BMG 744 Proteomics-Mass Spectrometry

Qualitative burrowing of the proteome

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1/24/14

Topics

Qualitative changes in the predicted proteome

- Digging deeper - SELDI/MALDI
- Posttranslational modifications
  - Isolation and characterization
  - Making using of the chemistry of the modification
  - Ubiquitination/SUMOylation
  - Global discovery
  - Targeted discovery

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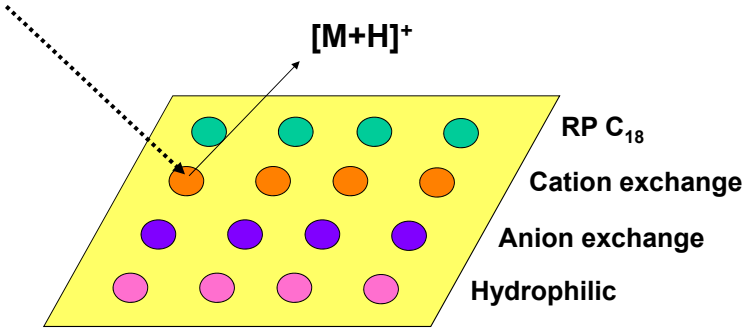
Surface enhanced laser desorption ionization (SELDI)



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

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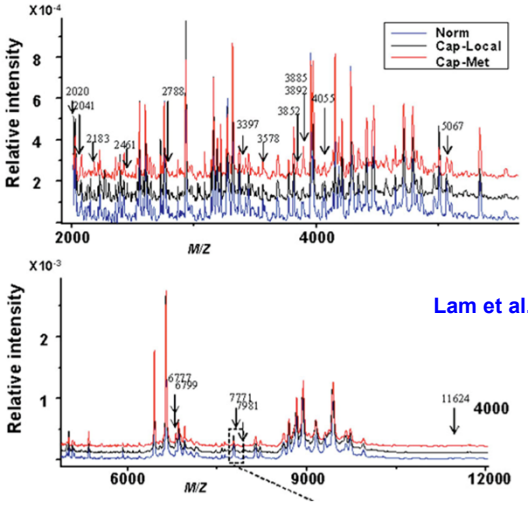
MALDI-TOF analysis of peptides recovered by C_{18} extraction of sera from prostate patients, with and without metastases, and controls



Lam et al., Proteomics 5, 2927

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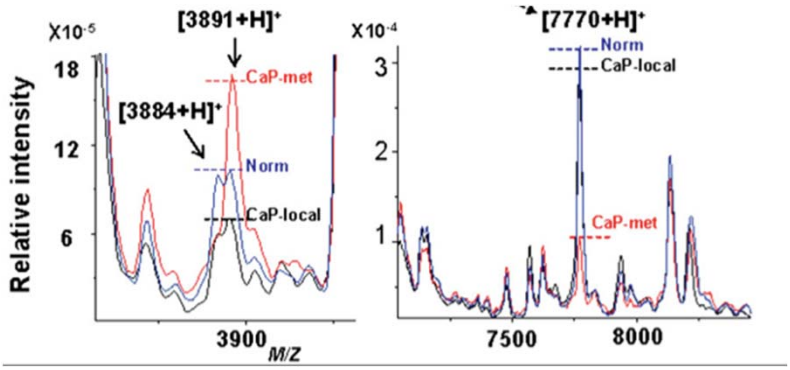
Identification of a significantly altered peptide in men with metastatic prostate cancer

Lam et al., Proteomics 5, 2927



But what is it?

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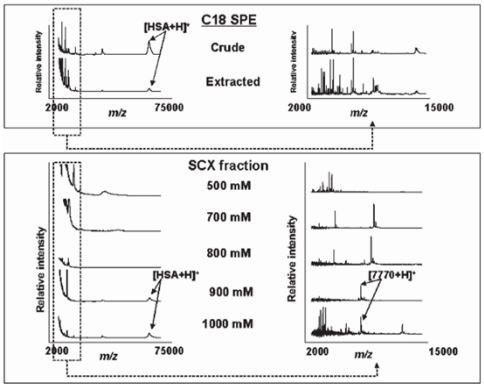
Purification of prostate peptide by SPE extraction and strong cation exchange

Lam et al., Proteomics 5, 2927



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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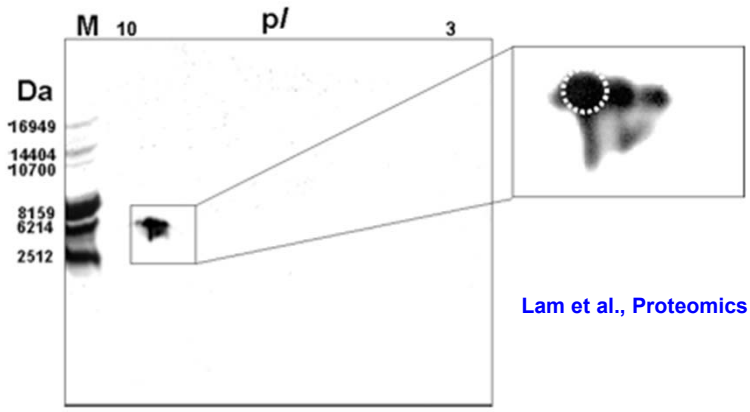
Purification of prostate peptide needed 2D-electrophoresis



Lam et al., Proteomics 5, 2927

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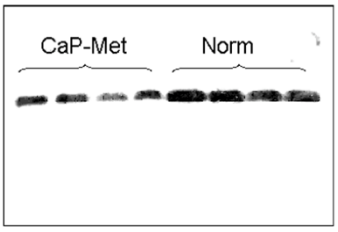
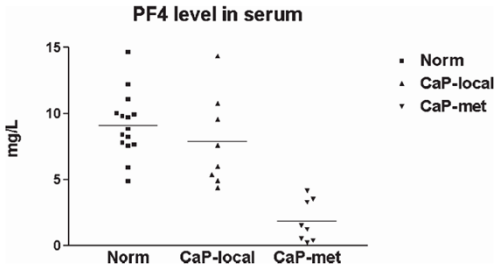
Non-mass spec verification of prostate peptide

Lam et al., Proteomics 5, 2927



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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Challenges in proteomics

- RNA can be amplified by PCR and converted to DNA (cDNA) - then it has been used to hybridize to DNA sequences on a microarray
  - Proteomics doesn't have an amplification system
- RNA-Seq sequences rather than hybridizes and dispenses with predetermined microarrays
  - Discovers new messages - signals not predicted by conventional gene annotations

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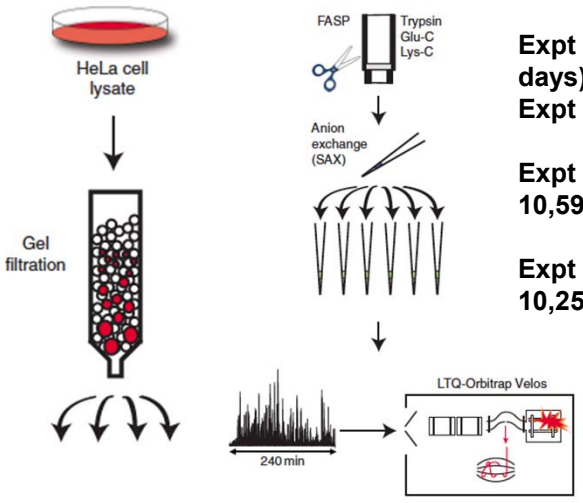
How deep can we go?



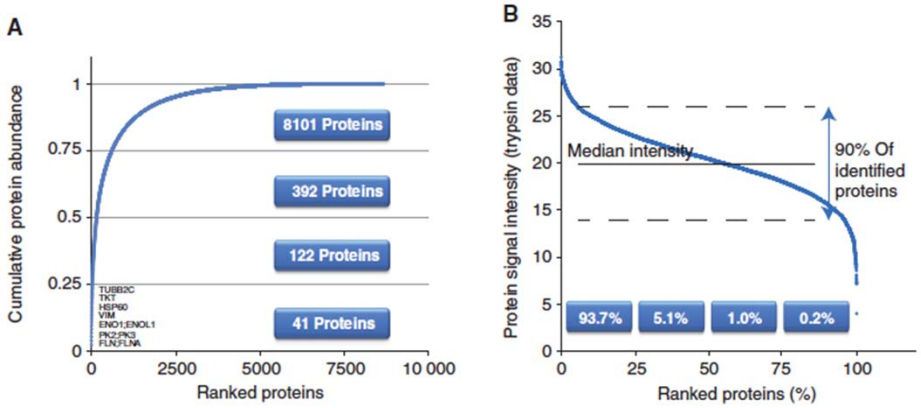
Expt 1 – 126 fractions (21 days)
Expt 2 – 72 fractions (12 days)

Expt 1 – 187,006 peptides and 10,596 proteins

Expt 2 – 163,784 peptides and 10,255 proteins

Nagara et al., Mol Sys Biol 7:548, (2011)

Description of protein abundances



Just 41 proteins accounted for 25% of the combined tryptic peptide ion intensities – 122 accounted for 50% and 392 for 75%.

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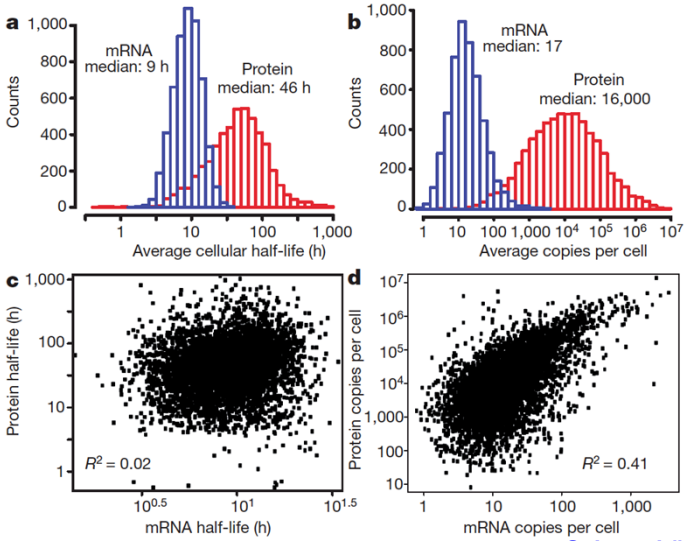
Nagara et al., Mol Sys Biol 7:548, (2011)

Protein – mRNA correlations



Schwanhäusser et al. Nature 473: 337 (2011)

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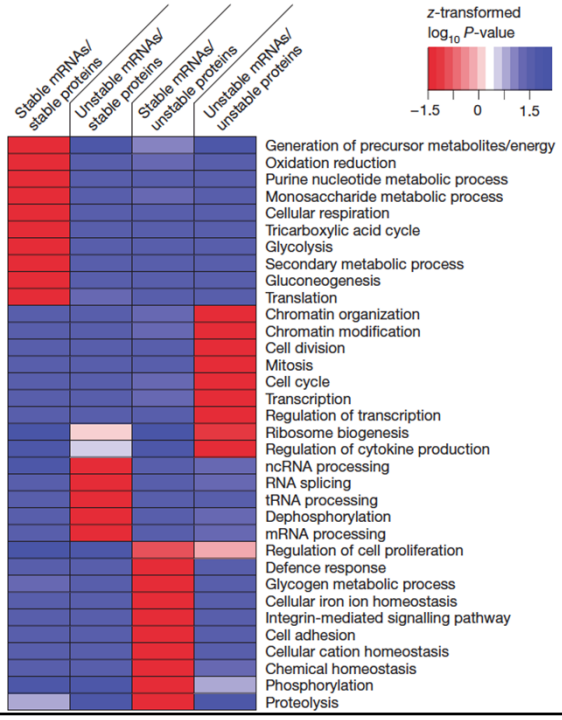

Proteins and mRNA have different stabilities and can be divided into 4 quadrants. The major cellular functions are divided as shown in the table.

Schwanhäusser et al.
Nature 473: 337 (2011)

Moral about protein discovery in 2014

- It is now possible to detect more proteins than there are separate forms of message
- However, such discovery is still "heroic"
- Proteomics does not have the equivalent of the PCR reaction
  - However, a cell does amplification of message
- Detecting the minor components requires being able to start with enough sample
  - 10 attomole is 6.023 million molecules
  - If present as 10 copies per cell, then minimum # of cells needed is 0.6×10^5

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How to dig deeper

- Sub-fractionate the sample being analyzed to enrich for the lower abundance components
  - Choose the right biofluid/tissue
  - Organelle isolation
  - Affinity chromatography
  - Antibody pulldowns
  - Abundant protein(s) depletion

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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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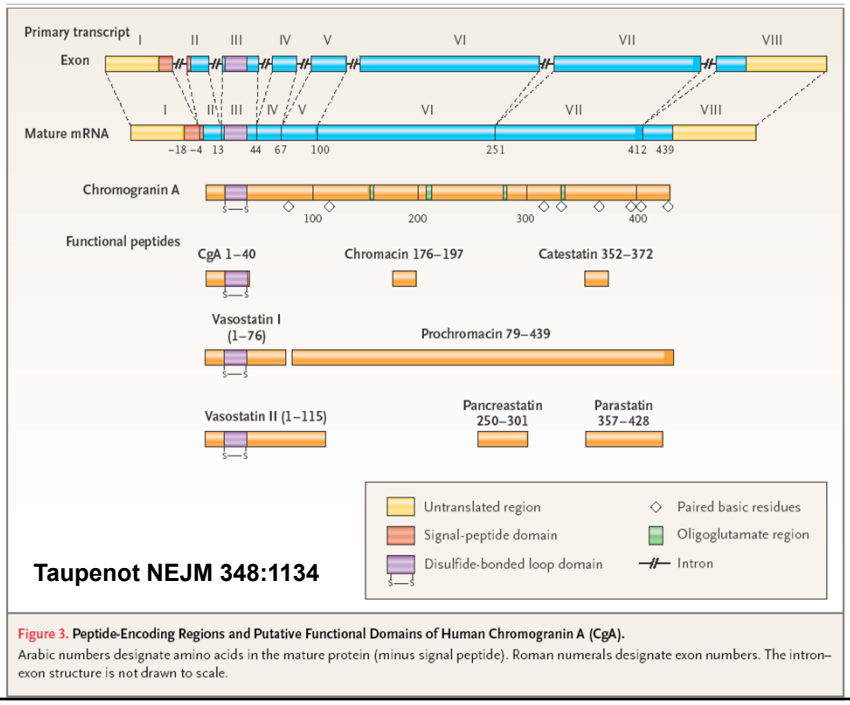
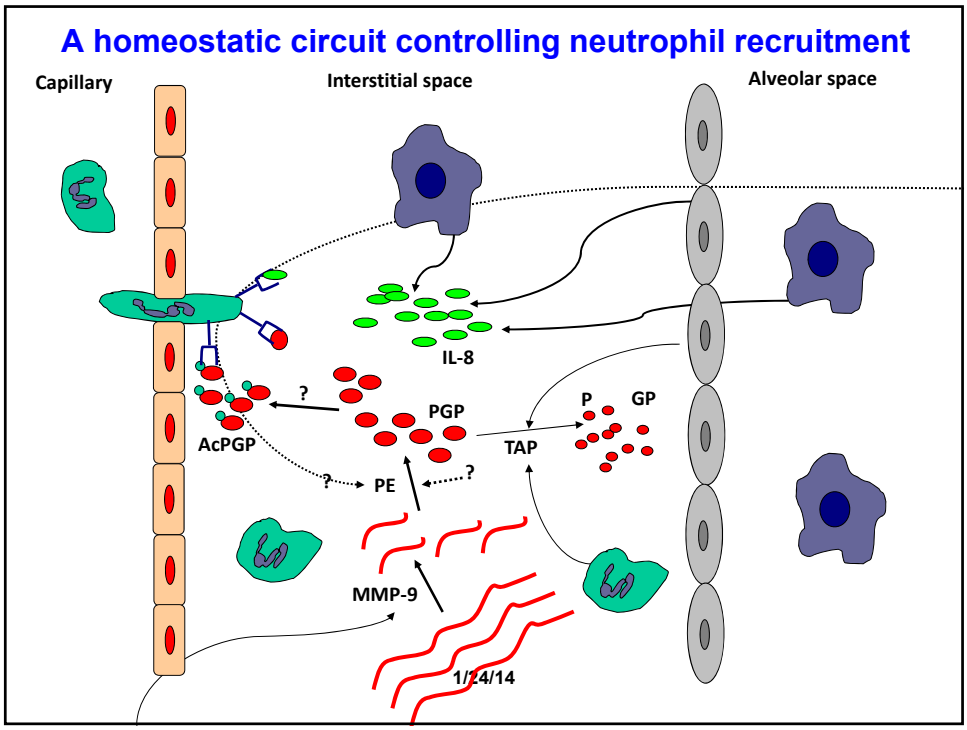


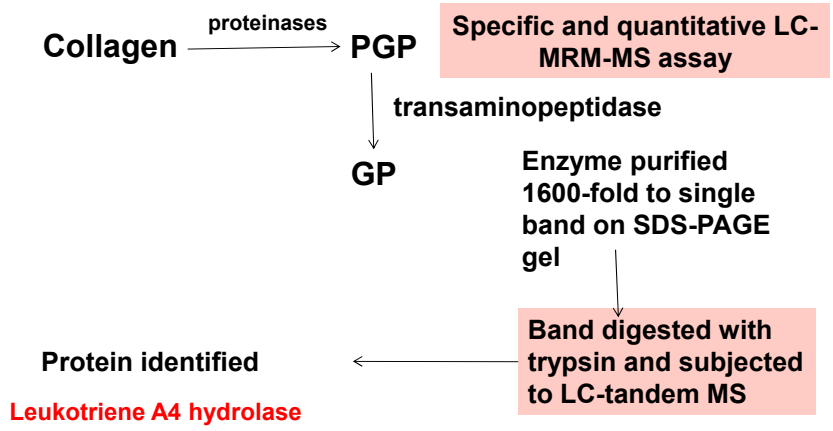
Figure 3. Peptide-Encoding Regions and Putative Functional Domains of Human Chromogranin A (CgA).
Arabic numbers designate amino acids in the mature protein (minus signal peptide). Roman numerals designate exon numbers. The intron–exon structure is not drawn to scale.



PGP is a common peptide in human collagen

MFSFVDLRLLLLLAATALLTHGQEEGQVEGQDEDIPPITCVQNGLRYHDRDVWKPEPCRI
CVCDNGKVLCDDVICDETKNCPGAEVPEGECCPVCPDGSESPTDQETTGVEGPKGDTGPR
GPRGPAGPPGRDGIPGQPGLPGPPGPPGPPGPPGPPGLGGNFAPQLSYGYDEKSTGGISVPGP
MGPSGPRGLPGPPGAPGPQGFQGPPGEPGEPGASGPMGPRGPPGPPGKNGDDGEAGKPGR
PGERGPPGPQGARGLPGTAGLPGMKGHRGFSGLDGAKGDAGPAGPKGEPGSPGENGAPGQ
MGPRGLPGERGRPGAPGPAGARGNDGATGAAGPPGPTGPAGPPGFPGAVGAKGEAGPQGP
RGSEGPQGVRGEPGPPGPAGAAGPAGNPGADGQPGAKGANGAPGIAGAPGFPGARGPSGP
QGPGGPPGPKGNSGEPGAPGSKGDTGAKGEPGPVGVQGPPGPAGEEGKRGARGEPGPTGL
PGPPGERGGPGSRGFPGADGVAGPKGPAGERGSPGPAGPKGSPGEAGRPGEAGLPGAKGL
TGSPGSPGPDGKTGPPGPAGQDGRPGPPGPPGARGQAGVMGFPGPKGAAGEPGKAGERGV
PGPPGAVGPAGKDGEAGAQGPPGPAGPAGERGEQGPAGSPGFQGLPGPAGPPGEAGKPGE
QGVPGDLGAPGPSGARGERGFPGERGVQGPPGPAGPRGANGAPGNDGAKGDAGAPGAPGS
QGAPGLQGMPGERGAAGLPGPKGDRGDAGPKGADGSPGKDGVRGLTGPIGPPGPAGAPGD
KGESGPSGPAGPTGARGAPGDRGEPGPPGPAGFAGPPGADGQPGAKGEPGDAGAKGDAGP
PGPAGPAGPPGPIGNVGAPGAKGARGSAGPPGATGFPGAAGRVGPPGPSGNAGPPGPPGP
AGKEGGKGPRGETGPAGRPGEVGPPGPPGPAGEKGSPGADGPAGAPGTPGPQGIAGQRGV
VGLPGQRGERGFPGLPGPSGEPGKQGPSGASGERGPPGPMGPPGLAGPPGESGREGAPGA
EGSPGRDGSPGAKGDRGETGPAGPPGAPGAPGAPGPVGPAGKSGDRGETGPAGPAGPVGP
VGARGPAGPQGPRGDKGETGEQGDRGIKGHRGFSGLQGPPGPPGSPGEQGPSGASGPAGP
RGPPGSAGAPGKDGLNGLPGPIGPPGPRGRTGDAGPVGPPGPPGPPGPPGPPSAGFDFSF
LPQPPQEKAHDGGRYYRADDANVVRDRDLEVDTTLKSLSQQIENIRSPEGSRKNPARTCR
DLKMCHSDWKSGEYWIDPNQGCNLDAIKVFCNMETGETCVYPTQPSVAQKNWYISKNPKD
KRHVWFGESMTDGFQFEYGGQGSDPADVAIQLTFLRLMSTEASQNITYHCKNSVAYMDQQ
TGNLKKALLLQGSNEIEIRAEGNSRFTYSVTVDGCTSHTGAWGKTVIEYKTTKTSRLPII
DVAPLDVGAPDQEFGFDVGPVCFL

Mass spec and PGP



(Robert Snelgrove et al., Science 2010)

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Enzymatic modifications

- Phosphorylation/dephosphorylation
  - On serine, threonine, tyrosine (also histidine)
- Glycosylation
  - N-glycosylation (asparagine-linked)
  - O-glycosylation (serine-, threonine-linked)
- N-Acetylation/deacetylation
  - On lysines – also higher acyl groups
- N-Methylation/demethylation
  - On lysines - mono-, di- and trimethylation

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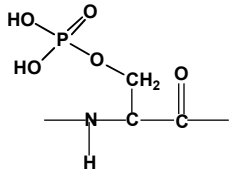
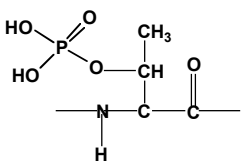
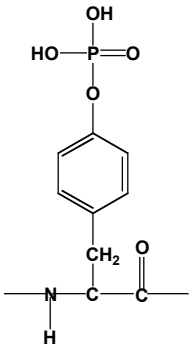
Chemistry of phosphorylation



Serine



Threonine



Tyrosine

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Mass spectrometry of phosphorylated 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 ^{79}Br, 79.9083 Da)

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Finding a phosphate group

Several methods are in use for detection of phosphopeptides

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

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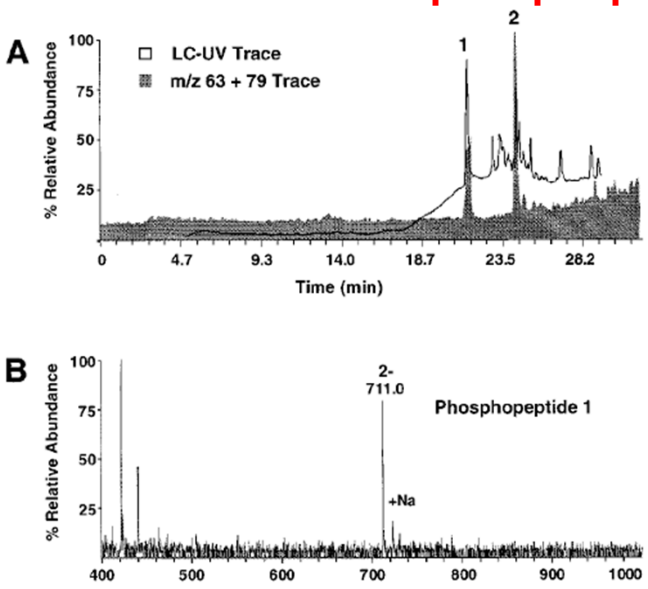
Parent ion scan to detect phosphopeptide



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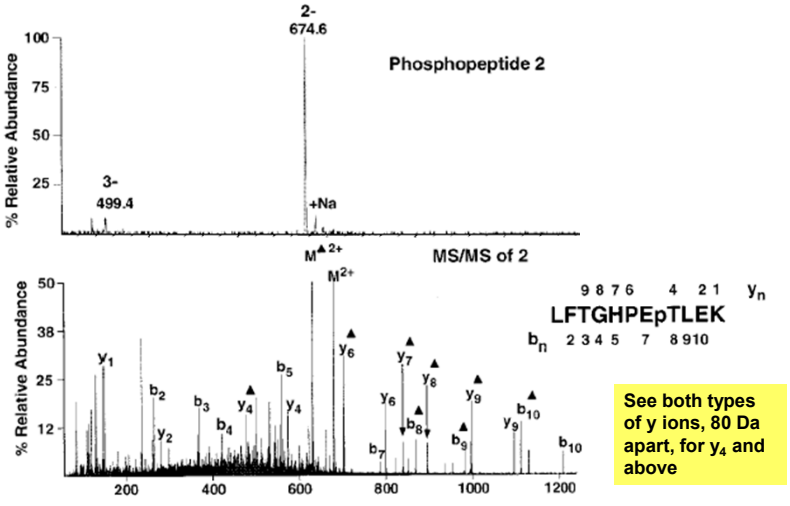
Annan Anal Chem 73:393

Phosphopeptide 2



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Annan Anal Chem 73:393

Neutral loss for phosphopeptides

- Based on the loss of phosphoric acid (H_3PO_4) - 97.98 Da
- If the peptide is doubly charged, then it's *m/z* 48.88
- If it's triply charged, then it's *m/z* 32.66
- Problems can occur if the peptide contains an N-terminal proline (97 Da) or valine (99 Da) and a low resolution instrument is used for the analysis

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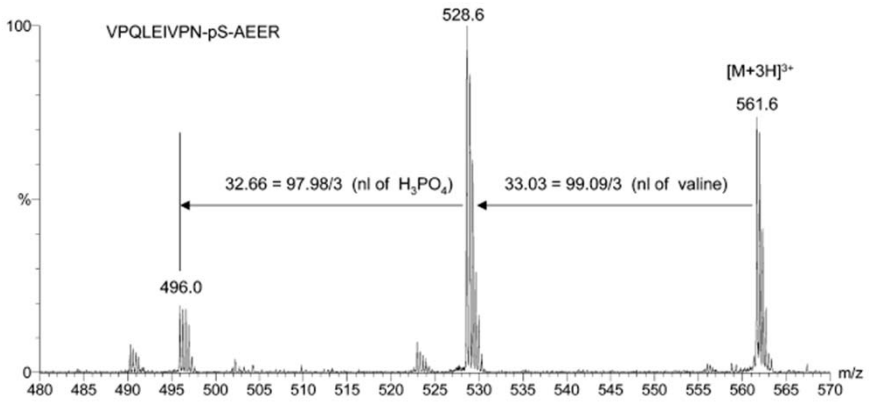
Casein peptide - mono- or diphosphate?



Lehmann et al., J Prot Res 7-2866

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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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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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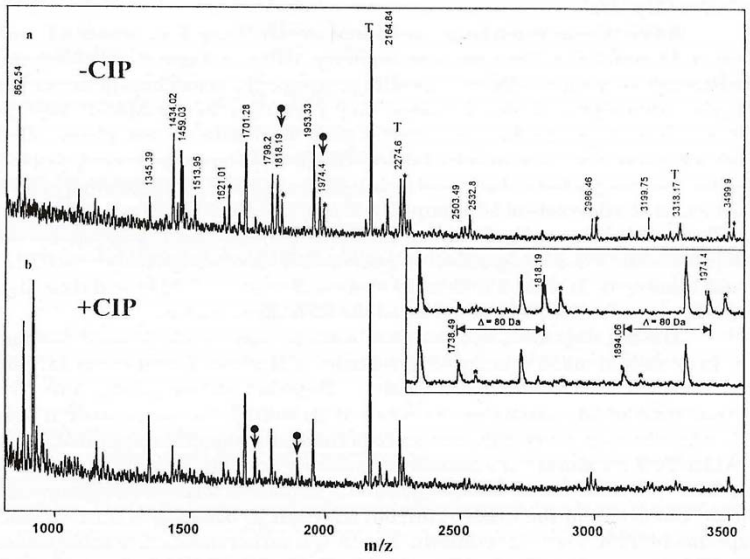
Detecting a phosphopeptide with alkaline phosphatase



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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
- TiO_2 is now being used with success

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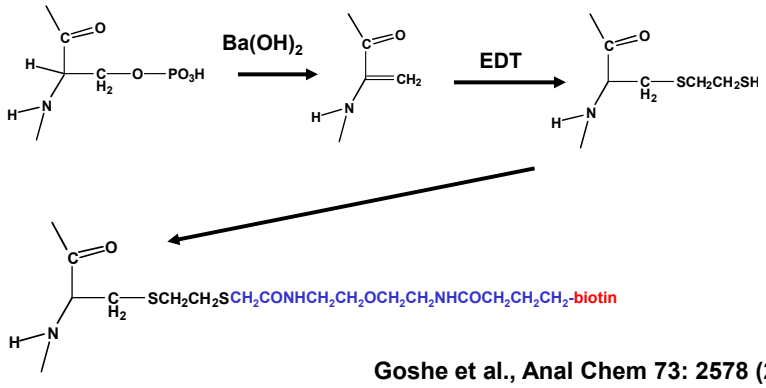
Selective biotinylation of phospho-groups



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

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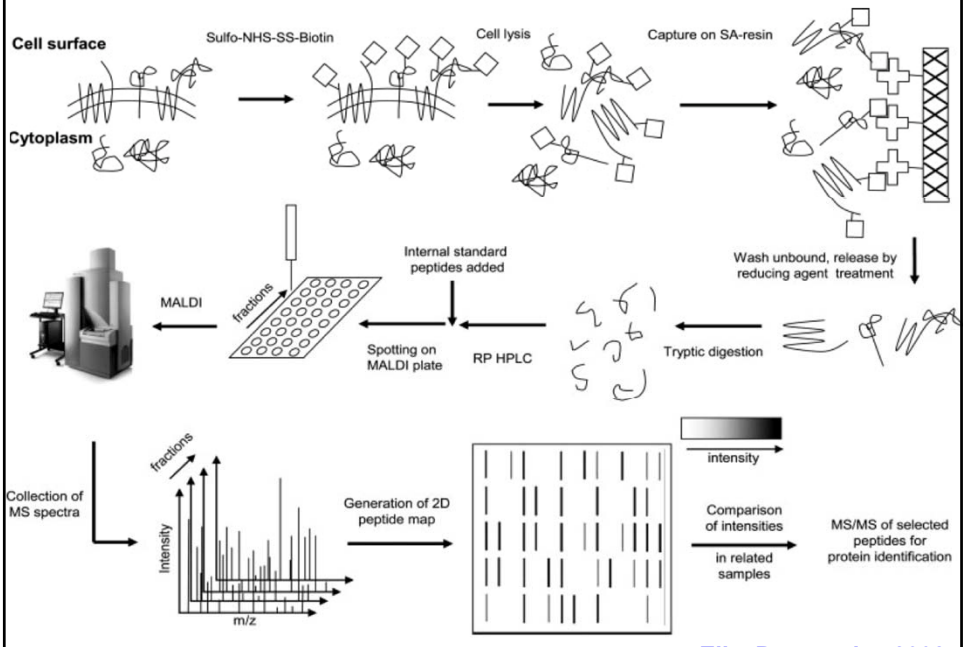

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Elia, Proteomics 2008

How to identify phosphorylated peaks by searching databases

- **The databases you have used earlier in this class 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/)**
  - **Phosphosite is more focused http://www.phosphosite.org/staticAboutPhosphosite.do**

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How to identify posttranslational modifications

FindMod at

http://www.expasy.org/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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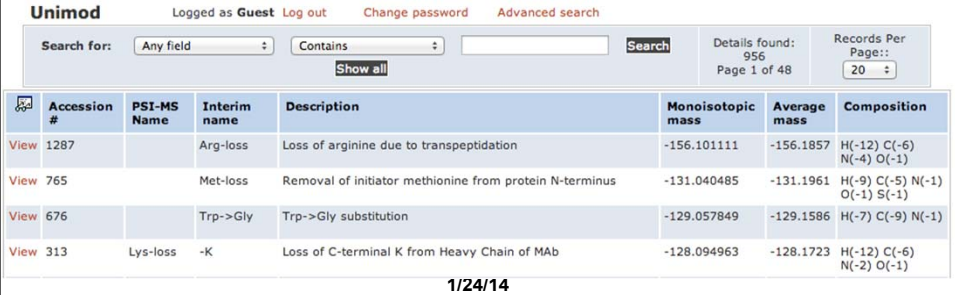
Unimod

- **As Matt Renfrow described on January 17th, Unimod**
  - **(http://www.unimod.org/modifications_list.php)**

provides a more detailed and more accurate list of possible modifications



	Accession #	PSI-MS Name	Interim name	Description	Monoisotopic mass	Average mass	Composition
View	1287		Arg-loss	Loss of arginine due to transpeptidation	-156.101111	-156.1857	H(-12) C(-6) N(-4) O(-1)
View	765		Met-loss	Removal of initiator methionine from protein N-terminus	-131.040485	-131.1961	H(-9) C(-5) N(-1) O(-1) S(-1)
View	676		Trp->Gly	Trp->Gly substitution	-129.057849	-129.1586	H(-7) C(-9) N(-1)
View	313	Lys-loss	-K	Loss of C-terminal K from Heavy Chain of MAb	-128.094963	-128.1723	H(-12) C(-6) N(-2) O(-1)

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FindMod

http://web.expasy.org/findmod/

Some of the modifications considered are:

acetylation	amidation	biotinylation
carboxymethyl cysteine	carboxyamidomethyl cysteine	
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		

Wilkins M.R., Gasteiger E., Gooley A., Herbert B., Molloy M.P., Binz P.A., Ou K., Sanchez J.-C., Bairoch A., Williams K.L, Hochstrasser D.F. *High-throughput Mass Spectrometric Discovery of Protein Post-translational Modifications.* Journal of Molecular Biology, 289, p. 645-657 (1999)

Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A.; *Protein Identification and Analysis Tools on the ExPASy Server;* (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005).

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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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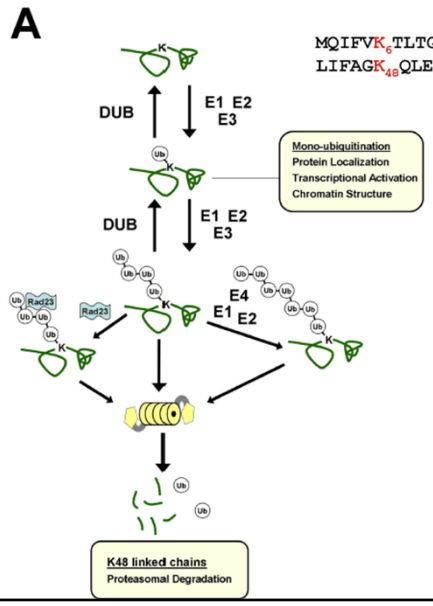
Ubiquitination



Ubiquitin (Human)

MQIFVK$_6$TLTGK$_{11}$TITLEVEPSDTIENVK$_{27}$AK$_{29}$IQDK$_{33}$EGIPPDQQR
LIFAGK$_{48}$QLEDGRTLSDYNIQK$_{63}$ESTLHLVLRLRGG

Ub adds to lysine residues on proteins; it can also add to itself at K_6, K_{11}, K_{21}, K_{29}, K_{33}, K_{46} and K_{66} to form polyubiquinated proteins

Kirkpatrick et al., 2005

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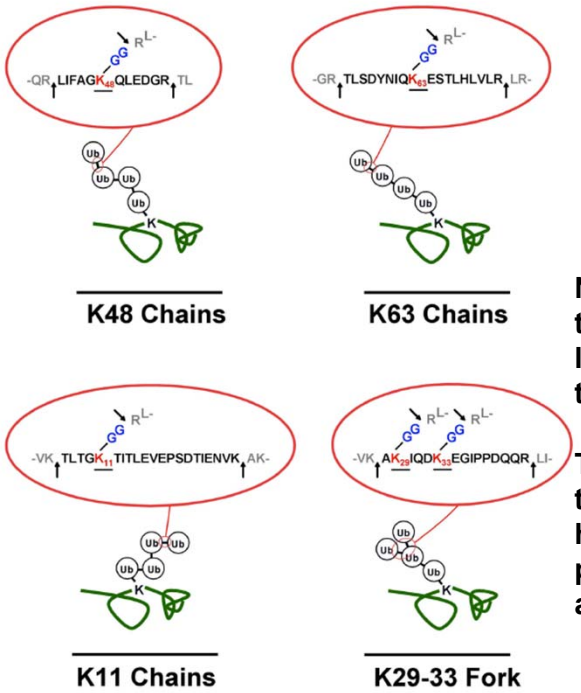
Sites of attachment of Ub groups

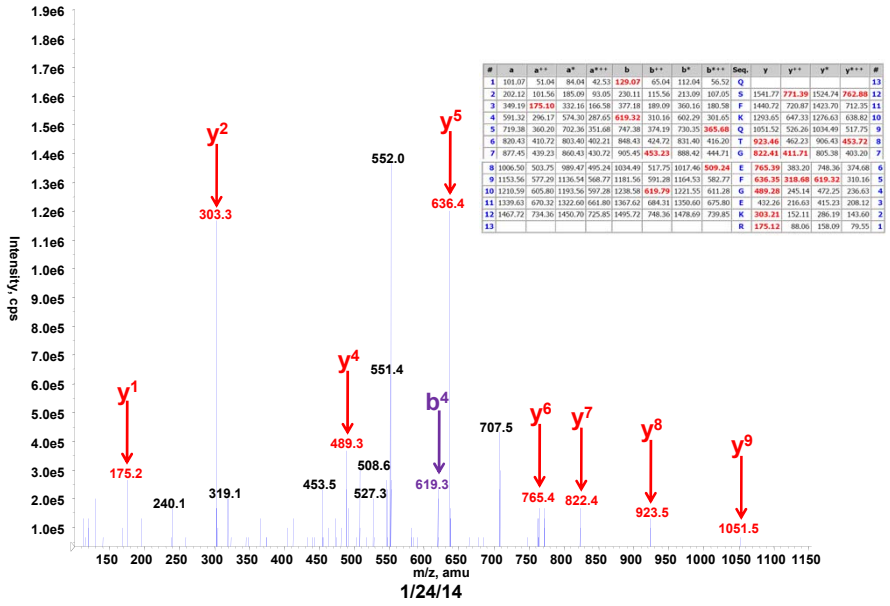


Note that Ub is attached through the side chain of lysine via the two C-terminal glycines of Ubq.

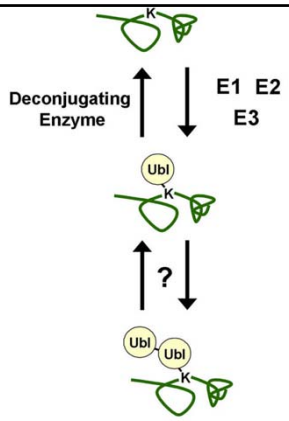
The next residue is Arg – therefore, trypsin hydrolysis will yield a peptide modified by the addition of 114 (*m/z* 261)

Kirkpatrick et al., 2005

Peptide Fragmentation Scan: QS(methylated)FK(ubiquitinated)QTGEFGEKR
Mass: 557.5(3+)



#	a	a++	a*	a*++	b	b++	b*	b*++	Seq.	y	y++	y*	y*++	#
1	101.07	51.04	84.04	42.53	129.07	65.04	112.04	56.52	Q					13
2	202.12	101.56	185.09	93.05	230.11	115.56	213.09	107.05	S	1541.77	771.39	1524.74	762.88	12
3	349.19	175.10	332.16	166.58	377.18	189.09	360.16	180.58	F	1440.72	720.87	1423.70	712.35	11
4	591.32	296.17	574.30	287.65	619.32	310.16	602.29	301.65	K	1293.65	647.33	1276.63	638.82	10
5	719.38	360.20	702.36	351.68	747.38	374.19	730.35	365.68	Q	1051.52	526.26	1034.49	517.75	9
6	820.43	410.72	803.40	402.21	848.43	424.72	831.40	416.20	T	923.46	462.23	906.43	453.72	8
7	877.45	439.23	860.43	430.72	905.45	453.23	888.42	444.71	G	822.41	411.71	805.38	403.20	7
8	1006.50	503.75	989.47	495.24	1034.49	517.75	1017.46	509.24	E	765.39	383.20	748.36	374.68	6
9	1153.56	577.29	1136.54	568.77	1181.56	591.28	1164.53	582.77	F	636.35	318.68	619.32	310.16	5
10	1210.59	605.80	1193.56	597.28	1238.58	619.79	1221.55	611.28	G	489.28	245.14	472.25	236.63	4
11	1339.63	670.32	1322.60	661.80	1367.62	684.31	1350.60	675.80	E	432.26	216.63	415.23	208.12	3
12	1467.72	734.36	1450.70	725.85	1495.72	748.36	1478.69	739.85	K	303.21	152.11	286.19	143.60	2
13									R	175.12	88.06	158.09	79.55	1



SUMO-modification
Protein Localization
Transcriptional Activation
Chromatin Structure
Prevent Ubiquitination
Poly-Sumoylation

Others
Immune Response (ISG15)
Ubiquitination (Nedd8)
Morphogenesis (Hub1)

SUMOylation

There are ubiquitin-like proteins (Ubls), one of which is Small Ubiquitin-like Modifier (SUMO)

Have different sequence, but similar structure. Recognize the consensus motif Ψ-K-x-D/E for SUMOylation. Connected via a Gly-Gly group to the lysine residue (just like ubiquitin)

http://sumosp.biocuckoo.org/faq.php

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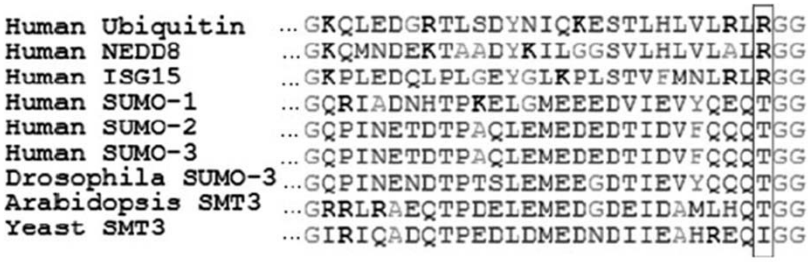
Kirkpatrick et al., 2005

Ubiquitin versus SUMO



So, trypsin will not cut SUMO sites (unlike for ubiquitin) to generate a Gly-Gly modified Lysine

Knuesel et al., 2005

Detecting PTMs

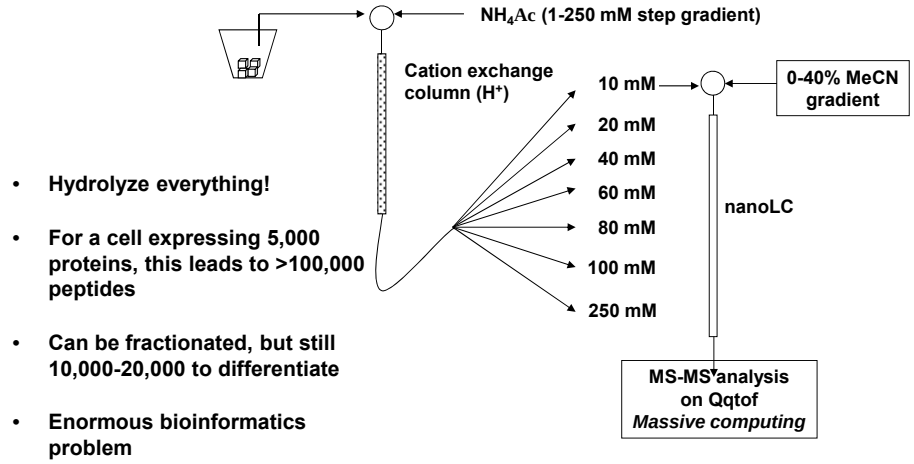
- If we don't have any idea about what the modification(s) is(are), how do we proceed?
- We won't have the modification on MASCOT or Protein Prospector
- No antibodies or convenient affinity phases

Can we approach this globally?

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

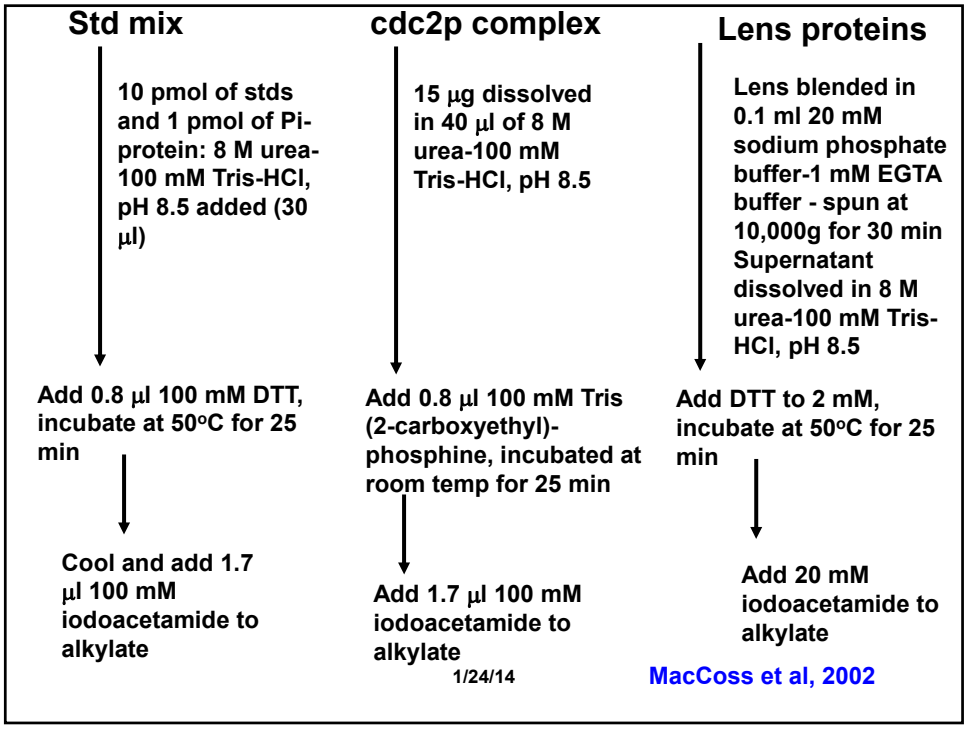
1/24/14

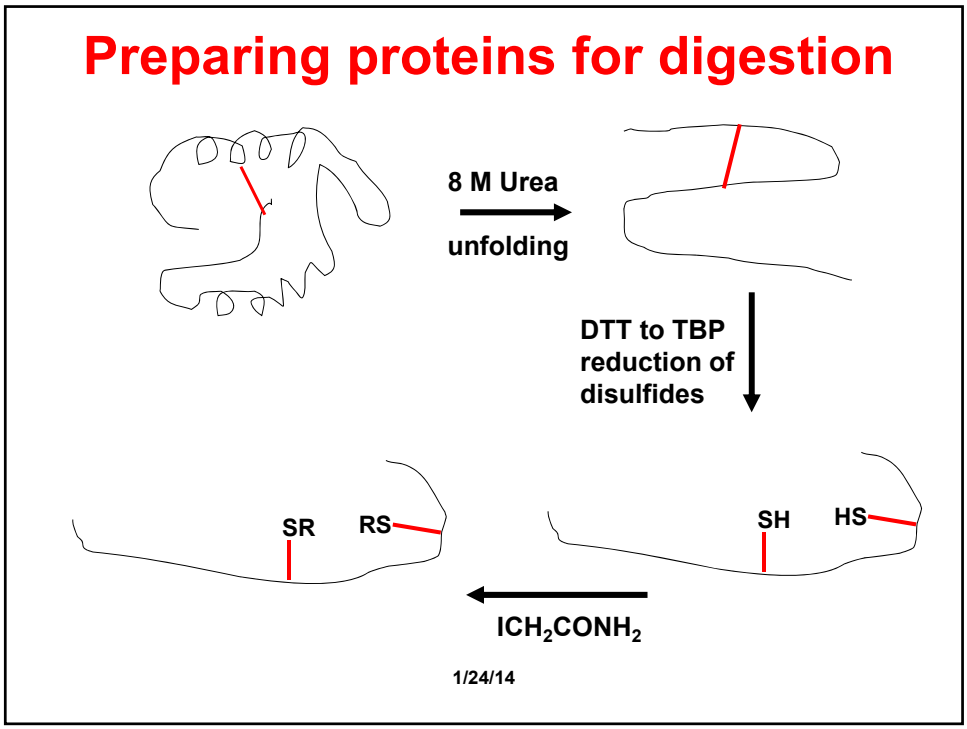
MudPIT - Multi-dimensional Protein Identification Technology



- 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

1/24/14 John Yates


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
1/24/14
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
1/24/14

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

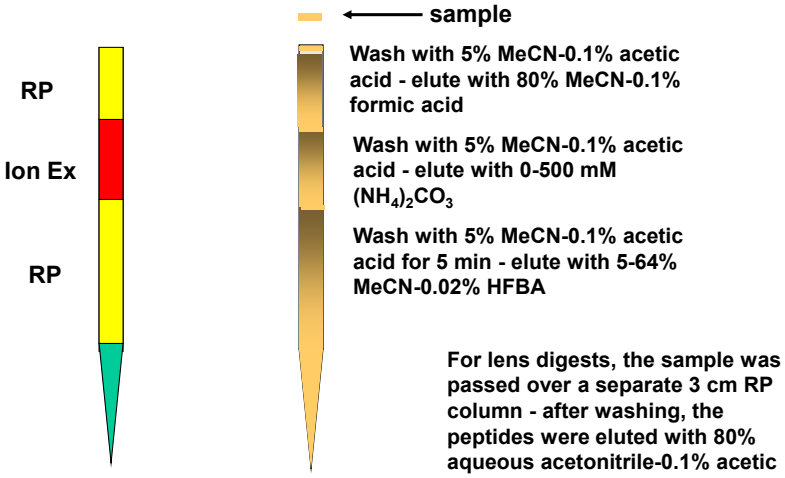
↓

quenched with 90% formic acid to 4%

1/24/14

MacCoss et al, 2002

Elution from a triphasic column



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

1/24/14

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)

1/24/14 MacCoss et al, 2002

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

1/24/14 MacCoss et al, 2002

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

 MacCoss et al, 2002

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

 MacCoss et al, 2002

Summary

- The proteome is very complex
- Some proteins are in pro-forms and can be found in many, often small, but active fragments
- In addition, proteins are heavily modified
- Modification can account for marked differences in turnover
- Selective fractionation is needed to demonstrate PTMs that are in low abundance

1/24/14

CFTR modifications

- McClure M, DeLucas LJ, Wilson L, Ray M, Rowe S, Wu X, Dai Q, Hong JS, Sorscher EJ, Kappes JC, Barnes S. Palmitoylation and other post-translational modifications of CFTR with relevance to gating and processing. *Protein Eng Des Sel. 2012 Jan;25(1):7-14*.

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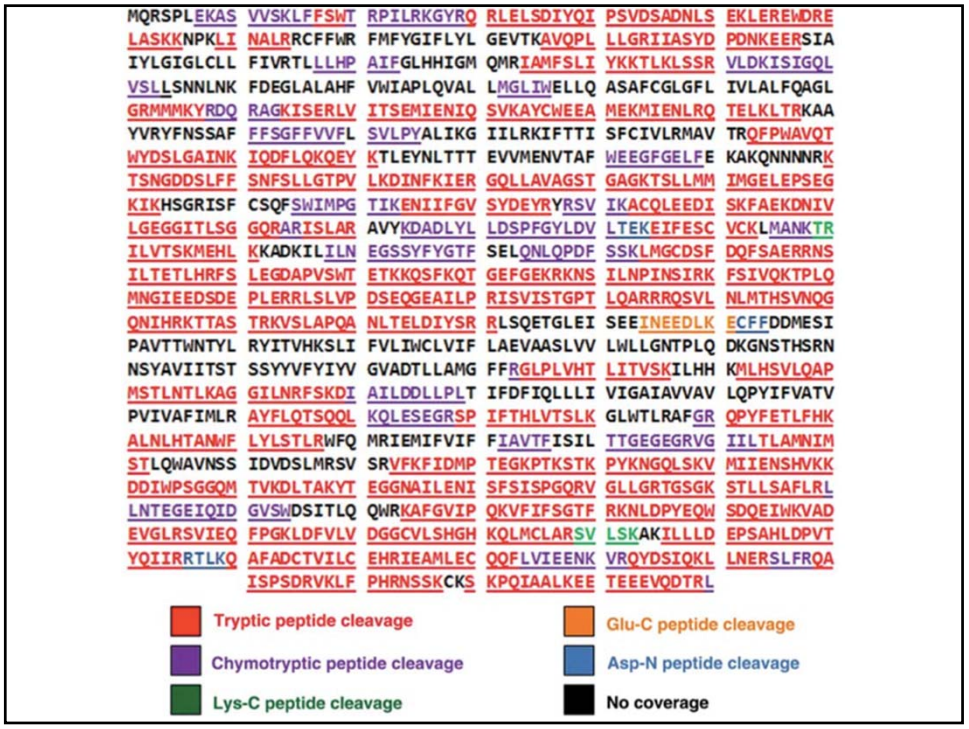
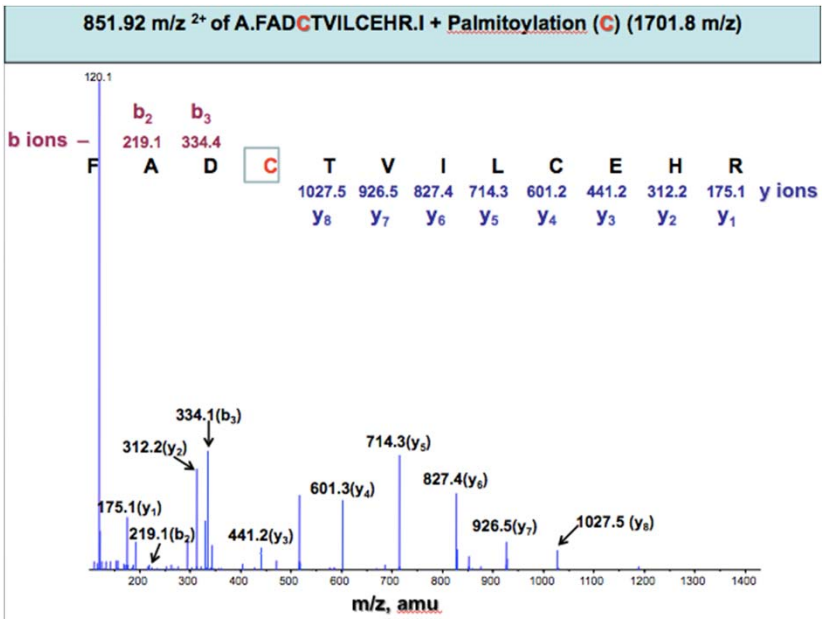


Sites of modification on CFTR

1/24/14



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Palmitoylation of CFTR

- Palmitoylation of Cys1395 was discovered in a "discovery" method, i.e., fully untargeted
- It opened the question as to (1) was it biologically important and (2) were other cysteine residues palmitoylated?
- This latter question was addressed by using "targeted" discovery using a MRM method on a triple quad mass spectrometer

1/24/14

Targeted MRM method

- We know the *m/z* value of the tryptic peptide that could become palmitoylated
- We add the mass of the palmitoyl group
- We also adjust the values of the b- and y-ions to reflect where the addition occurs

1/24/14

Ions for the peptide containing Cys524

ACQLEEDISK (*m/z* 596.8)

	232	360	473	602	731	845	958	1045
A	C*	Q	L	E	E	D	I	S	K
	971	843	720	591	462	347	234	147	

In this case, the Cys has been modified by IOA

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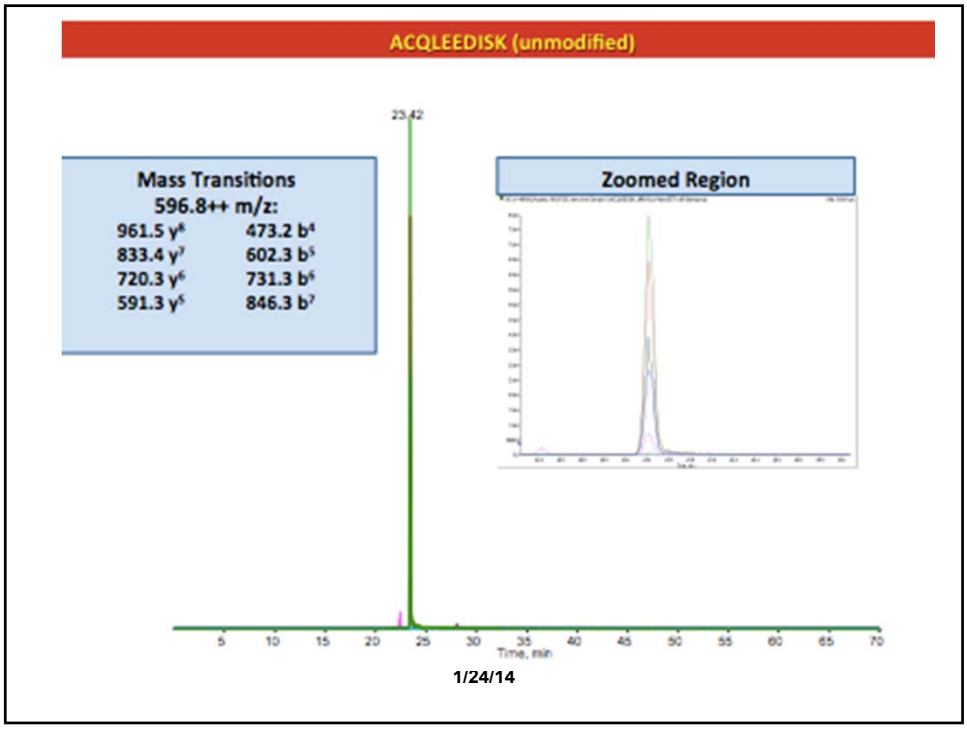


Palmitoylated peptide

ACQLEEDISK (*m/z* 687.4)

	413	541	654	783	912	1027	1140	1227
A	C*	Q	L	E	E	D	I	S	K
		971	843	720	591	462	347	234	147

In this case, the Cys has been modified by palmitate

1/24/14

ACQLEEDISK (palmitolylated)

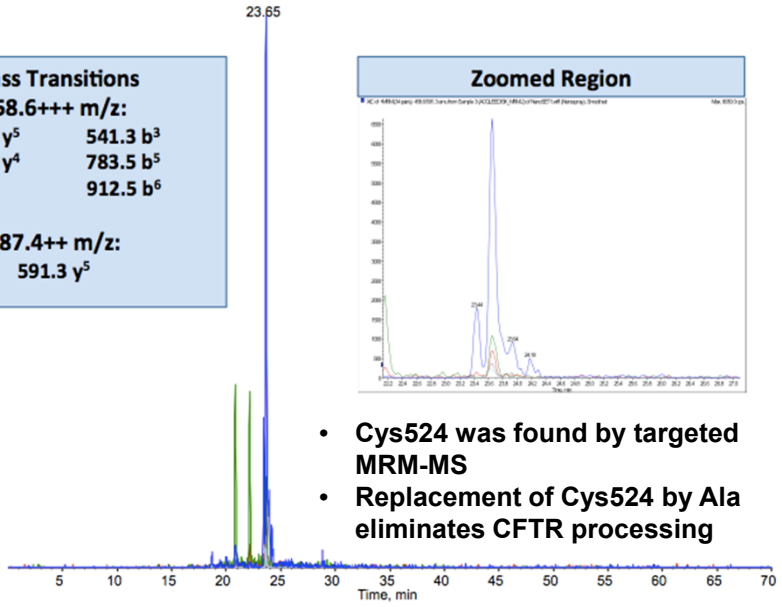
Mass Transitions

458.6+++ m/z:

591.3 y^5	541.3 b^3
462.3 y^4	783.5 b^5
	912.5 b^6

687.4++ m/z:

591.3 y^5



Zoomed Region

- **Cys524 was found by targeted MRM-MS**
- **Replacement of Cys524 by Ala eliminates CFTR processing**