

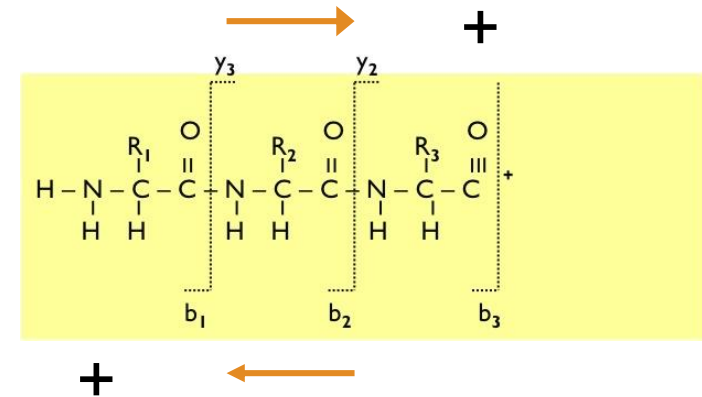
Tryp-N: a thermostable, N-terminal lys/arg protease

Lightning talk
US HUPO March 17, 2015

John P. Wilson
Pappin Laboratory

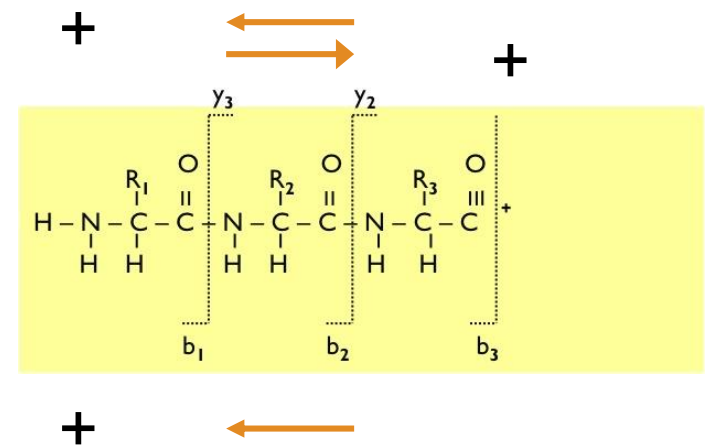
Proteomics enzyme tools

- Typically we use proteases
- We see what's charged
 - Positive ion mode: basic residues = K/R/N-terminus
- Trypsin
 - Cheap (moo), works great, cleaves C-terminal to K/R
 - K/R is basic residue number one (y-ions)
 - Amino terminus is basic residue number two (b-ions)
 - Thus get a series of y- and b-ions



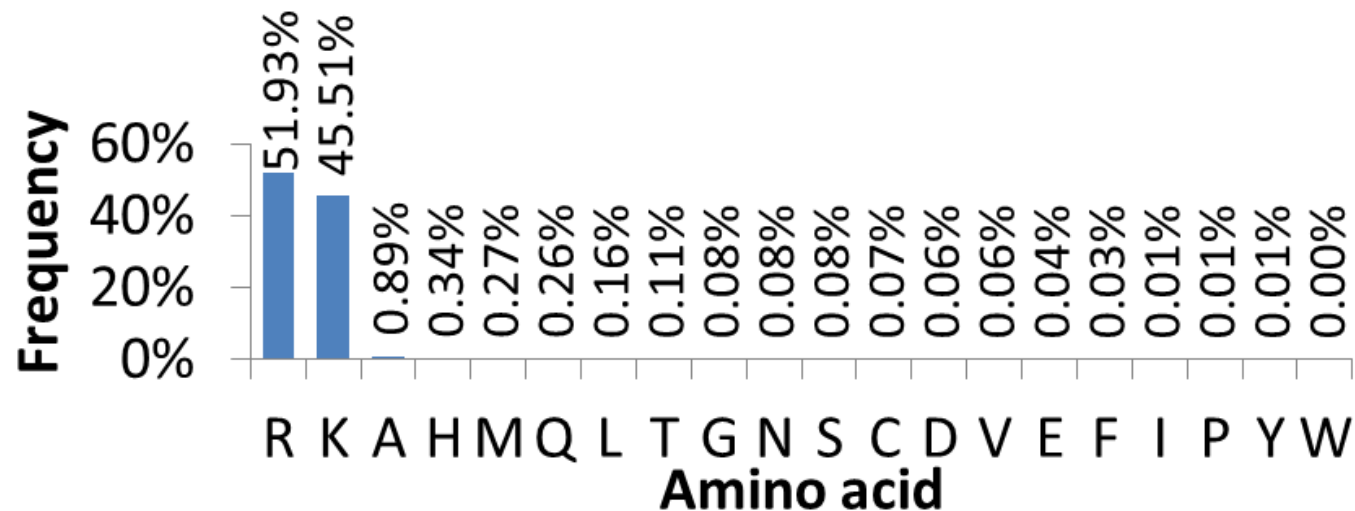
Simplification of MS/MS spectra by directed fragmentation

- Put charge with the fragments you want to see
 - Get bonds to break in a predetermined (simpler) order
- Peptides with charge solely at the N-terminus produce only b-ions
 - Creates an N->C sequence ladder
 - Ladders are directly readable
 - PTMs are easy to spot



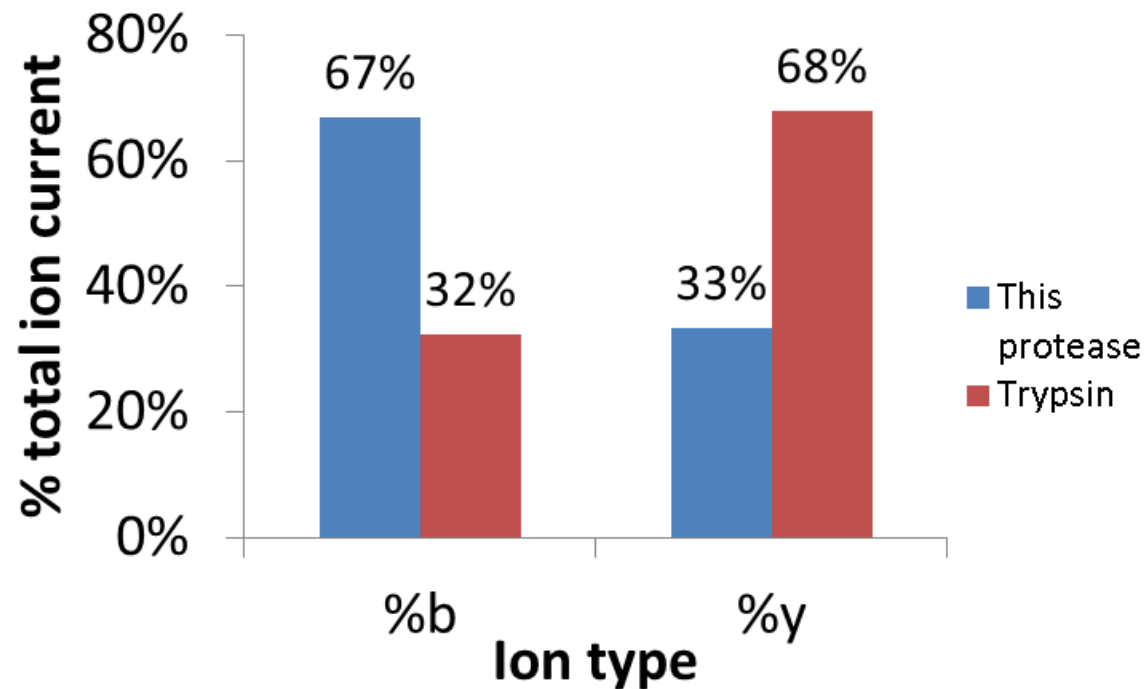
Tryp-N

- N-terminal specific R/K
- Same specificity as trypsin: ~95%.
- Proline = no care
- Methylation = no care



10,694 peptides from whole cell *E. coli* lysate digested at 60 ° C in ammonium acetate at pH 7.4.

Exactly inverts ion preference



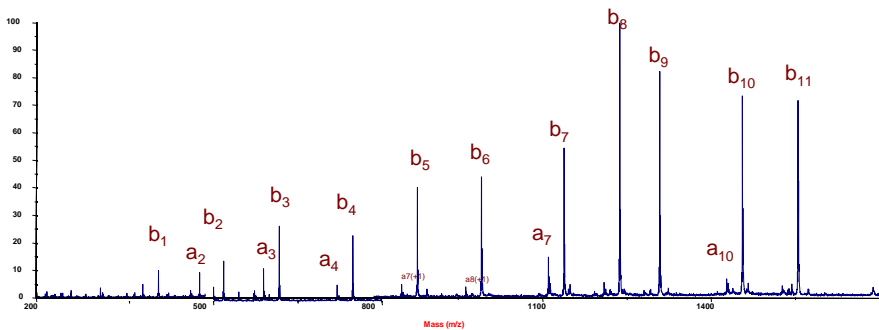
- Useful to get complimentary information
- Can sequence MALDI with a calculator

Directed fragmentation: Tryp-N vs. trypsin

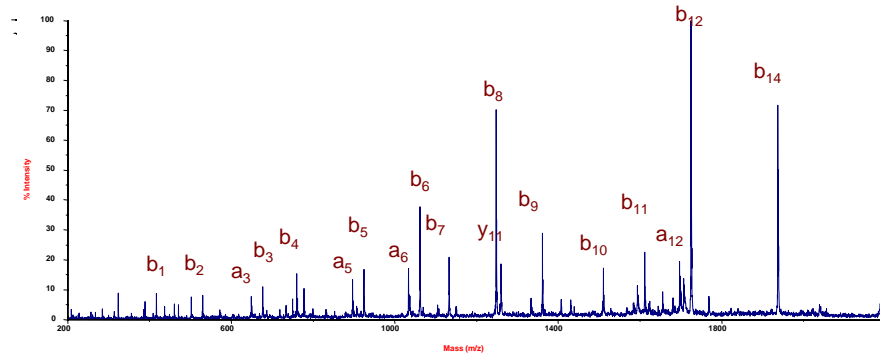


KTVMENFVAFVD

Tryp-N

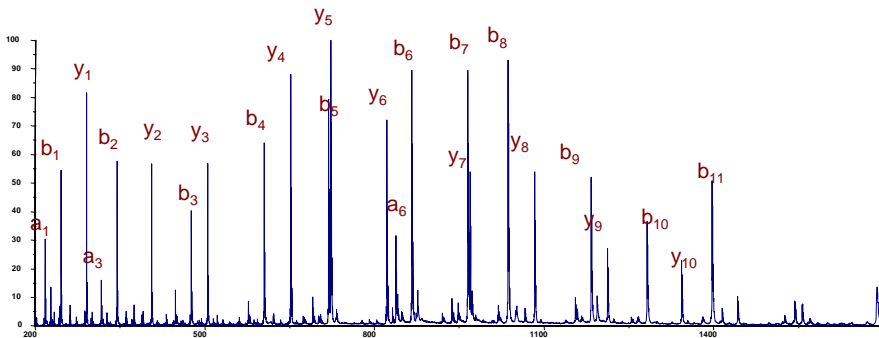


KLFTFHADICTLPDTE

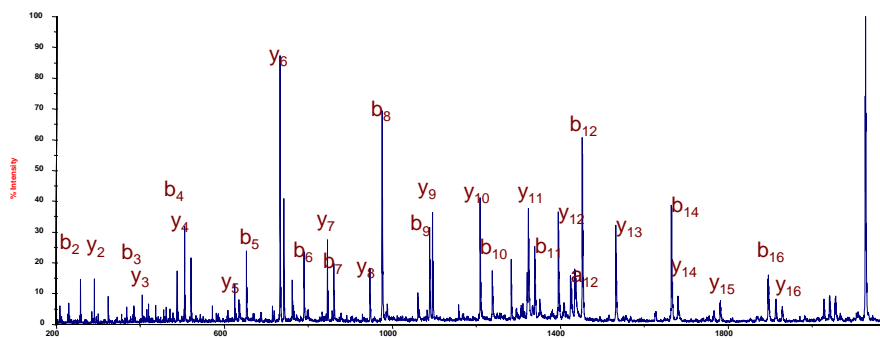


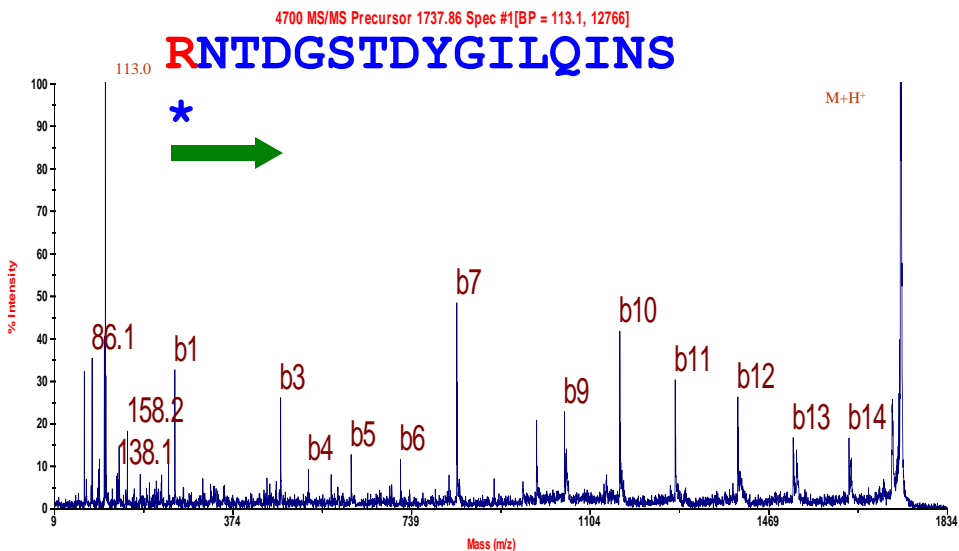
TVMENFVAFVD**K**

Trypsin

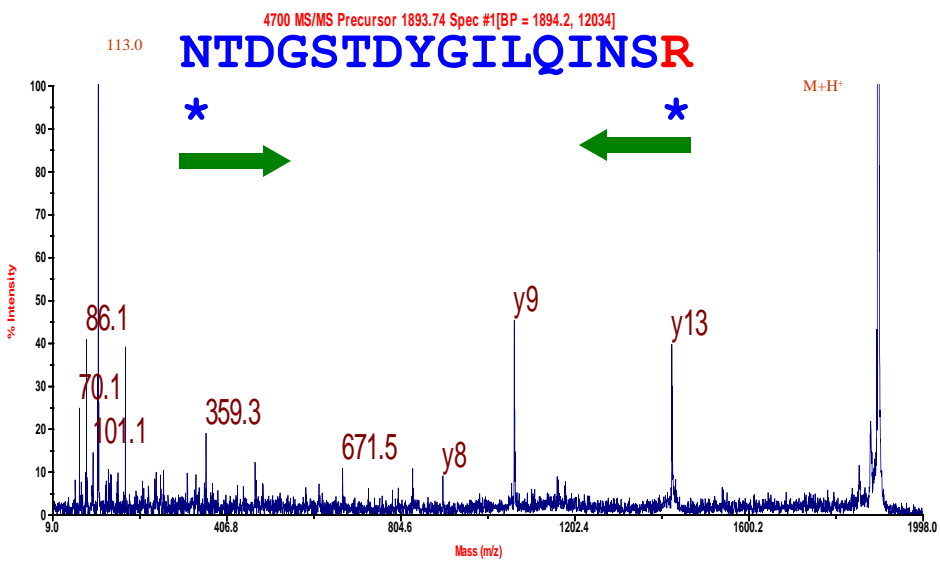


LFTFHADICTLPDTE**K**



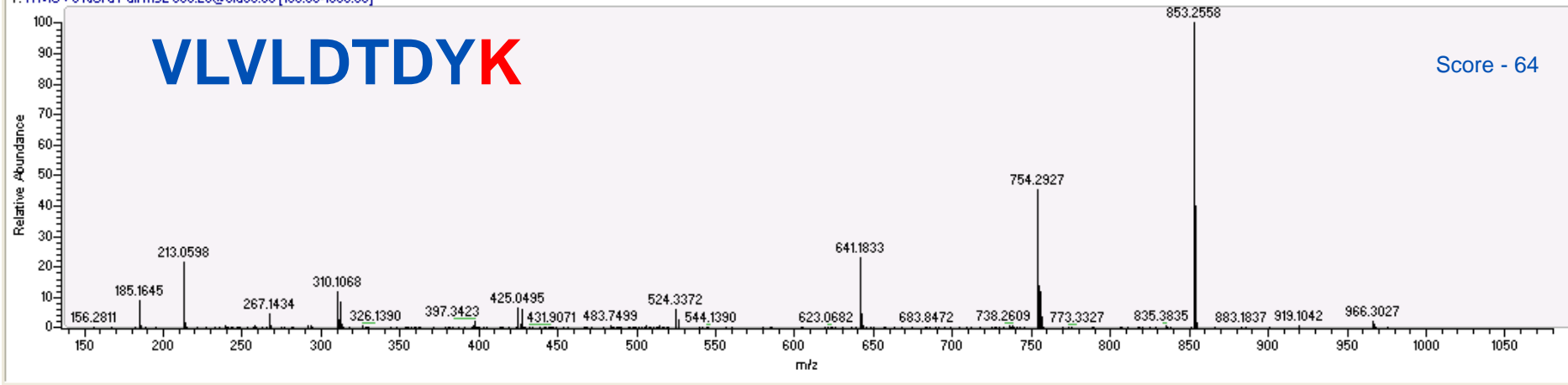


#	a	a*	b	b*	Seq.	y	y*	#
1	227.1	210.09	255.11	238.09	N			16
2	328.2	311.14	356.16	339.14	T	1483.7	1466.67	15
3	443.2	426.17	471.19	454.16	D	1382.6	1365.62	14
4	500.2	483.19	528.21	511.18	G	1267.6	1250.59	13
5	587.3	570.22	615.24	598.22	S	1210.6	1193.57	12
6	688.3	671.27	716.29	699.26	T	1123.6	1106.54	11
7	803.3	786.3	831.32	814.29	D	1022.5	1005.49	10
8	966.4	949.36	994.38	977.35	Y	907.49	890.46	9
9	1023	1006.38	1051.4	1034.4	G	744.43	727.4	8
10	1136	1119.46	1164.49	1147.5	I	687.4	670.38	7
11	1250	1232.55	1277.57	1260.5	L	574.32	557.29	6
12	1378	1360.61	1405.63	1388.6	Q	461.24	444.21	5
13	1491	1473.69	1518.71	1501.7	I	333.18	316.15	4
14	1605	1587.73	1632.76	1615.7	N	220.09	203.07	3
15	1692	1674.77	1719.79	1702.8	S	106.05	89.02	2
16					R	19.02	1.99	1

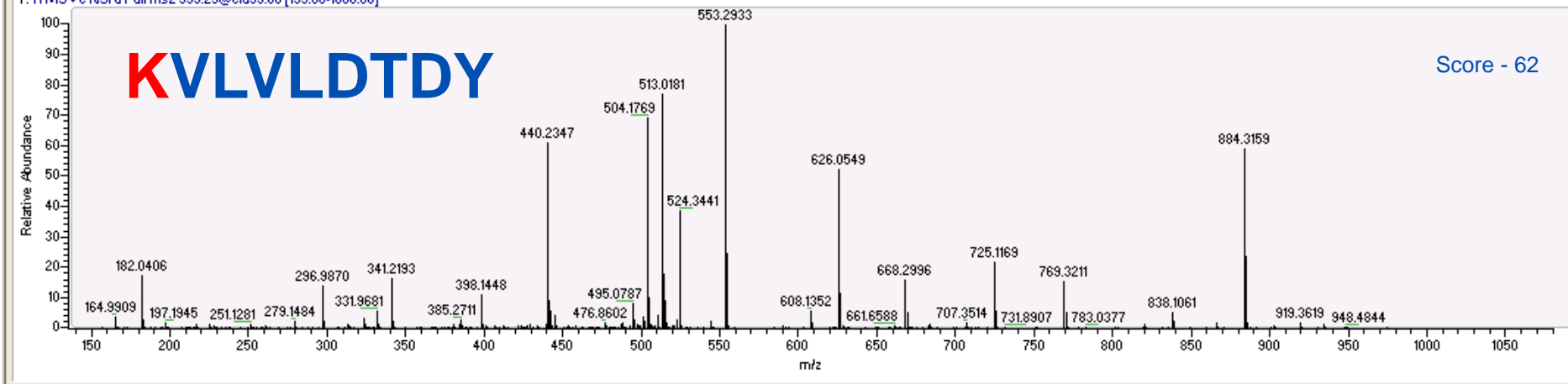


#	a	a*	b	b*	Seq.	y	y*	#
1	227.1	210.09	255.11	238.09	N			16
2	328.2	311.14	356.16	339.14	T	1639.8	1622.77	15
3	443.2	426.17	471.19	454.16	D	1538.8	1521.72	14
4	500.2	483.19	528.21	511.18	G	1423.7	1406.69	13
5	587.3	570.22	615.24	598.22	S	1366.7	1349.67	12
6	688.3	671.27	716.29	699.26	T	1279.7	1262.64	11
7	803.3	786.3	831.32	814.29	D	1178.6	1161.59	10
8	966.4	949.36	994.38	977.35	Y	1063.6	1046.56	9
9	1023	1006.38	1051.4	1034.4	G	900.53	883.5	8
10	1136	1119.46	1164.49	1147.5	I	843.51	826.48	7
11	1250	1232.55	1277.57	1260.5	L	730.42	713.39	6
12	1378	1360.61	1405.63	1388.6	Q	617.34	600.31	5
13	1491	1473.69	1518.71	1501.7	I	489.28	472.25	4
14	1605	1587.73	1632.76	1615.7	N	376.19	359.17	3
15	1692	1674.77	1719.79	1702.8	S	262.15	245.12	2

Casein-trypsin_allcharges #4557 RT: 43.62 AV: 1 NL: 2.50E4
 T: ITMS + c NSI d Full ms2 533.29@cid35.00 [135.00-1080.00]

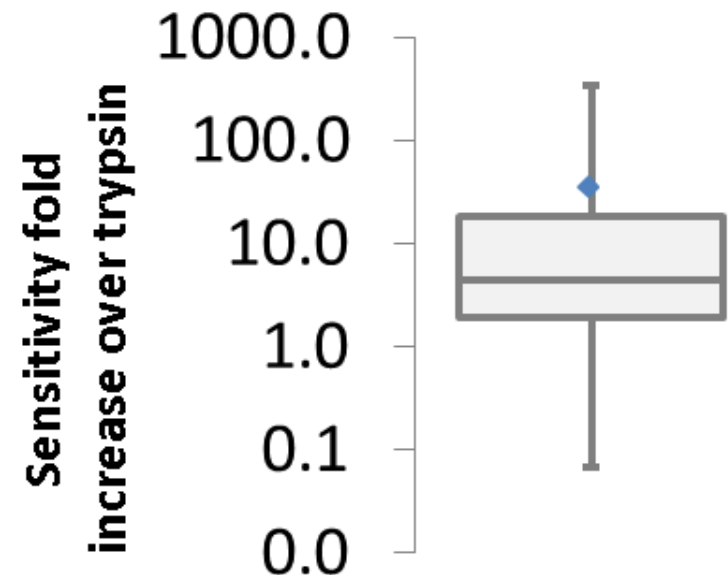


Casein-chtherm_allcharges #5482 RT: 47.56 AV: 1 NL: 1.51E4
 T: ITMS + c NSI d Full ms2 533.29@cid35.00 [135.00-1080.00]



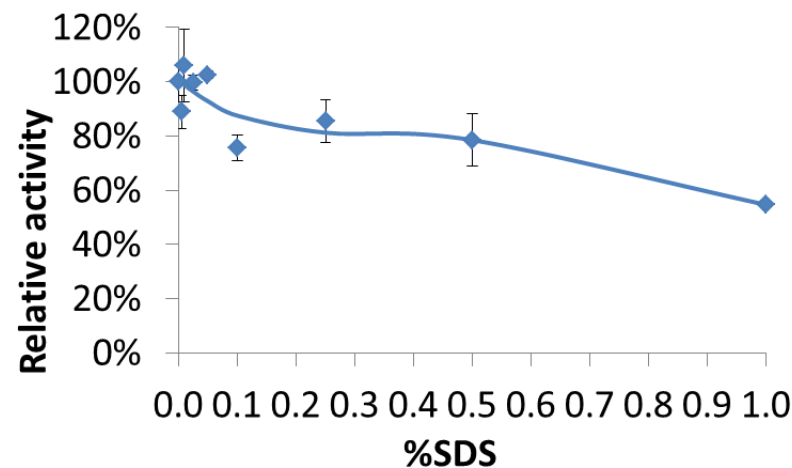
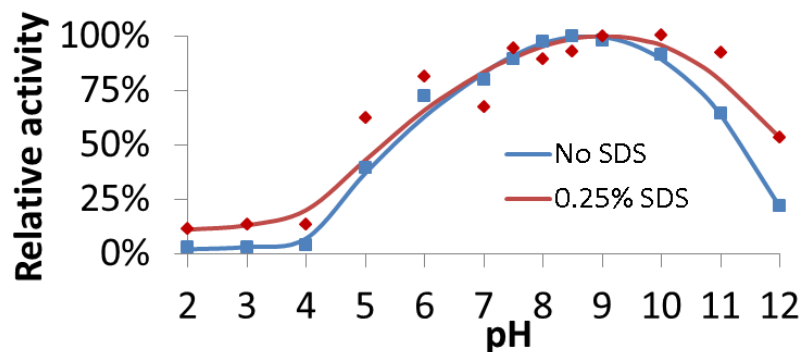
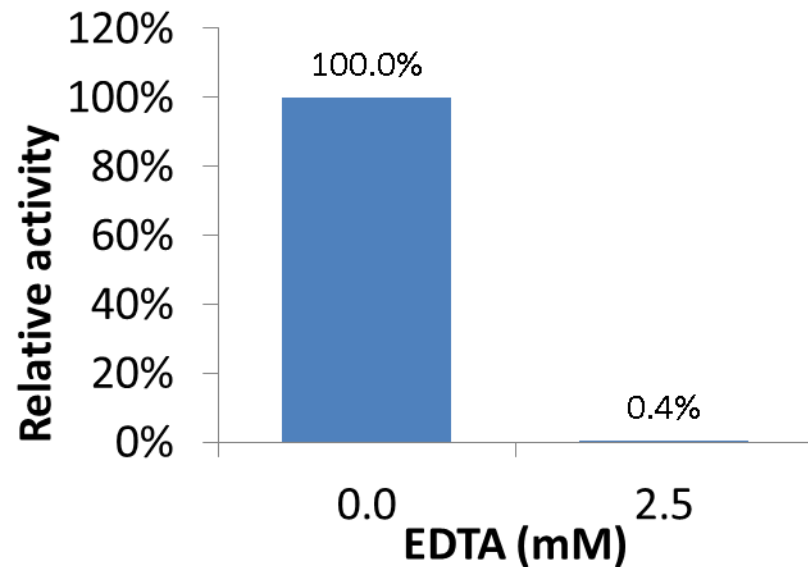
No ion current dilution between termini

- **Increased sensitivity in MRM**
 - Use b-ions
- Decreases in LOD as compared to trypsin:
 - Median fold increase in sensitivity = 4.5x.
 - Average = 35.4x.
 - 50% CI = 1.96x – 18.6x increase.
 - **We did one protein** (anybody want to do more?)



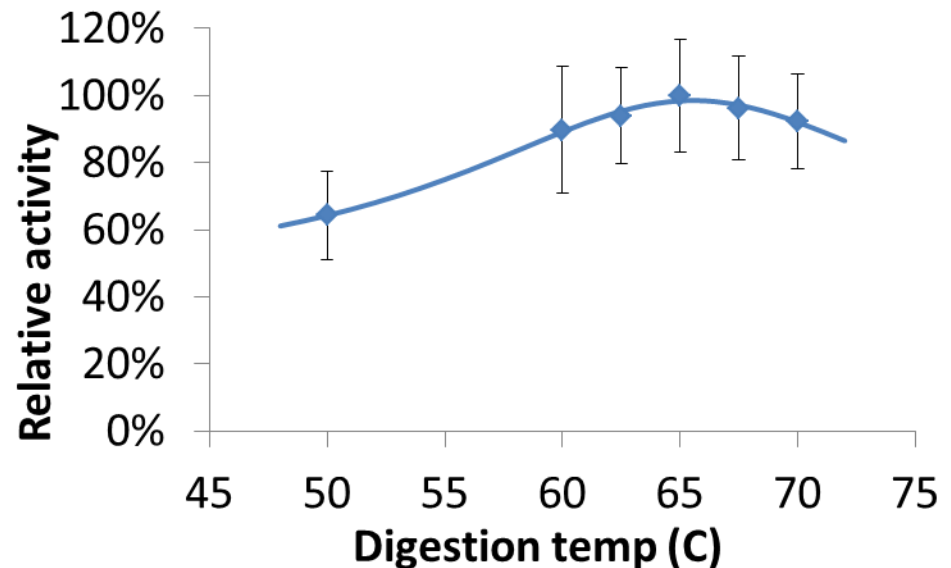
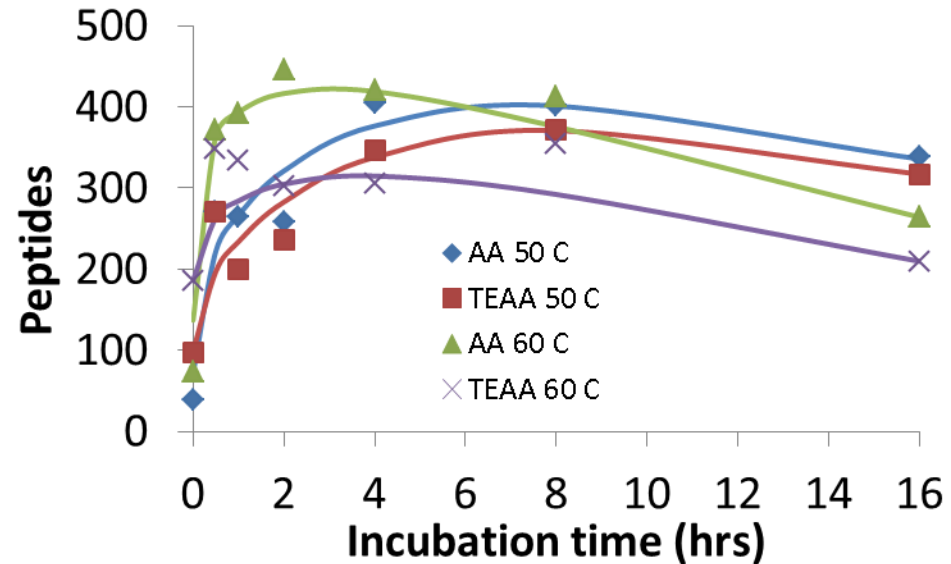
Tryp-N

- Metalloprotease
 - On/off with EDTA
 - Great for partial digestions
 - Increased sequence coverage
- Thermophile
 - $T_{opt} = 65^{\circ} C$
 - Really stable (salt, almost all buffers ok, etc.)
 - Doesn't autodigest



Tryp-N

- Hot = goes fast
 - 30 min or over lunch
- Hot = endogenous enzymes really dead
 - (E.g. snake venom anybody?)
- Hot = don't usually need e.g. detergents



Thank you!

- US HUPO
- Jonathan J. Ipsaro
- Samantha N. Peacock
- Keith D. Rivera
- Darryl J.C. Pappin



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