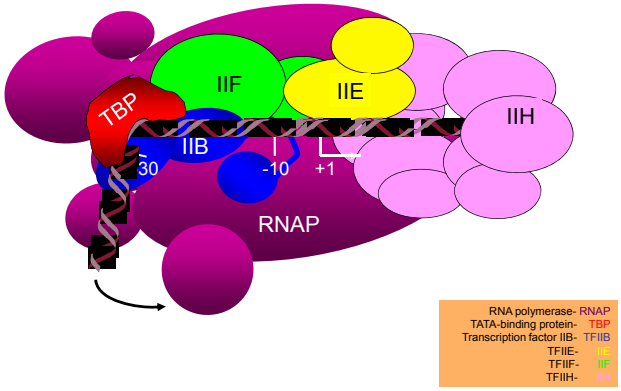
Biological Mass Spectrometry: Ionization, Calculating Mass, Charge

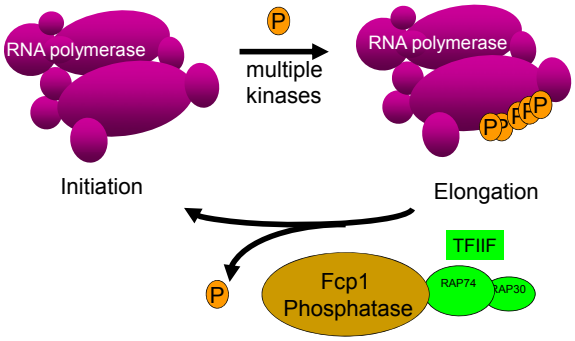
Matthew Renfrow, PhD
6-4681
renfrow@uab.edu

S. Barnes & M. Renfrow-UAB 1/14/12

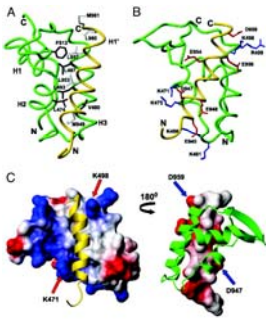
Eukaryotic Class II transcription preinitiation complex (PIC)



Dephosphorylation of RNA polymerase largest subunit

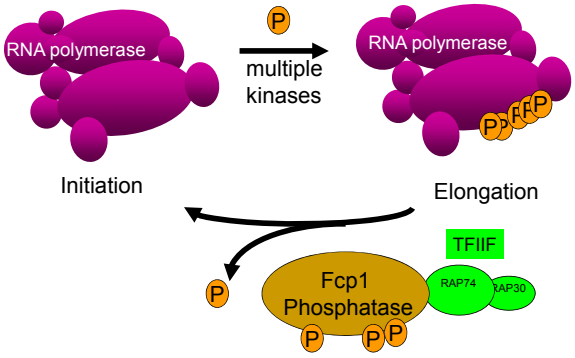


TFIIF (RAP74) – Fcp1 phosphatase interacting domain

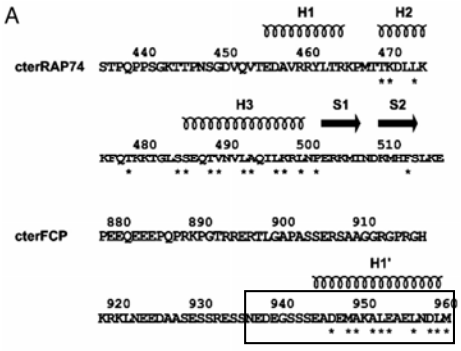


Nguyen BD, Abbott KL, Potempa K, Kobor MS, Archambault J, Greenblatt J, Legault P, Omichinski JG
Proc Natl Acad Sci U S A. 2003 May 13;100(10):5688-93

Dephosphorylation of
RNA polymerase largest subunit

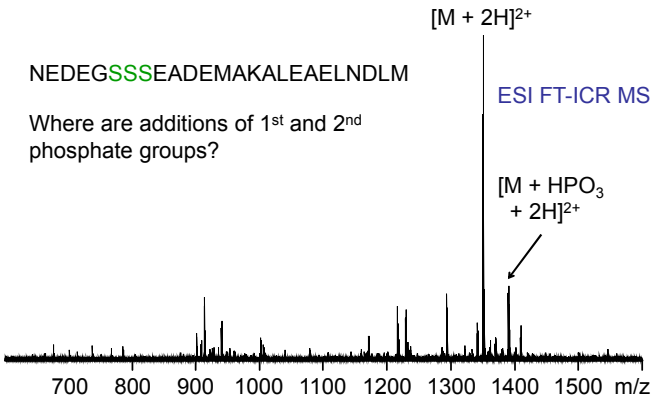


Fcp1 is a Phosphoprotein



Nguyen BD, Abbott KL, Potempa K, Kobor 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.avantilipids.com/index.php?option=com_content&view=article&id=2248&Itemid=415

Monoisotopic amino acid mass

http://www.abrf.org/researchgroups/massspectrometry/eposters/ms97quiz/residuemasses.html

Protein & peptide calculator

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

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

Unimod

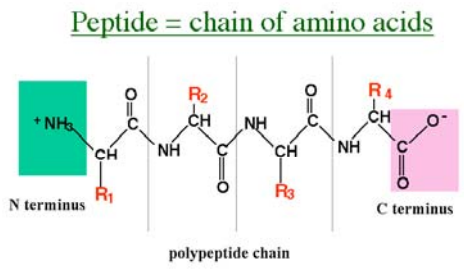
http://www.unimod.org/modifications_list.php

Lipid Calculator

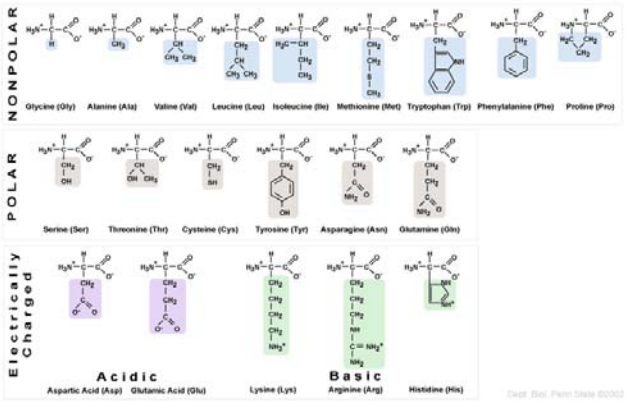
http://pharmacology.ucdenver.edu/lipidcalc/Default.aspx

Carbohydrate calculator

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



S. Barnes & M. Renfrow-UAB 1/12/11



S. Barnes & M. Renfrow-UAB 1/12/11

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

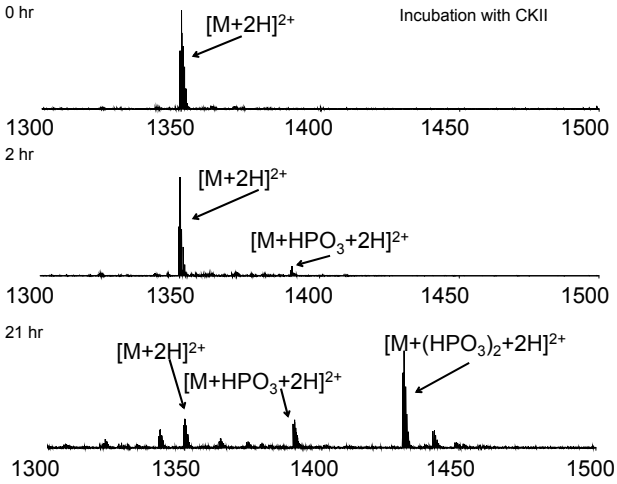
The m/z value of a peptide $[M+H]^+$ is the sum of the residue masses plus 18.015 for H_2O plus 1.008. So, what is it for ISLLD?

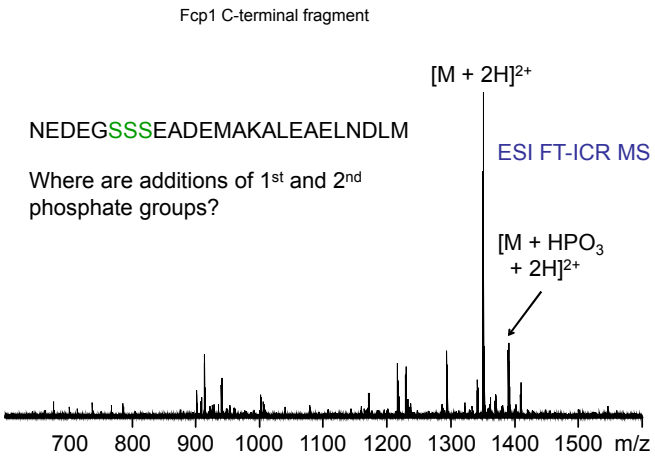
113.084 + 87.032 + 113.084 +113.084 + 115.027 + 18.015 + 1.008 = 560.334

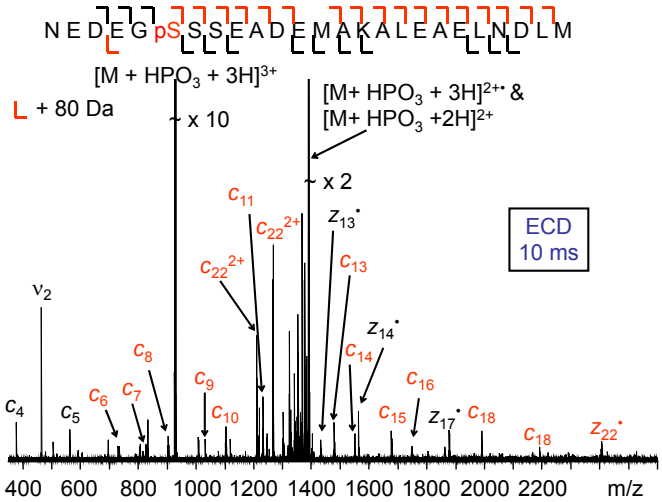
S. Barnes & M. Renfrow-UAB 1/12/11

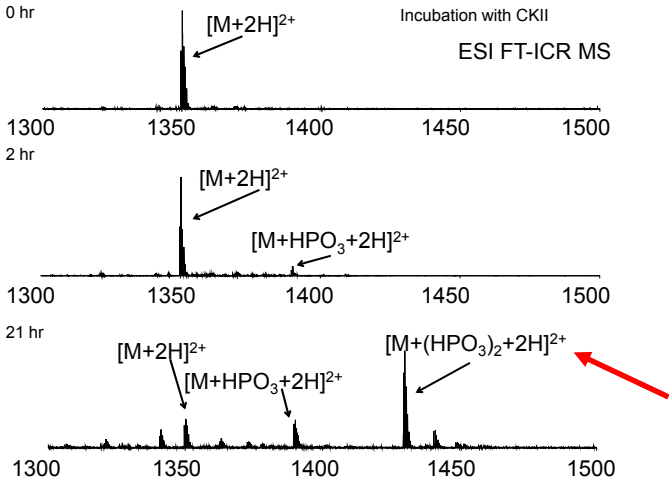
NEDEGSSSEADEMAKALEAELNDLM

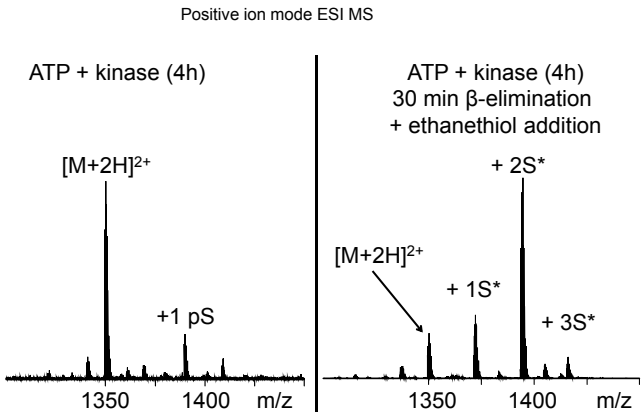
NEDEGSSSEADE	MAKALEAELNDLM
4 Glutamates	2 Methionines
1 Asparagine	3 Alanines
2 Aspartates	1 Lysine
1 Glycine	2 Glutamates
3 Serines	1 Aspartate
1 Alanine	1 Asparagine
	3 Leucines



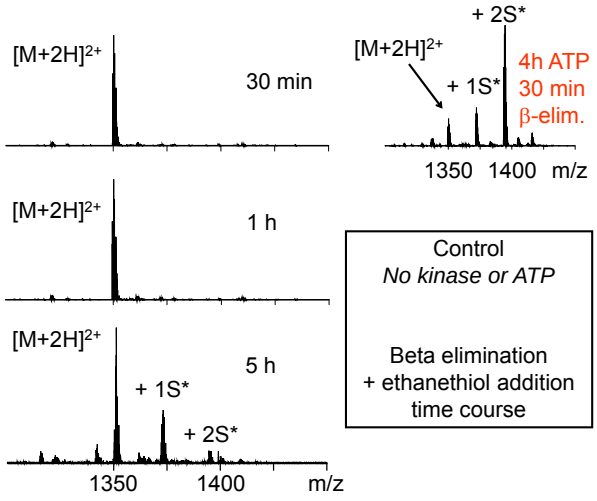


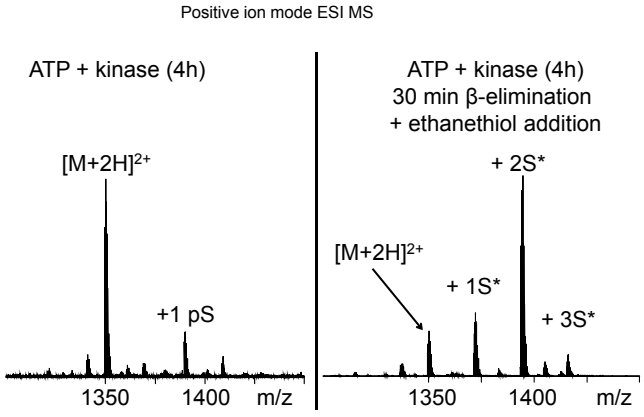




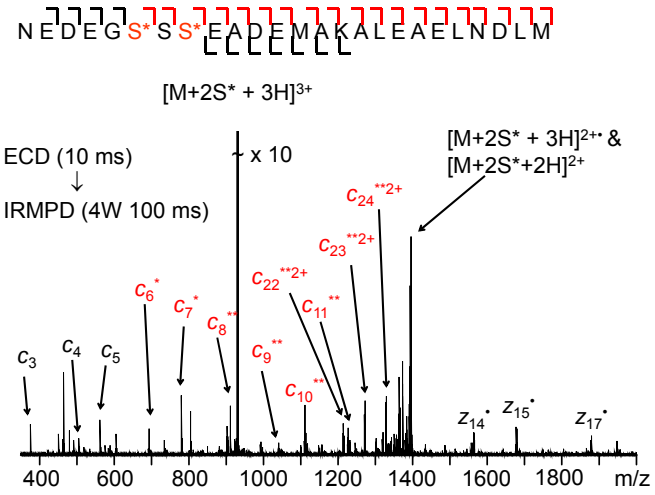


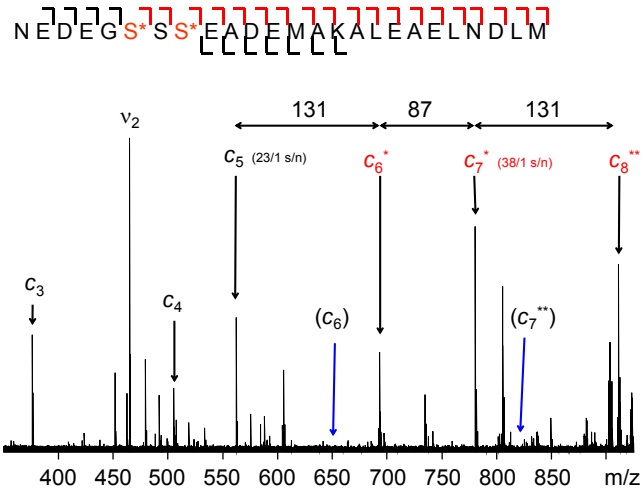
Molloy, M.P., Andrews, P.C. Phosphopeptide derivatization signatures to identify serine and threonine phosphorylated peptides by mass spectrometry. Anal. Chem. 2001, 73, 22, 5387-5394

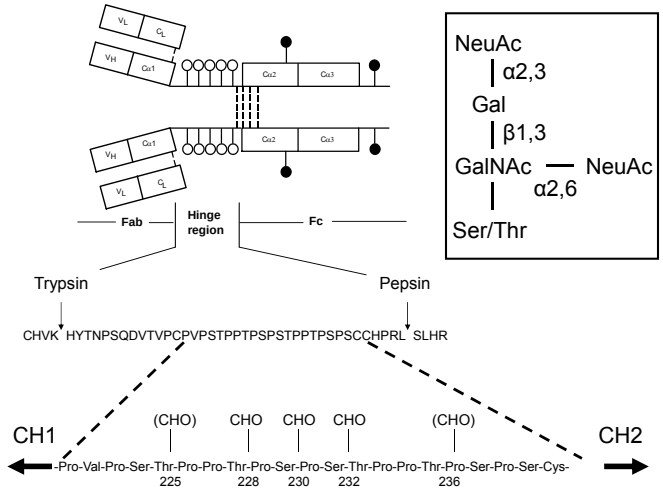


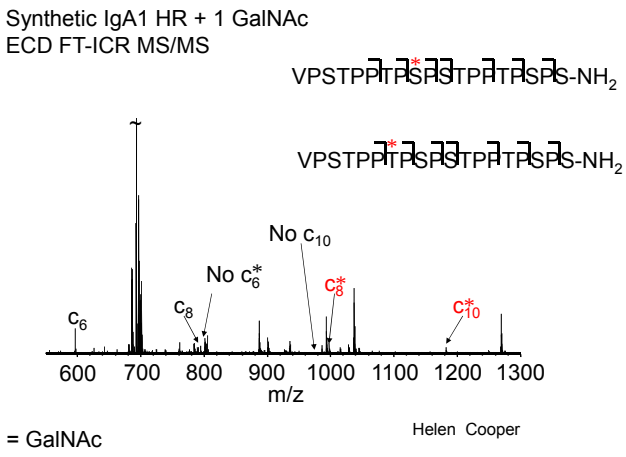


Molloy, M.P., Andrews, P.C. Phosphopeptide derivatization signatures to identify serine and threonine phosphorylated peptides by mass spectrometry. Anal. Chem. 2001, 73, 22, 5387-5394

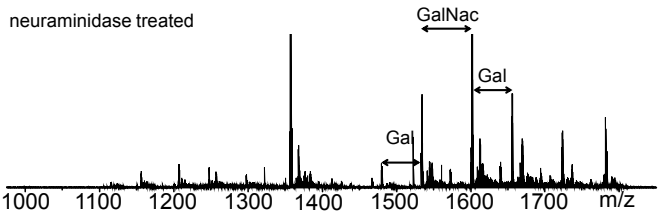

N E D E G S* S S* E A D E M A K A L E A E L N D L M
[M+2S* + 3H]3+
ECD (10 ms)
IRMPD (4W 100 ms)
x 10
[M+2S* + 3H]2+• &
[M+2S*+2H]2+
c3 c4 c5 c6* c7* c8** c9** c10** c11** c22**2+ c23**2+ c24**2+ z14• z15• z17•
400 600 800 1000 1200 1400 1600 1800 m/z

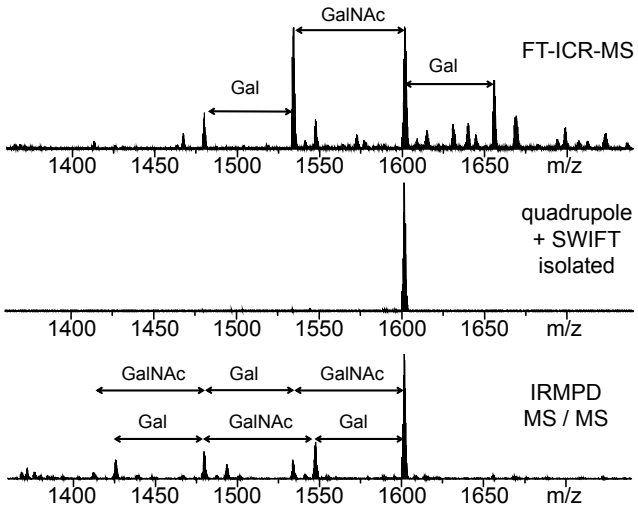

N E D E G S* S S* E A D E M A K A L E A E L N D L M
131 87 131
v2
c5 (23/1 s/n)
c6*
c7* (38/1 s/n)
c8**
c3 c4 (c6) (c7**)
400 450 500 550 600 650 700 750 800 850 m/z


Fab
Hinge region
Fc
NeuAc
α2,3
Gal
β1,3
GalNAc — NeuAc
α2,6
Ser/Thr
Trypsin
Pepsin
CHVK HYTNPSQDVTVPCPVPSTPPTPSPSTPPTPSPSCCHPRL SLHR
CH1
CH2
(CHO) CHO CHO CHO (CHO)
-Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Cys-
225 228 230 232 236

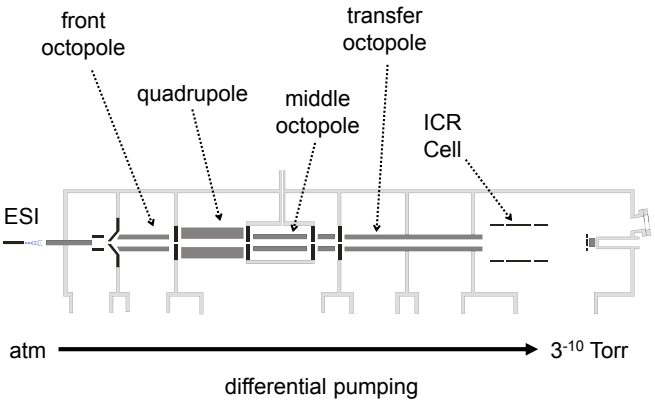

Synthetic IgA1 HR + 1 GalNAc
ECD FT-ICR MS/MS
VPSTPPTPSPSTPPTPSPS-NH2
VPSTPPTPSPSTPPTPSPS-NH2
No c10
No c6*
c6
c8
c8*
c10*
600 700 800 900 1000 1100 1200 1300
m/z
* = GalNAc
Helen Cooper

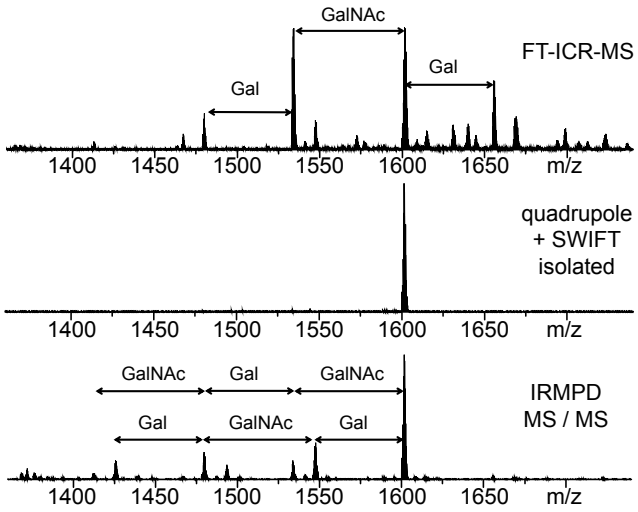
IgA1 hinge region *O*-glycosylation



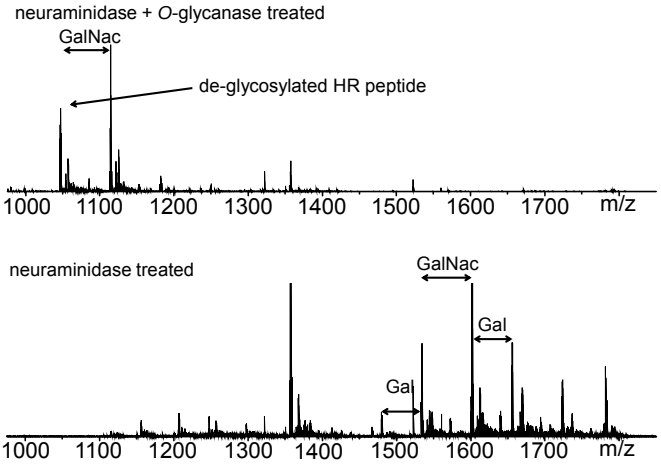


NHMFL ESI FT-ICR Instrument (9.4 T)

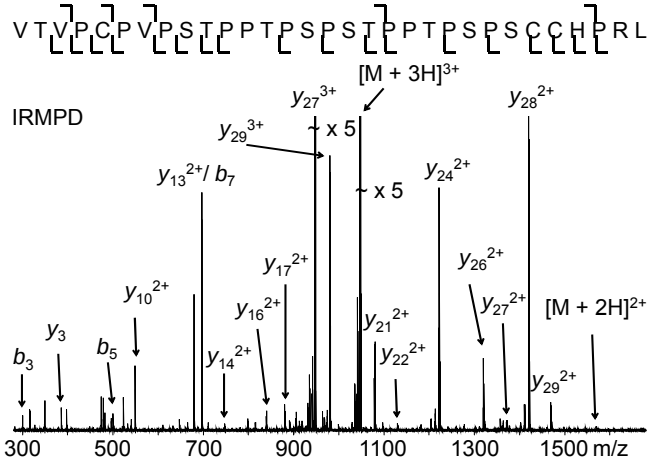




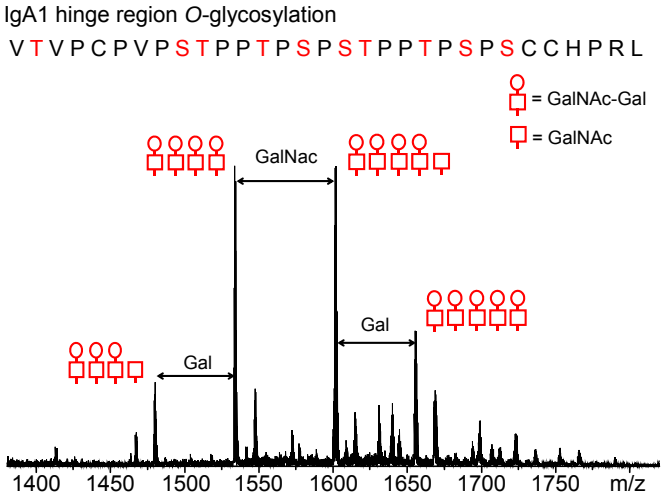
IgA1 hinge region *O*-glycosylation

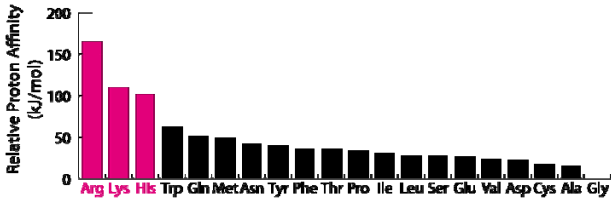


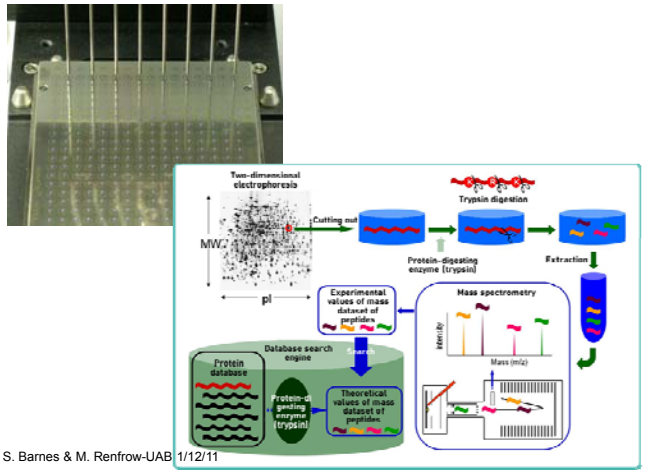
IgA1 hinge region *O*-glycosylation



IgA1 hinge region *O*-glycosylation

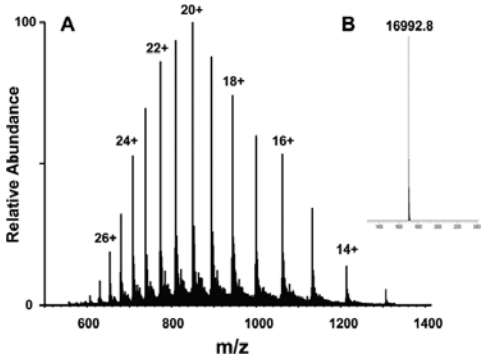






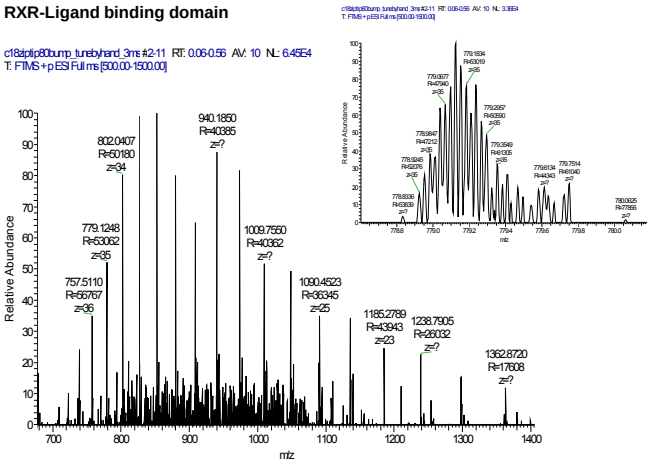
S. Barnes & M. Renfrow-UAB 1/12/11

Myoglobin

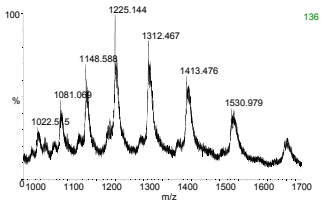


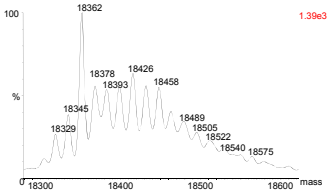
S. Barnes & M. Renfrow-UAB 1/12/11

RXR-Ligand binding domain



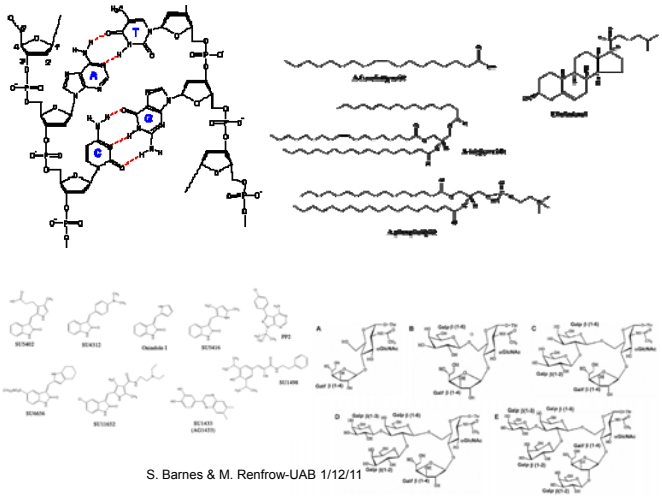
Deconvolution of oxidized forms of β-lactoglobulin



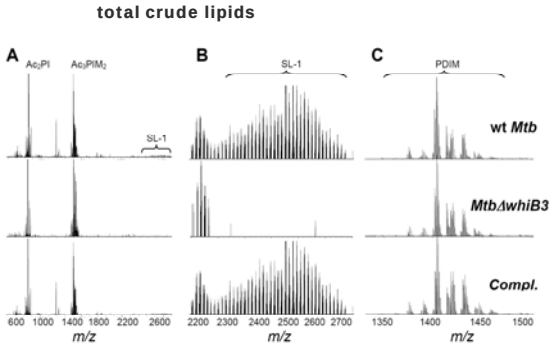


S. Barnes & M. Renfrow-UAB 1/12/11

Junlong Shao

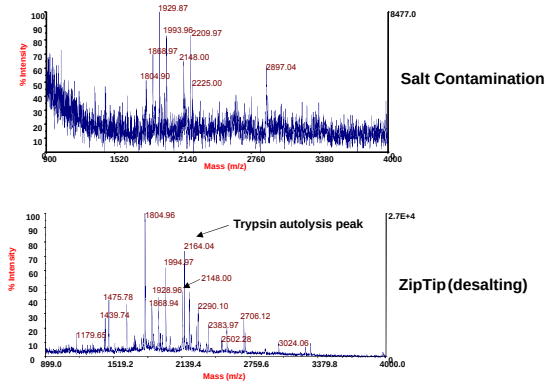


S. Barnes & M. Renfrow-UAB 1/12/11

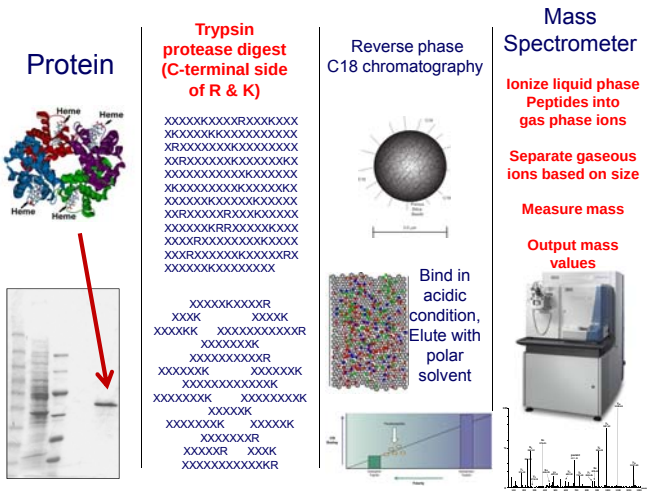


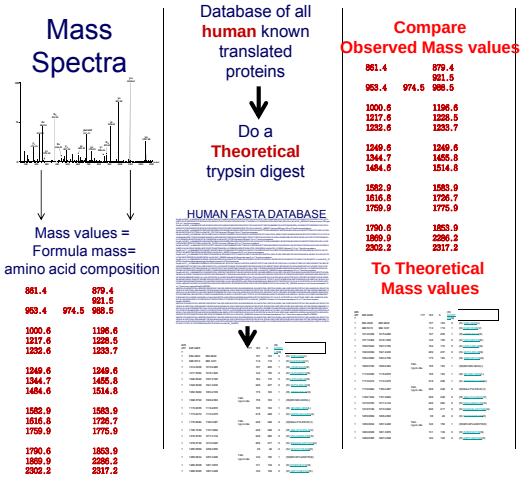
FT-ICR mass spectra of total crude lipids derived from *MtbΔwhiB3* growing in macrophages demonstrate the absence of SL-1. (A) Total crude lipid extracts were prepared from *Mtb* growing inside macrophages and analyzed in the negative ion mode by FT-ICR MS. We observed the presence of Ac_2PI species at *m/z* 835.5261 and 851.5566, which corresponds to their reported theoretical masses. The dimannose specie esterified to three acyl chains (Ac_2PIM_2) corresponds to mass *m/z* 1413.8888 was also detected in all the strains tested. Note that the multiple lipoforms 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.

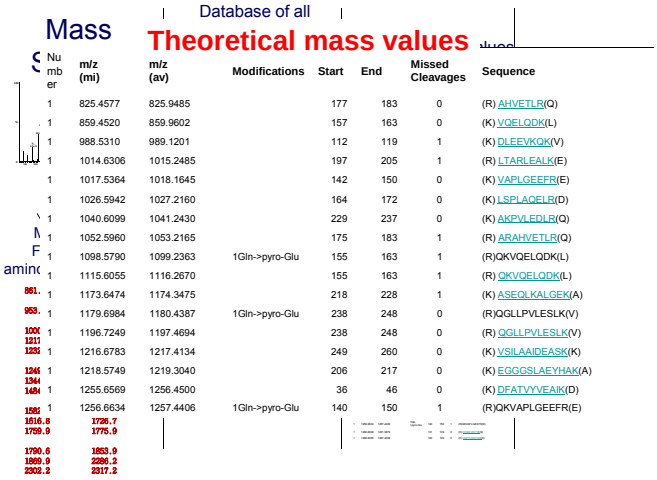
Benefit of removing salt from tryptic digest

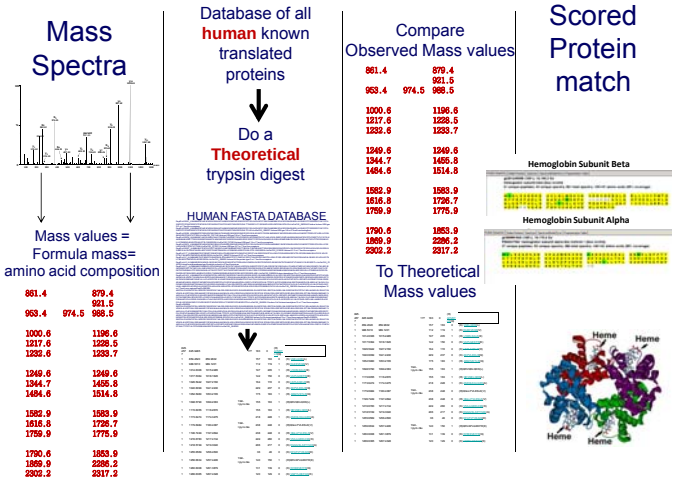


S. Barnes & M. Renfrow-UAB 1/12/11

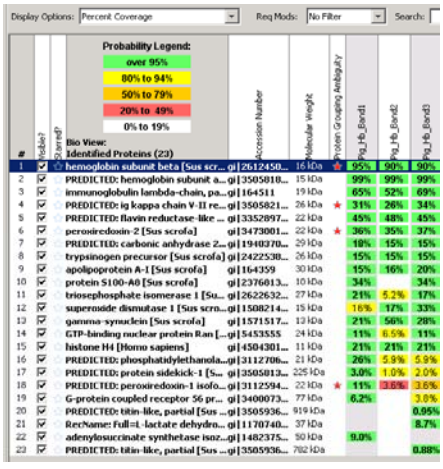






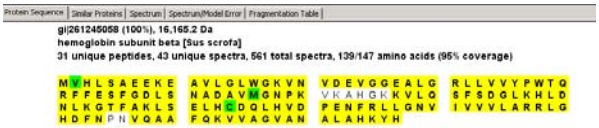


% Protein Coverage from Fetal Hemoglobin Gel Band

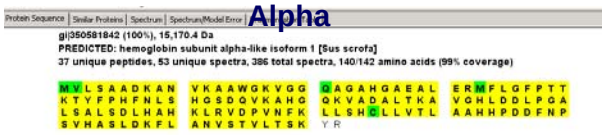


Protein Sequence

Hemoglobin Subunit Beta



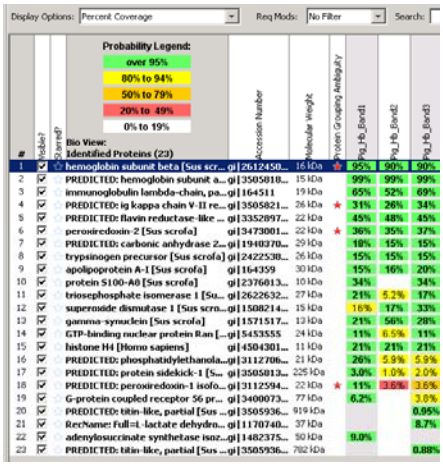
Hemoglobin Subunit Alpha



Peptides from Hemoglobin Subunit Beta

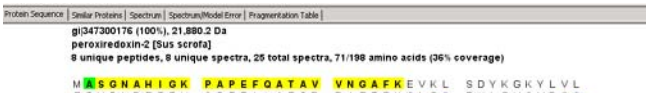
Good?	...	Sequence	Prob	SEQU...	SEQU...	Xl Tan...	NTT	Modifications	Observed	Actual Mass	Charg
☑	✓	(V)AGVANALAHK(Y)	95%			5.59	1		476.27	950.52	2
☑	✓	(V)AGVANALAHK(Y)	95%			7.54	1		476.27	950.52	2
☑	✓	(K)EAVLGLWGK(V)	95%	2.64	0.41	3.00	2		972.55	971.54	1
☑	✓	(K)EAVLGLWGK(V)	95%	2.54	0.43	1.92	2		972.55	971.54	1
☑	✓	(K)EAVLGLWGK(V)	95%	2.76	0.43	3.24	2		972.55	971.54	1
☑	✓	(K)EAVLGLWGK(V)	95%	2.56	0.46	2.49	2		972.55	971.54	1
☑	✓	(K)EAVLGLWGK(V)	95%	2.79	0.44	5.05	2		972.55	971.54	1
☑	✓	(K)EAVLGLWGK(V)	95%	2.68	0.45	3.24	2		972.55	971.54	1
☑	✓	(K)EAVLGLWGK(V)	95%	2.82	0.46	3.54	2		972.55	971.54	1
☑	✓	(K)EAVLGLWGK(V)	95%	2.70	0.45	2.57	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	2.98	0.44	3.05	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	2.80	0.50	2.89	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	2.81	0.51	3.06	2		486.70	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	3.10	0.47	3.32	2		486.70	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	3.03	0.49	3.09	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	3.02	0.50	2.72	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	2.83	0.55	2.72	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	3.17	0.50	3.96	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	3.18	0.52	3.68	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	3.08	0.54	3.30	2		486.78	971.54	2
☑	✓	(K)EAVLGLWGK(V)	95%	3.11	0.54	3.59	2		486.78	971.54	2
☑	✓	(D)EVGGEALGR(L)	77%			3.02	1		444.23	886.45	2
☑	✓	(R)FFESFGDLSNADAVMGNP(V)	95%			4.37	1		674.79	1,347.57	2
☑	✓	(R)FFESFGDLSNADAVMGN(P)	95%	6.59	0.69	10.77	1		910.89	1,819.77	2
☑	✓	(R)FFESFGDLSNADAVMGN(P)	95%	6.26	0.72	14.41	1		910.90	1,819.78	2
☑	✓	(R)FFESFGDLSNADAVMGN(P)	95%	6.49	0.70	8.24	1		910.89	1,819.77	2
☑	✓	(R)FFESFGDLSNADAVMGN(P)	95%	6.55	0.70	9.15	1		910.89	1,819.77	2
☑	✓	(R)FFESFGDLSNADAVMGN(P)	95%	6.66	0.70	15.64	1		910.90	1,819.78	2
☑	✓	(R)FFESFGDLSNADAVMGN(P)	95%	6.40	0.74	9.15	1		910.90	1,819.78	2
☑	✓	(R)FFESFGDLSNADAVM%GNPK(V)	95%	5.99	0.62	13.43	2	Oxidation (+16)	1,031.47	2,060.92	2
☑	✓	(R)FFESFGDLSNADAVM%GNPK(V)	95%	6.20	0.63	11.92	2	Oxidation (+16)	1,031.47	2,060.92	2
☑	✓	(R)FFESFGDLSNADAVM%GNPK(V)	95%	6.47	0.64	15.74	2	Oxidation (+16)	1,031.47	2,060.92	2
☑	✓	(R)FFESFGDLSNADAVM%GNPK(V)	95%	6.10	0.66	6.59	2		1,023.47	2,044.92	2

% Protein Coverage from Fetal Hemoglobin Gel Band

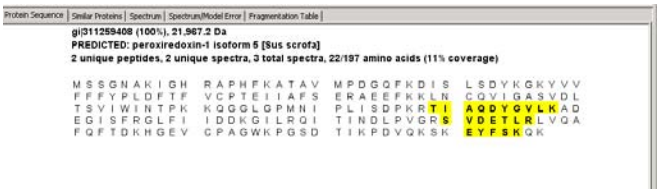


Protein Sequence

Peroxiredoxin - 2



Peroxiredoxin-1 isoform 5



- **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 it's 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.**