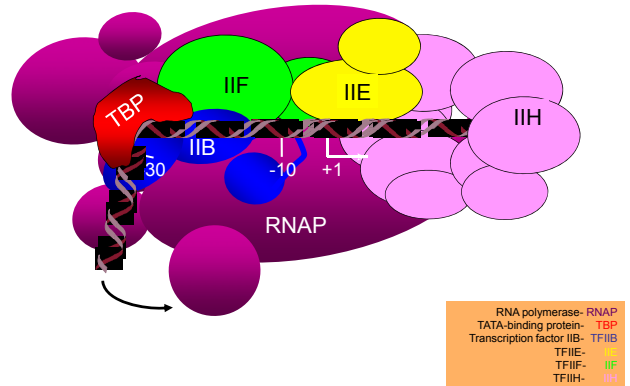


Biological Mass Spectrometry: Ionization, Calculating Mass, Charge

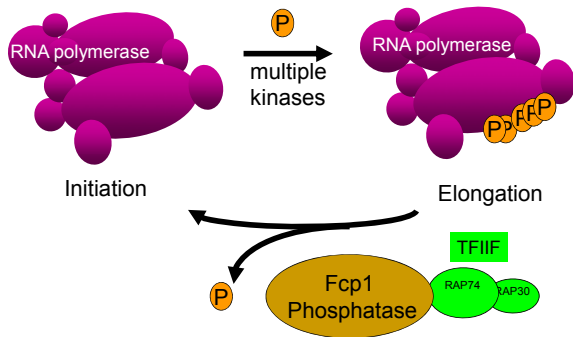
Matthew Renfrow, PhD
6-4681
renfrow@uab.edu

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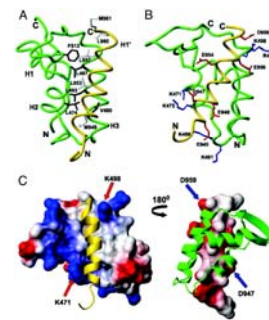
Eukaryotic Class II transcription preinitiation complex (PIC)



Dephosphorylation of RNA polymerase largest subunit

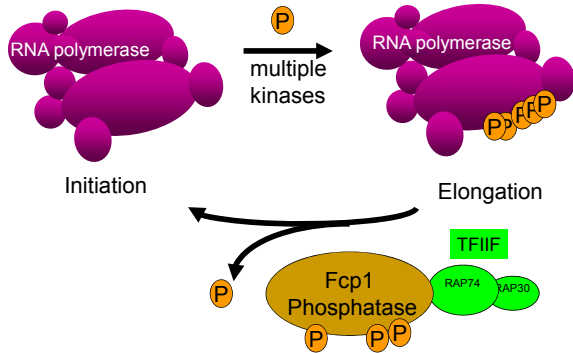


TFIIF (RAP74) – Fcp1 phosphatase interacting domain

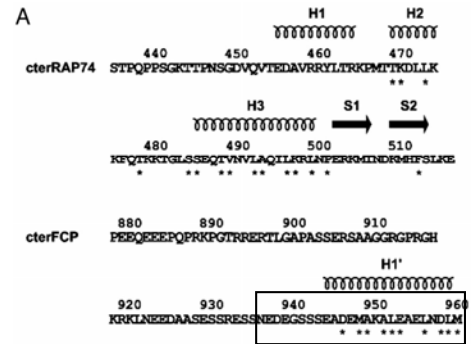


Nguyen BD, Abbott KL, Potempa K, Kobor MS, Archambault J, Greenblatt J, Legault P, Omichinski JG
Proc Natl Acad Sci U S A. 2003 May 13;100(10):5688-93

Dephosphorylation of RNA polymerase largest subunit

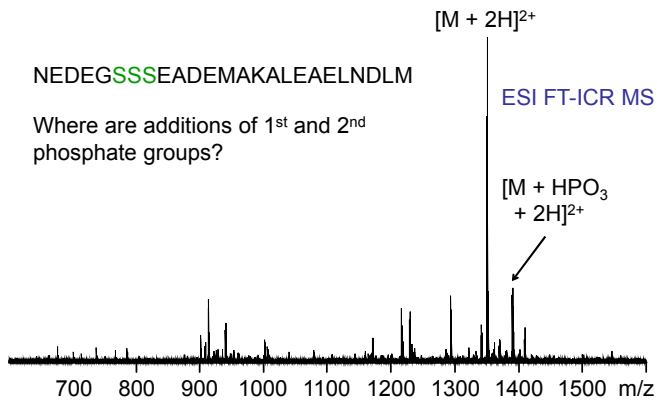


Fcp1 is a Phosphoprotein



Nguyen BD, Abbott KL, Potempa K, Kobar MS, Archambault J, Greenblatt J, Legault P, Omichinski JG Proc Natl Acad Sci U S A. 2003 May 13;100(10):5688-93

Fcp1 C-terminal fragment after 4 h incubation with CKII



Monoisotopic elemental mass

http://www.avantlipids.com/index.php?option=com_content&view=article&id=2248&Itemid=415

Monoisotopic amino acid mass

<http://www.abrf.org/researchgroups/massspectrometry/eposters/ms97quiz/re siduemasses.html>

Protein & peptide calculator

<http://prospector.ucsf.edu/prospector/mshome.htm>

http://web.expasy.org/peptide_mass/

Unimod

http://www.unimod.org/modifications_list.php

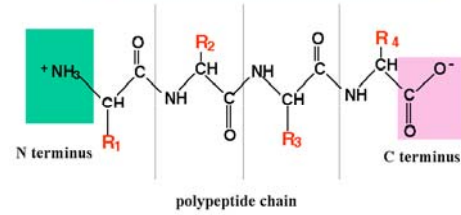
Lipid Calculator

<http://pharmacology.ucdenver.edu/lipidcalc/Default.aspx>

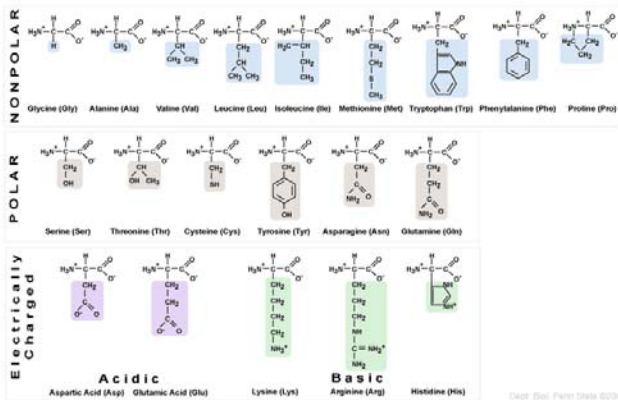
Carbohydrate calculator

<http://web.expasy.org/glycanmass/>

Peptide = chain of amino acids



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Amino acid residue masses

Alanine	71.037	Leucine	113.084
Arginine	156.101	Lysine	128.094
Asparagine	114.043	Methionine	131.040
Aspartic acid	115.027	Phenylalanine	147.068
Cysteine	103.009	Proline	97.053
Glutamic acid	129.043	Serine	87.032
Glutamine	128.058	Threonine	101.048
Glycine	57.021	Tryptophan	186.079
Histidine	137.059	Tyrosine	163.063
Isoleucine	113.084	Valine	99.068

The m/z value of a peptide [M+H]⁺ is the sum of the residue masses plus 18.015 for H₂O plus 1.008. So, what is it for ISLLD?

$$113.084 + 87.032 + 113.084 + 113.084 + 115.027 + 18.015 + 1.008 = 560.334$$

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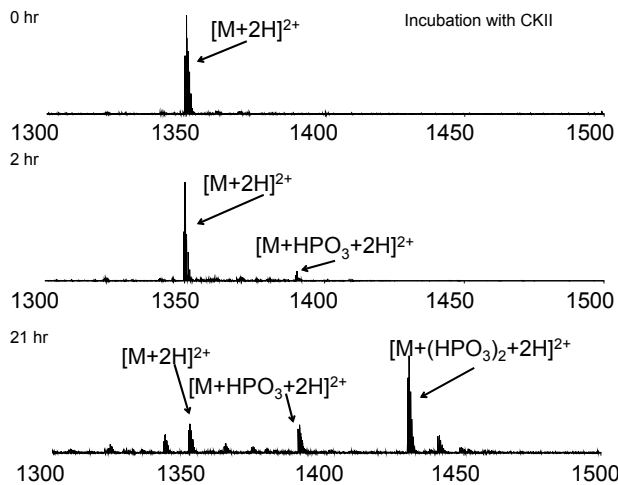
NEDEGSSSEADEMAKALEAELNDLM

NEDEGSSSEADE

MAKALEAELNDLM

- 4 Glutamates
- 1 Asparagine
- 2 Aspartates
- 1 Glycine
- 3 Serines
- 1 Alanine

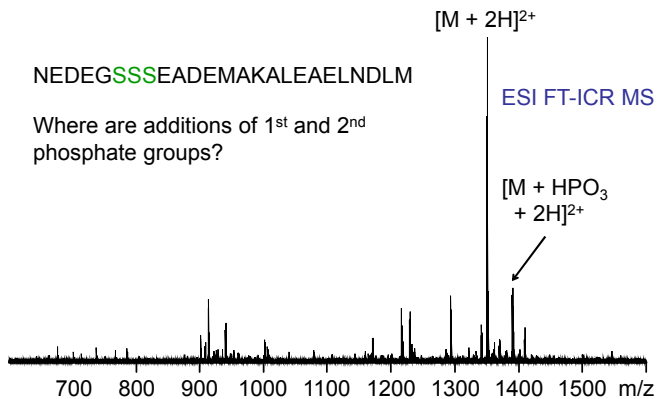
- 2 Methionines
- 3 Alanines
- 1 Lysine
- 2 Glutamates
- 1 Aspartate
- 3 Leucines



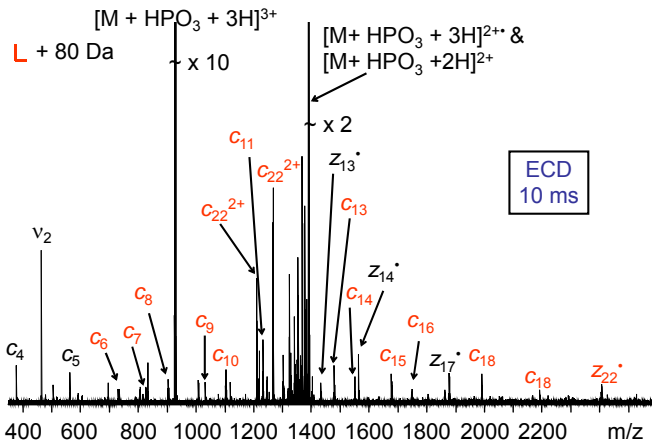
Fcp1 C-terminal fragment

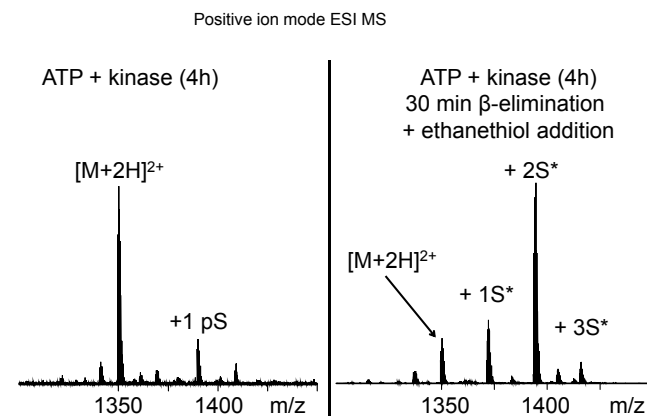
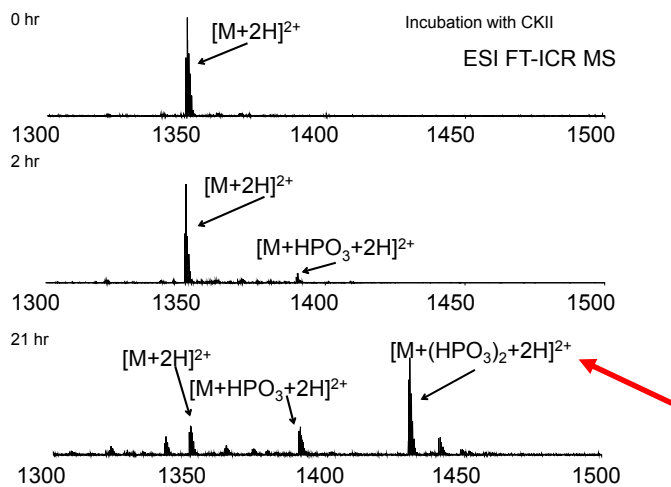
NEDEGSSSEADEMAKALEAELNDLM

Where are additions of 1st and 2nd phosphate groups?

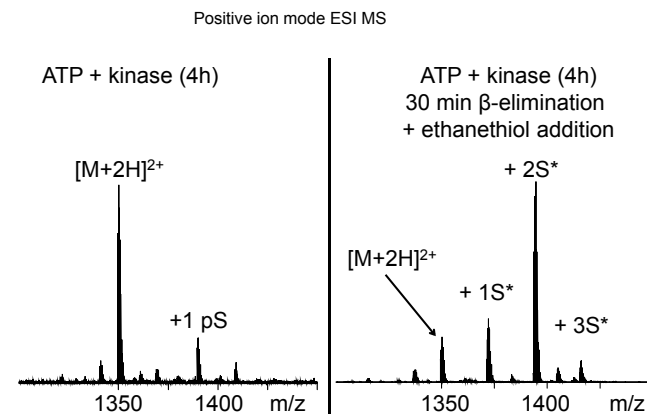
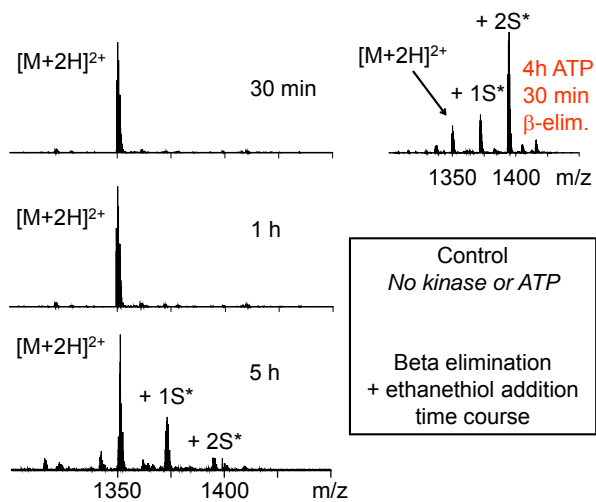


NEDEGSSSEADEMAKALEAELNDLM

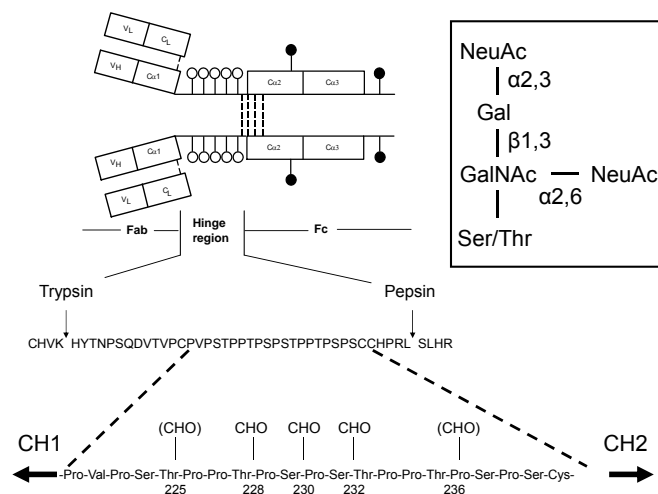
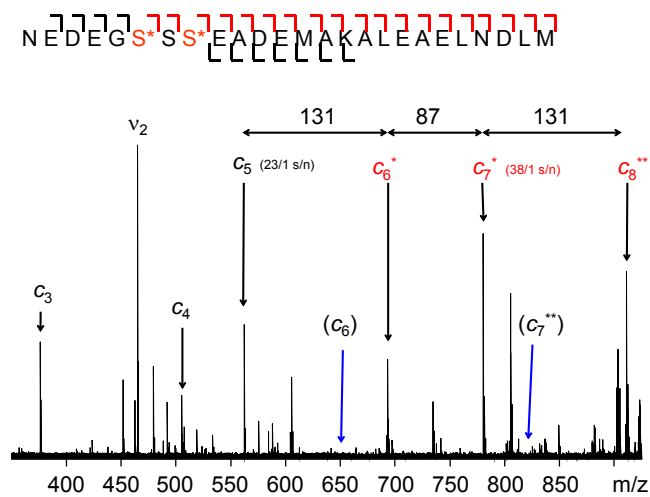
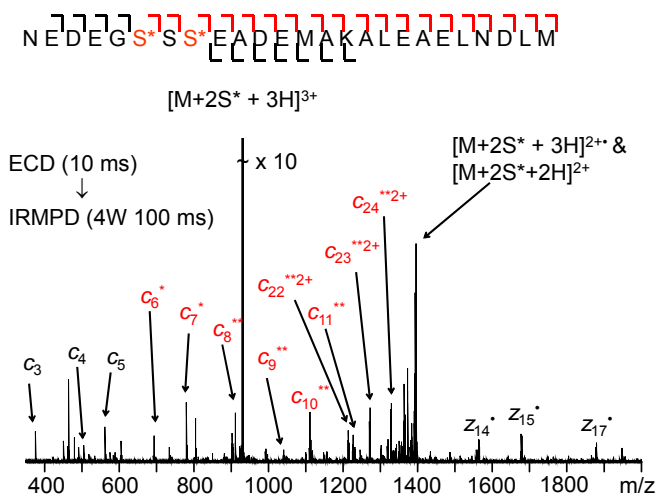




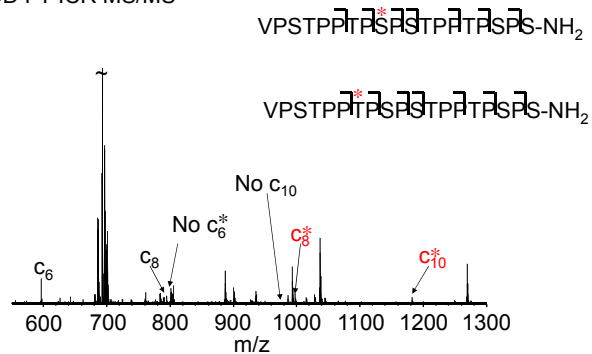
Molloy, M.P., Andrews, P.C. Phosphopeptide derivatization signatures to identify serine and threonine phosphorylated peptides by mass spectrometry. *Anal. Chem.* 2001, 73, 22, 5387-5394



Molloy, M.P., Andrews, P.C. Phosphopeptide derivatization signatures to identify serine and threonine phosphorylated peptides by mass spectrometry. *Anal. Chem.* 2001, 73, 22, 5387-5394



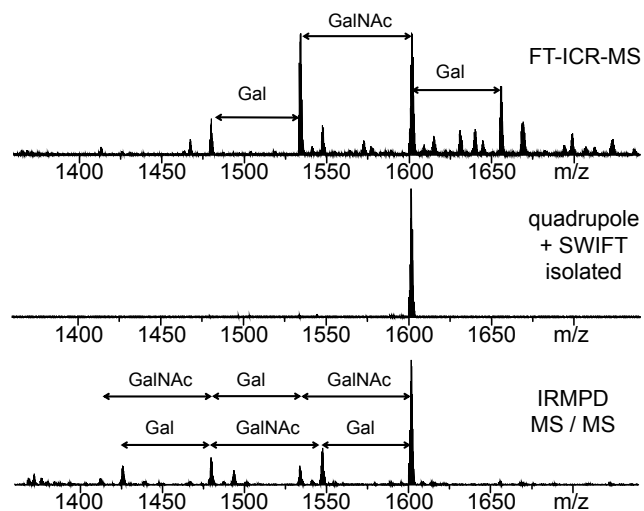
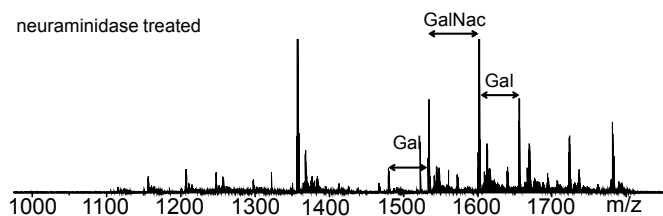
Synthetic IgA1 HR + 1 GalNAc
ECD FT-ICR MS/MS



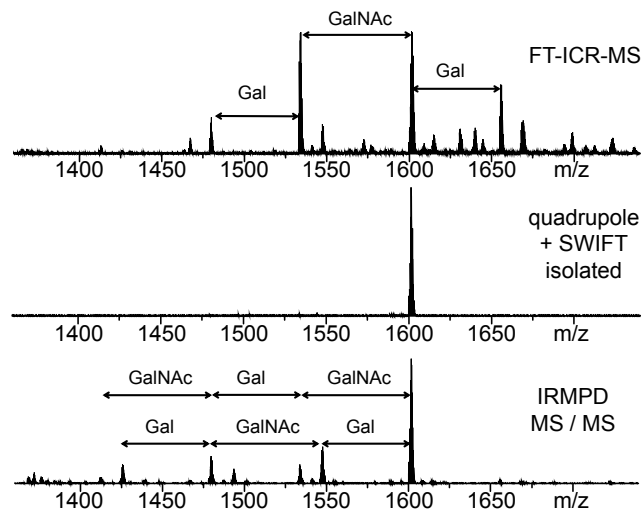
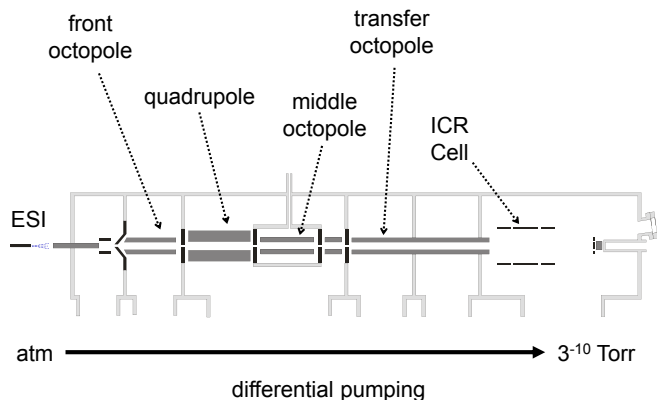
* = GalNAc

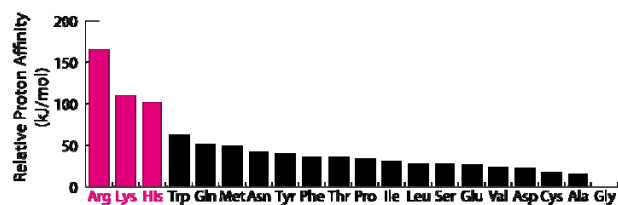
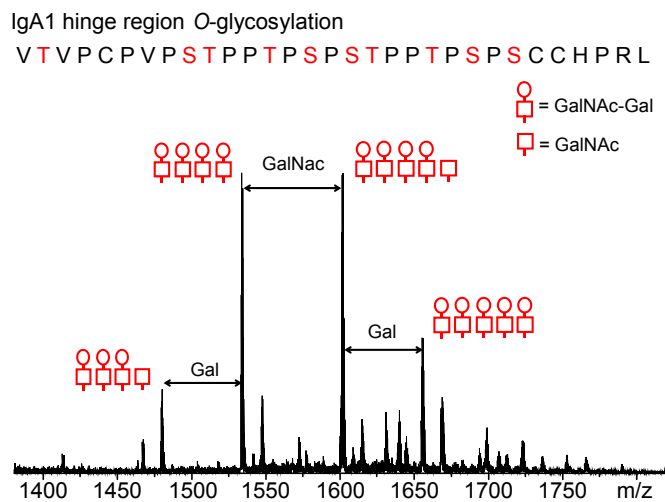
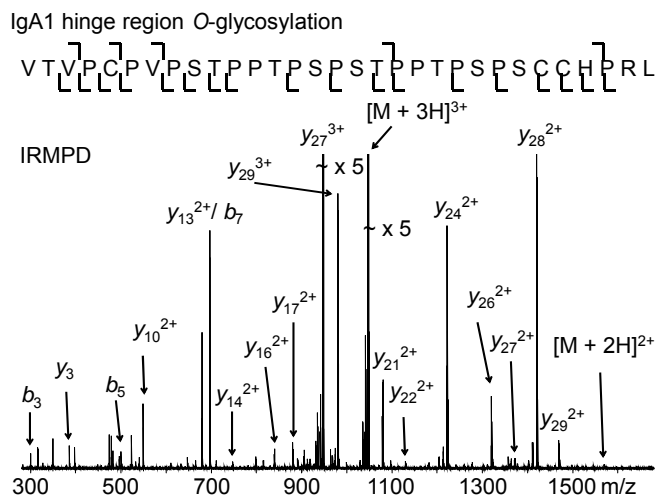
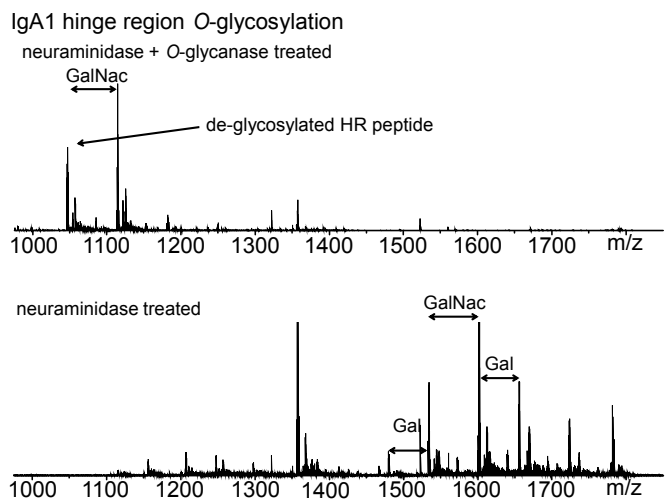
Helen Cooper

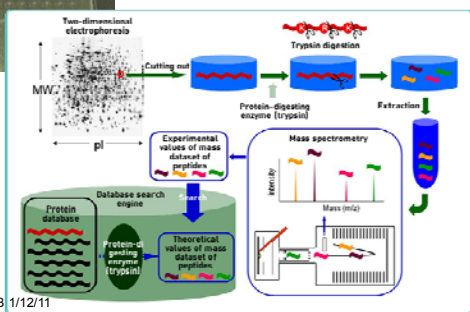
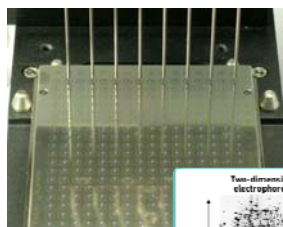
IgA1 hinge region O-glycosylation



NHMFL ESI FT-ICR Instrument (9.4 T)

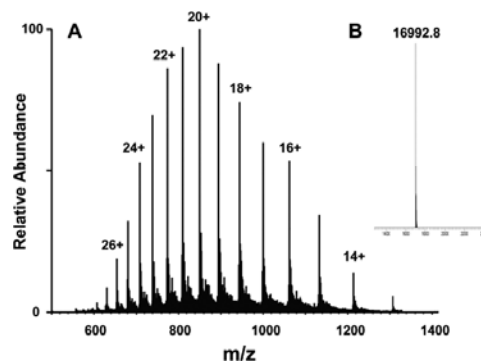






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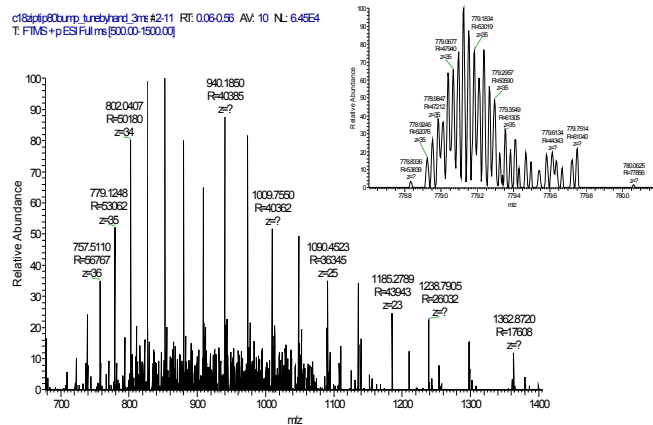
Myoglobin



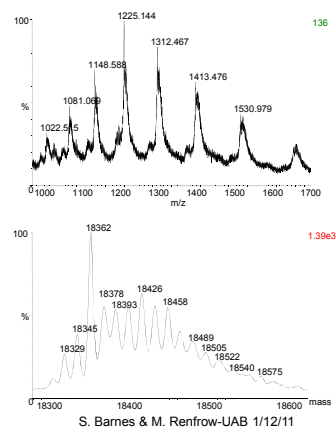
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RXR-Ligand binding domain

138650966.Ligand_Bind_3m4211.RT:036026 AV:10 NL:3354
T: FIMS+pES/Full.ms[500.00-1500.00]

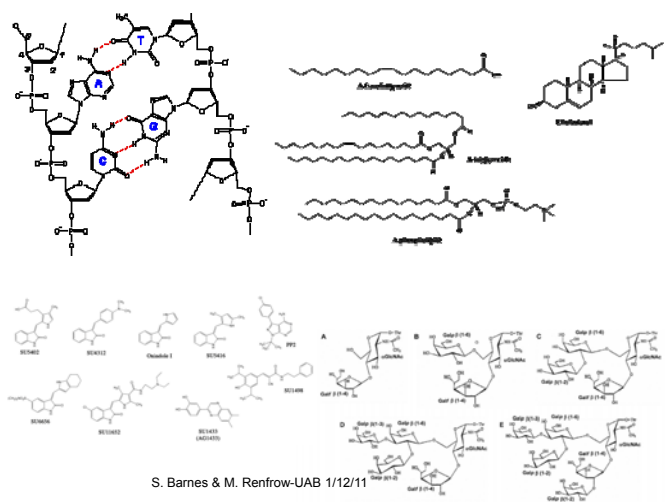


Deconvolution of oxidized forms of β -lactoglobulin

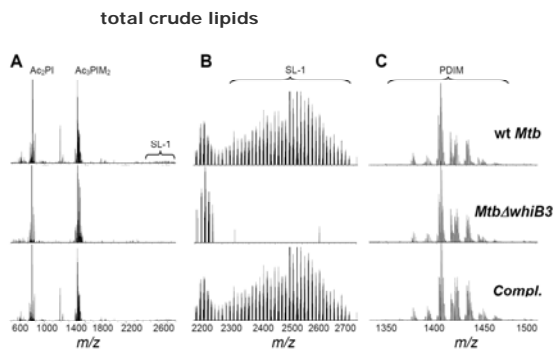


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Junlong Shao

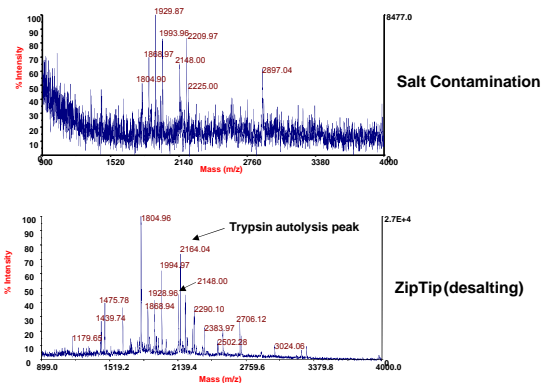


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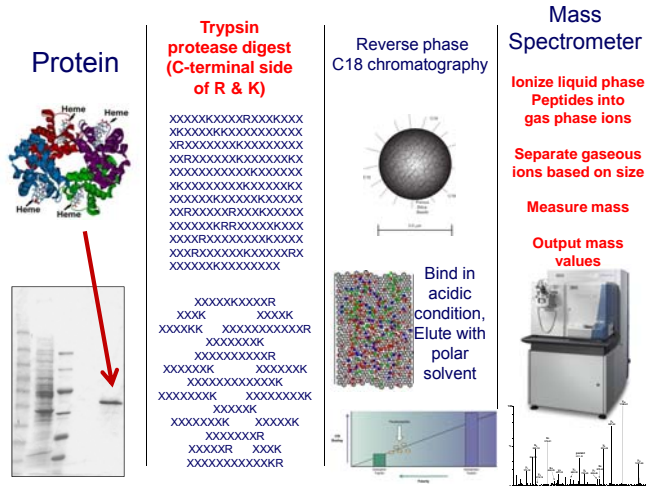


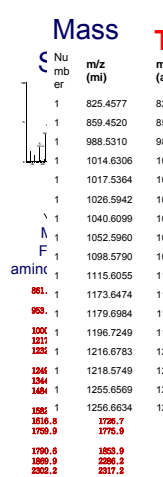
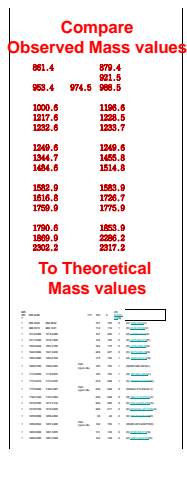
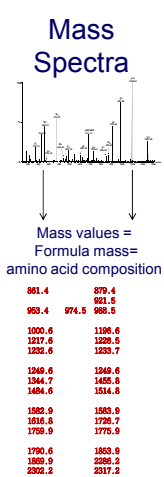
FT-ICR mass spectra of total crude lipids derived from *MtbΔwhiB3* growing in macrophages demonstrate the absence of SL-1. (A) Total crude lipid extracts were prepared from *Mtb* growing inside macrophages and analyzed in the negative ion mode by FT-ICR MS. We observed the presence of Ac₂PI species at *m/z* 835.5261 and 851.8566, which corresponds to their reported theoretical masses. The dimannose specie esterified to three acyl chains (Ac₂PIM₂) corresponds to mass *m/z* 1413.8888 was also detected in all the strains tested. Note that the multiple lipofoms of SL-1 were absent in *MtbΔwhiB3*. (B) The SL-1 region of FT-ICR mass spectrum showed a complete absence of this class of lipids (~*m/z* 2300 to 2600) in *MtbΔwhiB3*. (C) FT-ICR mass spectra of the PDIM region.

Benefit of removing salt from tryptic digest



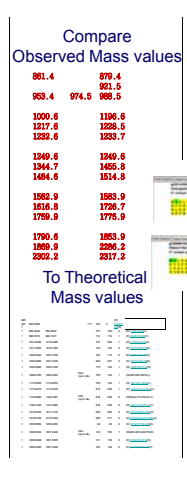
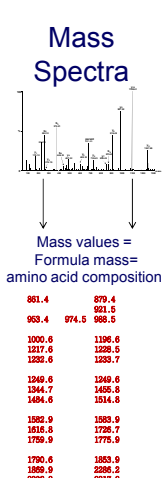
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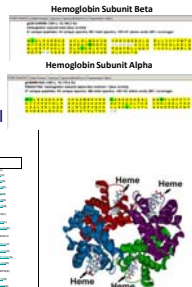


Theoretical mass values

Modifications	Start	End	Missed Cleavages	Sequence
	177	183	0	(R) AHVETLR(Q)
	157	163	0	(K) VQELQDK(L)
	112	119	1	(K) DLEEVKQK(V)
	197	205	1	(R) LTAELVAK(E)
	142	150	0	(K) VAPLGEFFR(E)
	164	172	0	(K) LSPVLADELQ(D)
	229	237	0	(K) AKVPLLEDLR(Q)
	175	183	1	(R) ARAHVETLR(Q)
1Gln->pyro-Glu	155	163	1	(R)QKQVQELQDK(L)
	155	163	1	(R) QKQVQELQDK(L)
	218	228	1	(K) ASQKALGEEK(A)
1Gln->pyro-Glu	238	248	0	(R)QGLPLVLESLEK(V)
	238	248	0	(R) QGLPLVLESLEK(V)
	249	260	0	(K) VSLAAIDAEASK(K)
	206	217	0	(K) EGGSLAEAYIAK(A)
	36	46	0	(K) DFATVYVVEAIK(D)
1Gln->pyro-Glu	140	150	1	(R)QKQVAPLGEFFR(E)



Scored Protein match



% Protein Coverage from Fetal Hemoglobin Gel Band

Display Options: Percent Coverage Req. Mods: No Filter Search:

#	Label	Accession Number	Molecular Weight	Protein Grouping	Antibody	Req. Mods	Search
1	hemoglobin subunit beta [Sus scr...	gi 2612450...	16 kDa	95%	95%	95%	
2	PREDICTED: hemoglobin subunit a...	gi 3505010...	15 kDa	99%	99%	99%	
3	immunoglobulin lambda-chain, pa...	gi 164511...	19 kDa	85%	52%	69%	
4	PREDICTED: ig kappa chain V-II re...	gi 3505021...	26 kDa	31%	28%	34%	
5	PREDICTED: flavin reductase-like ...	gi 3352997...	22 kDa	45%	48%	45%	
6	peroxiredoxin-2 [Sus scrofa]	gi 13473001...	22 kDa	36%	35%	37%	
7	PREDICTED: carbonic anhydrase 2...	gi 1940270...	29 kDa	16%	15%	15%	
8	trypsinogen precursor [Sus scrofa]	gi 2422530...	25 kDa	15%	15%	15%	
9	apolipoprotein A-I [Sus scrofa]	gi 1164359...	30 kDa	15%	16%	20%	
10	protein S100-A0 [Sus scrofa]	gi 2376013...	10 kDa	34%	34%	34%	
11	triosephosphate isomerase 1 [Su...	gi 2622632...	27 kDa	21%	5.2%	17%	
12	superoxide dismutase 1 [Sus scro...	gi 1508214...	15 kDa	16%	17%	33%	
13	genomus myxacin [Sus scrofa]	gi 1571517...	13 kDa	21%	56%	26%	
14	GTP-binding nuclear protein Ran [L...	gi 5433355...	24 kDa	11%	6.5%	11%	
15	histone H4 (Homo sapiens)	gi 4504301...	11 kDa	21%	21%	21%	
16	PREDICTED: phosphatidylethanolam...	gi 3112706...	21 kDa	26%	5.9%	5.9%	
17	PREDICTED: protein sidekick-1 [Su...	gi 3505012...	22 kDa	3.0%	1.0%	2.0%	
18	PREDICTED: peroxiredoxin-1 (isofo...	gi 3112594...	22 kDa	11%	3.0%	3.0%	
19	G-protein coupled receptor 56 pr...	gi 3400073...	37 kDa	6.2%	3.0%	3.0%	
20	PREDICTED: bin-like, partial [Sus...	gi 3505036...	819 kDa	0.5%	0.5%	0.5%	
21	RecName: Full=1-lactate dehydro...	gi 1170740...	37 kDa	0.0%	0.0%	8.7%	
22	adenylosuccinate synthetase (iso...	gi 1482375...	50 kDa	0.0%	0.0%	0.0%	
23	PREDICTED: bin-like, partial [Sus...	gi 3505036...	702 kDa	0.0%	0.0%	0.88%	

Protein Sequence

Hemoglobin Subunit Beta

Protein Sequence | Similar Proteins | Spectrum | Spectrum/Model Error | Fragmentation Table

gi|261245098 (100%), 16,165.2 Da
 hemoglobin subunit beta [Sus scrofa]
 31 unique peptides, 43 unique spectra, 561 total spectra, 139/147 amino acids (95% coverage)

```

M W H L S A E E K E A V L G L W Q K V N V D E V G G E A L G R R L L Y V P F N T Q
R F F E S F Q D L S N A D A V G G N P K V K A H G K K V L S F S D G L K H L D
N L K G T F A K L S E L N G D Q L H V D P E N F R L L G N V I V V L L A R R L G
H D F N P N V Q A A F Q K V V A G V A N A L A H K Y H
    
```

Hemoglobin Subunit Alpha

Protein Sequence | Similar Proteins | Spectrum | Spectrum/Model Error

gi|350581842 (100%), 15,170.4 Da
 PREDICTED: hemoglobin subunit alpha-like isoform 1 [Sus scrofa]
 37 unique peptides, 53 unique spectra, 386 total spectra, 140/142 amino acids (99% coverage)

```

M V L S A A D K A N V K A A W G K V G G G A G A H G A E A L E R M F L G F P T T
R T Y F P F H N L S H O S D G V K A H G Q R V A D A L T A V Q H L D D L P G A
L S A L S D L R A H K L R V D P V N F K L L S H G L L V T L A A H H P D F N P
S V H A S L D K F L A N V S Y L T L S K Y R
    
```

Peptides from Hemoglobin Subunit Beta

GoodP	Sequence	Prob.	SECU	SECU	X1 Tan.	NTT	Modifications	Observed	Actual Mass	Charg
✓	VVAGVAMLAHK(V)	100%			7.54	1		476.27	950.52	2
✓	VQKAVGLWQ(V)	95%	2.64	0.41	3.00	2		972.55	971.54	1
✓	VQKAVGLWQ(V)	95%	2.54	0.43	1.92	2		972.55	971.54	1
✓	VQKAVGLWQ(V)	95%	2.76	0.43	3.24	2		972.55	971.54	1
✓	VQKAVGLWQ(V)	95%	2.56	0.46	2.49	2		972.55	971.54	1
✓	VQKAVGLWQ(V)	95%	2.79	0.44	3.05	2		972.55	971.54	1
✓	VQKAVGLWQ(V)	95%	2.68	0.45	3.24	2		972.55	971.54	1
✓	VQKAVGLWQ(V)	95%	2.62	0.46	3.54	2		972.55	971.54	1
✓	VQKAVGLWQ(V)	95%	2.70	0.45	2.57	2		486.70	971.54	2
✓	VQKAVGLWQ(V)	95%	2.98	0.44	3.05	2		486.78	971.54	2
✓	VQKAVGLWQ(V)	95%	2.80	0.50	2.89	2		486.78	971.54	2
✓	VQKAVGLWQ(V)	95%	2.81	0.51	3.06	2		486.70	971.54	2
✓	VQKAVGLWQ(V)	95%	3.10	0.47	3.32	2		486.70	971.54	2
✓	VQKAVGLWQ(V)	95%	3.03	0.48	3.89	2		486.70	971.54	2
✓	VQKAVGLWQ(V)	95%	3.02	0.50	2.72	2		486.78	971.54	2
✓	VQKAVGLWQ(V)	95%	2.83	0.55	2.72	2		486.78	971.54	2
✓	VQKAVGLWQ(V)	95%	3.17	0.50	3.96	2		486.70	971.54	2
✓	VQKAVGLWQ(V)	95%	3.18	0.52	3.68	2		486.78	971.54	2
✓	VQKAVGLWQ(V)	95%	3.08	0.54	3.30	2		486.78	971.54	2
✓	VQKAVGLWQ(V)	95%	3.11	0.54	3.59	2		486.78	971.54	2
✓	VEVGEALGPL	97%			3.02	1		444.23	886.45	2
✓	VYTFSPQLSNADAVWQNP	99%			4.97	1		674.79	1349.57	2
✓	VYTFSPQLSNADAVWQNP	95%	6.59	0.69	10.77	1		910.89	1,819.77	2
✓	VYTFSPQLSNADAVWQNP	95%	6.26	0.72	14.41	1		910.90	1,819.78	2
✓	VYTFSPQLSNADAVWQNP	95%	6.49	0.70	8.24	1		910.89	1,819.77	2
✓	VYTFSPQLSNADAVWQNP	95%	6.58	0.70	9.18	1		910.89	1,819.77	2
✓	VYTFSPQLSNADAVWQNP	95%	6.66	0.70	15.64	1		910.90	1,819.78	2
✓	VYTFSPQLSNADAVWQNP	95%	6.40	0.74	9.15	1		910.90	1,819.78	2
✓	VYTFSPQLSNADAVWQNP(V)	95%	5.99	0.62	13.43	2	Oxidation (+16)	1,021.47	2,040.92	2
✓	VYTFSPQLSNADAVWQNP(V)	95%	6.20	0.63	11.92	2	Oxidation (+16)	1,031.47	2,060.92	2
✓	VYTFSPQLSNADAVWQNP(V)	95%	6.47	0.64	15.74	2	Oxidation (+16)	1,031.47	2,060.92	2
✓	VYTFSPQLSNADAVWQNP(V)	95%	6.18	0.66	6.59	2		1,023.47	2,044.92	2

% Protein Coverage from Fetal Hemoglobin Gel Band

Display Options: Percent Coverage Req. Mode: No Filter Search:

Probability Legend:
 over 95%
 80% to 94%
 50% to 79%
 20% to 49%
 0% to 19%

#	Protein	Accession Number	Molecular Weight	Protein Coverage	Identified Proteins (23)
1	hemoglobin subunit beta [Sus scrofa]	gi 261245098	16.165 kDa	95%	1
2	PREDICTED: hemoglobin subunit alpha-like isoform 1 [Sus scrofa]	gi 350581842	15.170 kDa	99%	99%
3	immunoglobulin lambda chain, constant 1 [Sus scrofa]	gi 164511	19.1 kDa	65%	52%
4	PREDICTED: ig kappa chain V H1 [Sus scrofa]	gi 3505821	26.4 kDa	31%	28%
5	PREDICTED: flavin reductase-like [Sus scrofa]	gi 3352897	22.1 kDa	45%	48%
6	peroxiredoxin-2 [Sus scrofa]	gi 3473001	22.1 kDa	36%	35%
7	PREDICTED: carbonic anhydrase 2 [Sus scrofa]	gi 1948370	29.1 kDa	18%	15%
8	trypsinogen precursor [Sus scrofa]	gi 2422538	26.1 kDa	15%	15%
9	apolipoprotein A-1 [Sus scrofa]	gi 114359	30.1 kDa	15%	20%
10	protein S100-A0 [Sus scrofa]	gi 2376813	10.1 kDa	34%	34%
11	triosephosphate isomerase 1 [Sus scrofa]	gi 2622632	27.1 kDa	21%	52%
12	superoxide dismutase 1 [Sus scrofa]	gi 1588214	15.1 kDa	16%	17%
13	gamma-synuclein [Sus scrofa]	gi 1571517	13.1 kDa	21%	56%
14	GTP-binding nuclear protein Ran [Sus scrofa]	gi 543355	24.1 kDa	11%	65%
15	Nucleosome H4 (Homo sapiens)	gi 4504301	11.1 kDa	21%	21%
16	PREDICTED: phosphatidylethanolamine 3-phosphate phosphatase [Sus scrofa]	gi 3112706	21.1 kDa	26%	53%
17	PREDICTED: protein sidekick-1 [Sus scrofa]	gi 3505813	22.5 kDa	3.0%	1.0%
18	PREDICTED: peroxiredoxin-1 isoform 1 [Sus scrofa]	gi 3112594	22.1 kDa	11%	3.0%
19	G-protein coupled receptor 56 [Sus scrofa]	gi 3408073	77.1 kDa	6.2%	3.0%
20	PREDICTED: beta-like, partial [Sus scrofa]	gi 3505936	51.9 kDa	0.0%	0.0%
21	RecName: Full-L. lactate dehydrogenase [Sus scrofa]	gi 1170740	37.1 kDa	0.0%	8.7%
22	adenylsuccinate synthetase isoform 1 [Sus scrofa]	gi 1482375	30.1 kDa	0.0%	0.0%
23	PREDICTED: beta-like, partial [Sus scrofa]	gi 3505936	70.2 kDa	0.0%	0.0%

Protein Sequence

Peroxioredoxin - 2

Protein Sequence | Similar Proteins | Spectrum | Spectrum/Model Error | Fragmentation Table

gi|347300176 (100%), 21,880.2 Da
 peroxiredoxin-2 [Sus scrofa]
 8 unique peptides, 9 unique spectra, 25 total spectra, 71/198 amino acids (35% coverage)

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M E S Q N A H I G K P A P E F Q A T A V V N G A F K V K L S D Y K G K Y L V L
F F Y P L D F T F V C P T E I I A F S D R A E E F H G L G C E V L G V S V D S Q
F T H A W I N T P R K E G C L G P L K I P L L A D V T R N L S L D Y Q V L K E
D E G I I A Y R K G L F I I D Q K G Y L R D I T N D L P V G R S V D E A L R L V Q
A F Q Y T D E H C E V C P A G W K P G S D T I K P H V D D S K E Y F S K H N
    
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Peroxioredoxin-1 isoform 5

Protein Sequence | Similar Proteins | Spectrum | Spectrum/Model Error | Fragmentation Table

gi|31259408 (100%), 21,887.2 Da
 PREDICTED: peroxiredoxin-1 isoform 5 [Sus scrofa]
 2 unique peptides, 2 unique spectra, 3 total spectra, 22/197 amino acids (11% coverage)

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M E S Q N A H I G H R A P H F K A T A V M P D G Q F K D I S L S D Y K G R Y V V
F F Y P L D F T F V C P T E I I A F S E R A E E F K L N C Q V I G A S V D L
T S Y I W I N T P K K Q G L O P M N I P L I S D P K R T I A O D Y G V L K A D
E G I S F R G L F I I D Q K G L R Q I T I N D L P V G R S V D E T L R L V Q A
F G F T D R K H G E V C P A G W K P G S D T I K P D Y G K S H E Y F S K Q K
    
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- Assigning charge to an aqueous peptide at a given pH is based on the
- Ionization state of the side chains that have a proton that can ionize.

- Assigning how many charges will be on a given GAS PHASE peptide ion for mass spectrometry is SIMILAR BUT DIFFERENT.

- The “rule of thumb” is that the number of basic sites (side chains and amino terminus) is an estimate of how many protons will associate “be attached” to a peptide ion and therefore what its charge state will be.

- Protons that associate (not a covalent bond) with a peptide in a volatile polar solvent do not go to a fixed spot. They can move around

- PLUS.....the presence of a negatively charged side chain doesn't necessarily “negate” a charge state.....then you are mixing two different “exercises” that are similar and have similar terminology but are two different processes.