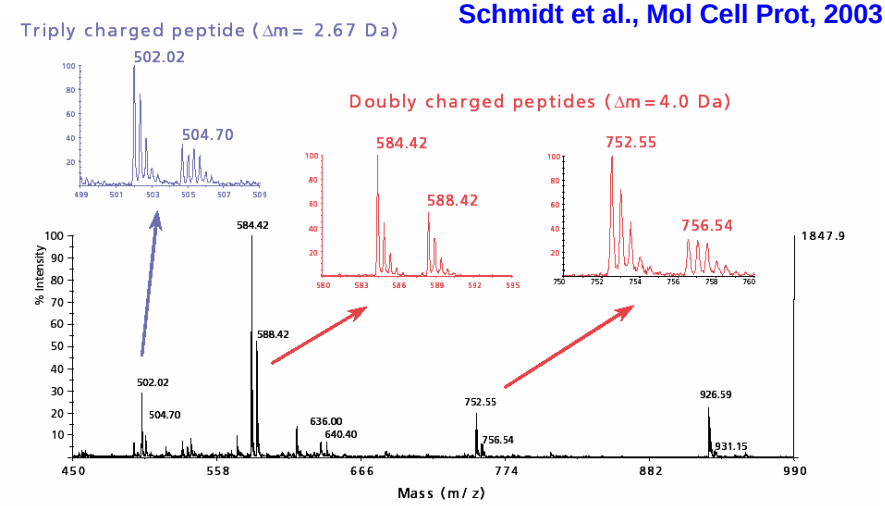
Quantitation from ESI-mass spectrum

Schmidt et al., Mol Cell Prot, 2003



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Other non-isotopic quantitative methods in proteomics

- The coverage (the number of peptides observed for a protein) is sensitive to the amount of the protein
  - This can be used to calculate whether a treatment affects the abundance of a protein
- Peptides of interest can be analyzed like small molecules
  - Choose the parent molecular ion, collide with argon gas and select a unique fragment

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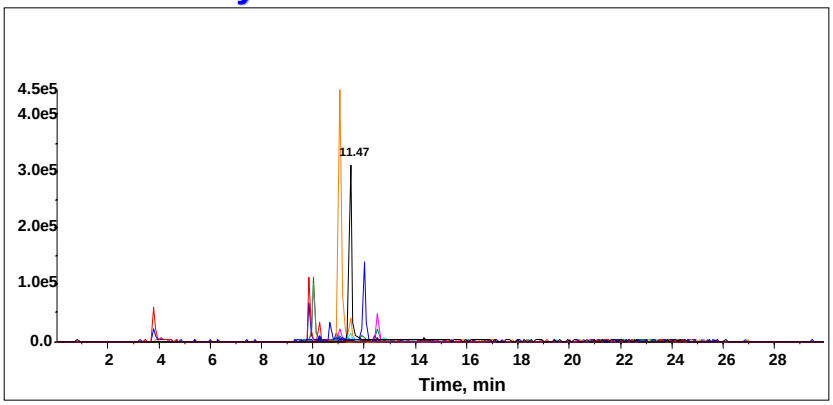
Quantitation experiment for biotinylated cytochrome c

MRM analysis monitored in 50 channels



Each colored peak represents a different biotinylated peptide

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References for this talk

- Annan RS, Hudleston MJ, Verma R, Deshaies RJ, Carr SA. A multidimensional electrospray MS-based approach to phosphopeptide mapping. Anal. Chem. 73:393, 2001.
- Flory MR, Griffin TJ, Martin D, Aebersold R. Advances in quantitative proteomics using stable isotope tags. Trends in Biotechnology 20: S23, 2002.
- Taupenot L, Harper KL, O'Connor DT. The chromogranin-secretogranin family. New Engl. J. Med. 348: 1134, 2003.
- Lam YW, Mobley JA, Evans JE, Carmody JF, Ho S-M. Mass profiling-directed isolation and identification of a stage-specific serologic protein biomarker of advanced prostate cancer. Proteomics 5: 2927, 2005.

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