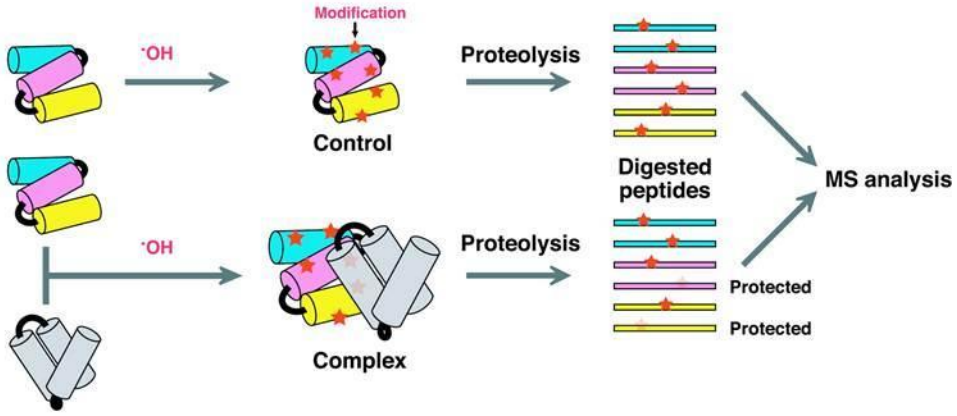
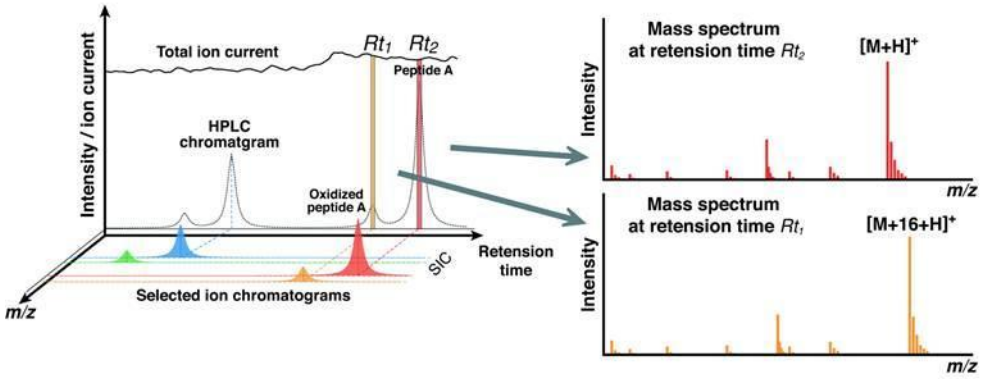
ProtMapMS: Integrated Algorithms for High-Throughput Examination of Biomolecules by Structural Mass Spectrometry

Parminder Kaur

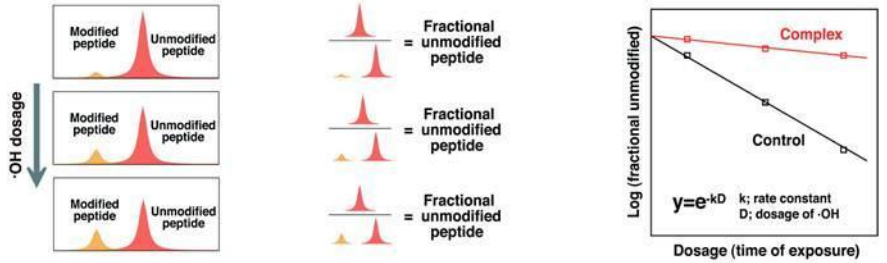
Exposure to hydroxyl radicals and subsequent proteolysis



HPLC chromatogram, ion chromatogram, and mass spectrum



Calculation of rate constants from dose response curve

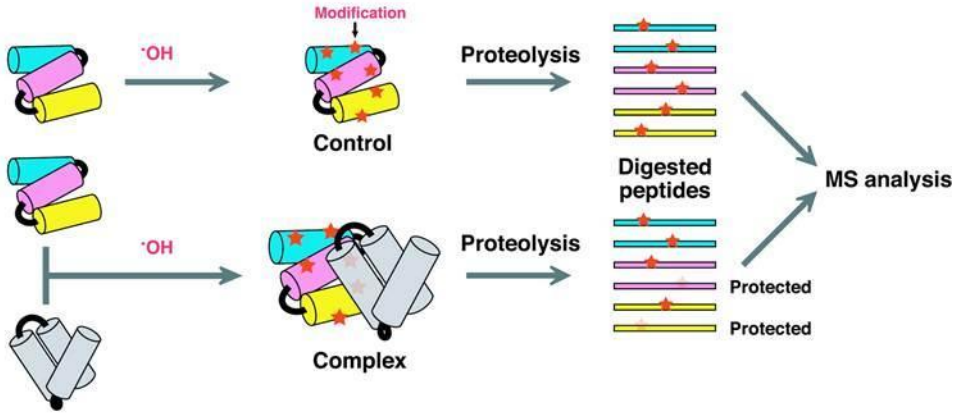


Takamoto K, Chance MR. 2006.
Annu. Rev. Biophys. Biomol. Struct. 35:251–76

side chain	side chain modification and mass changes
Cys	sulfonic acid (+48), sulfinic acid (+32), hydroxy (−16)
cystine	sulfonic acid, sulfinic acid
Met	sulfoxide (+16), sulfone (+32), aldehyde (−32)
Trp	hydroxy- (+16, +32, +48, etc.), pyrrol ring-open (+32, etc.)
Tyr	hydroxy- (+16, +32, etc.)
Phe	hydroxy- (+16, +32, etc.)
His	oxo- (+16), ring-open (−22, −10, +5)
Leu[a]	hydroxy- (+16), carbonyl (+14)
Ile[a]	hydroxy- (+16), carbonyl (+14)
Val[a]	hydroxy- (+16), carbonyl (+14)
Pro	hydroxy- (+16), carbonyl (+14)
Arg	deguanidination (−43), hydroxy- (+16), carbonyl (+14)
Lys	hydroxy- (+16), carbonyl (+14)
Glu	decarboxylation (−30), hydroxy- (+16), carbonyl (+14)
Gln	hydroxy- (+16), carbonyl (+14)
Asp	decarboxylation (−30), hydroxy- (+16)
Asn	hydroxy- (+16)
Scr[b]	hydroxy- (+16), carbonyl (−2- or +16-H_2O)
Thr[b]	hydroxy- (+16), carbonyl (−2- or +16-H_2O)
Ala	hydroxy- (+16)

Xu, Chance,
Chem Reviews (2007),
107 (8)

Exposure to hydroxyl radicals and subsequent proteolysis



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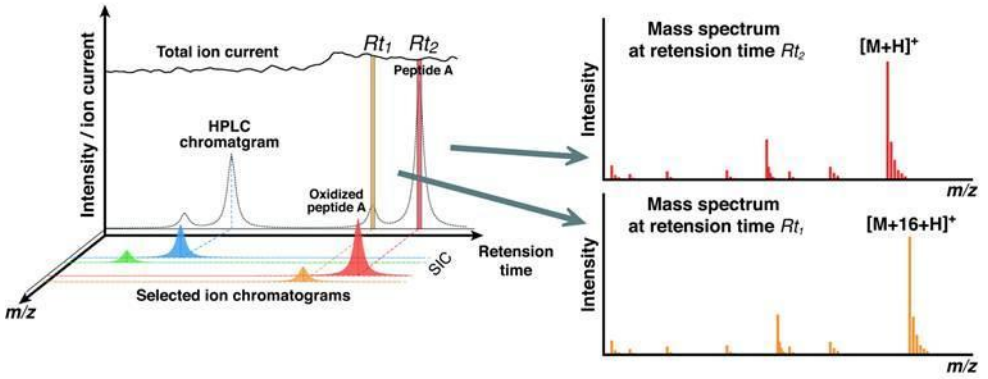

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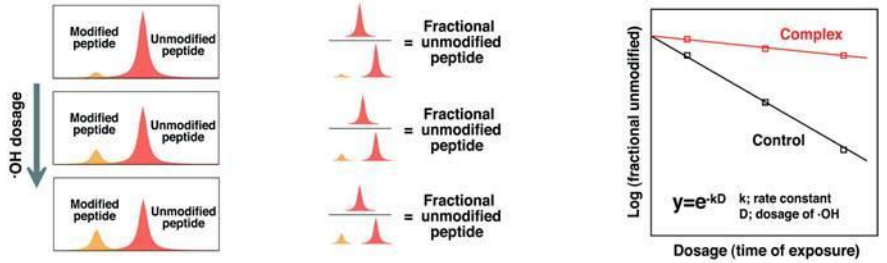

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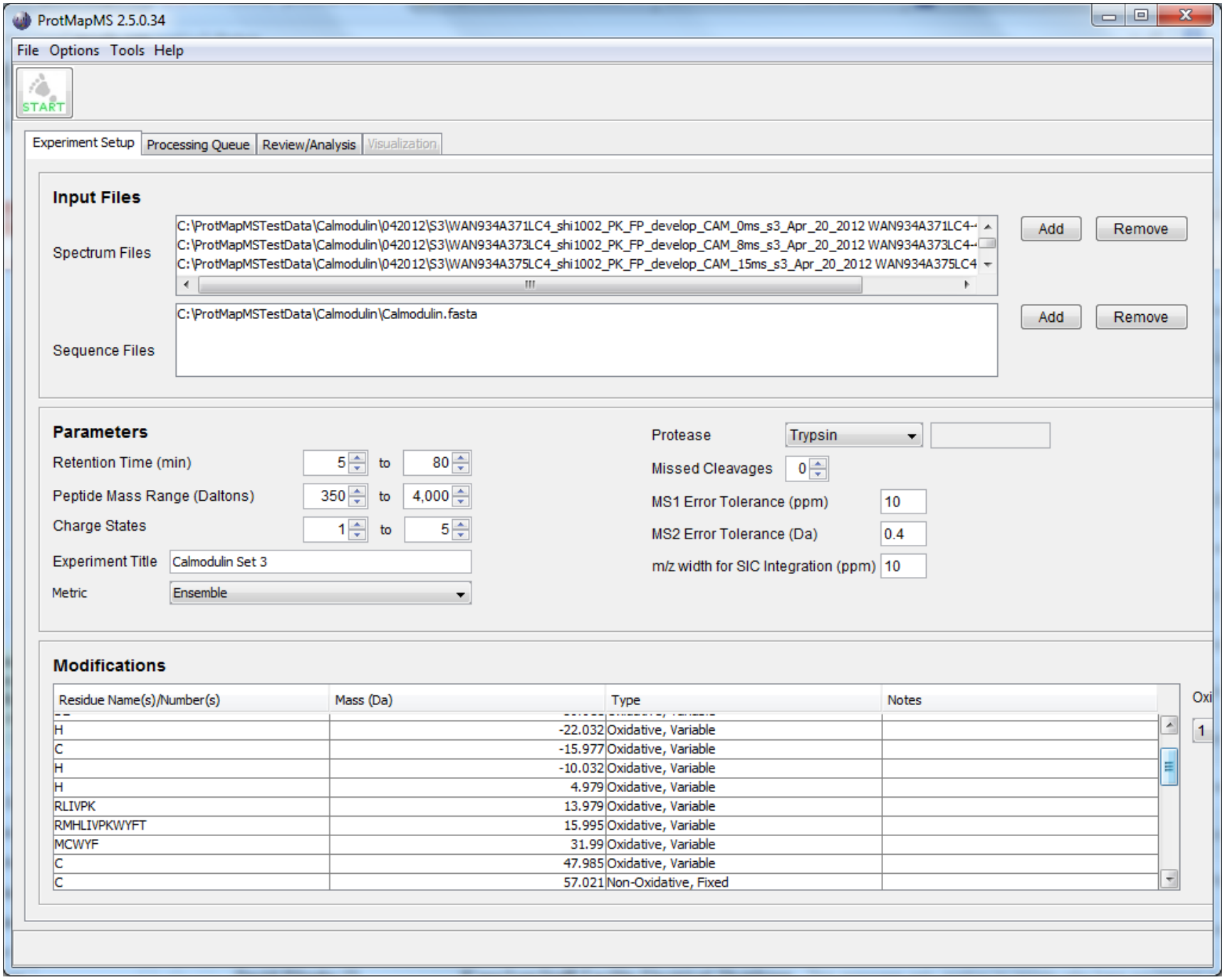
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Processing Queue
Review/Analysis
Visualization
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Remove
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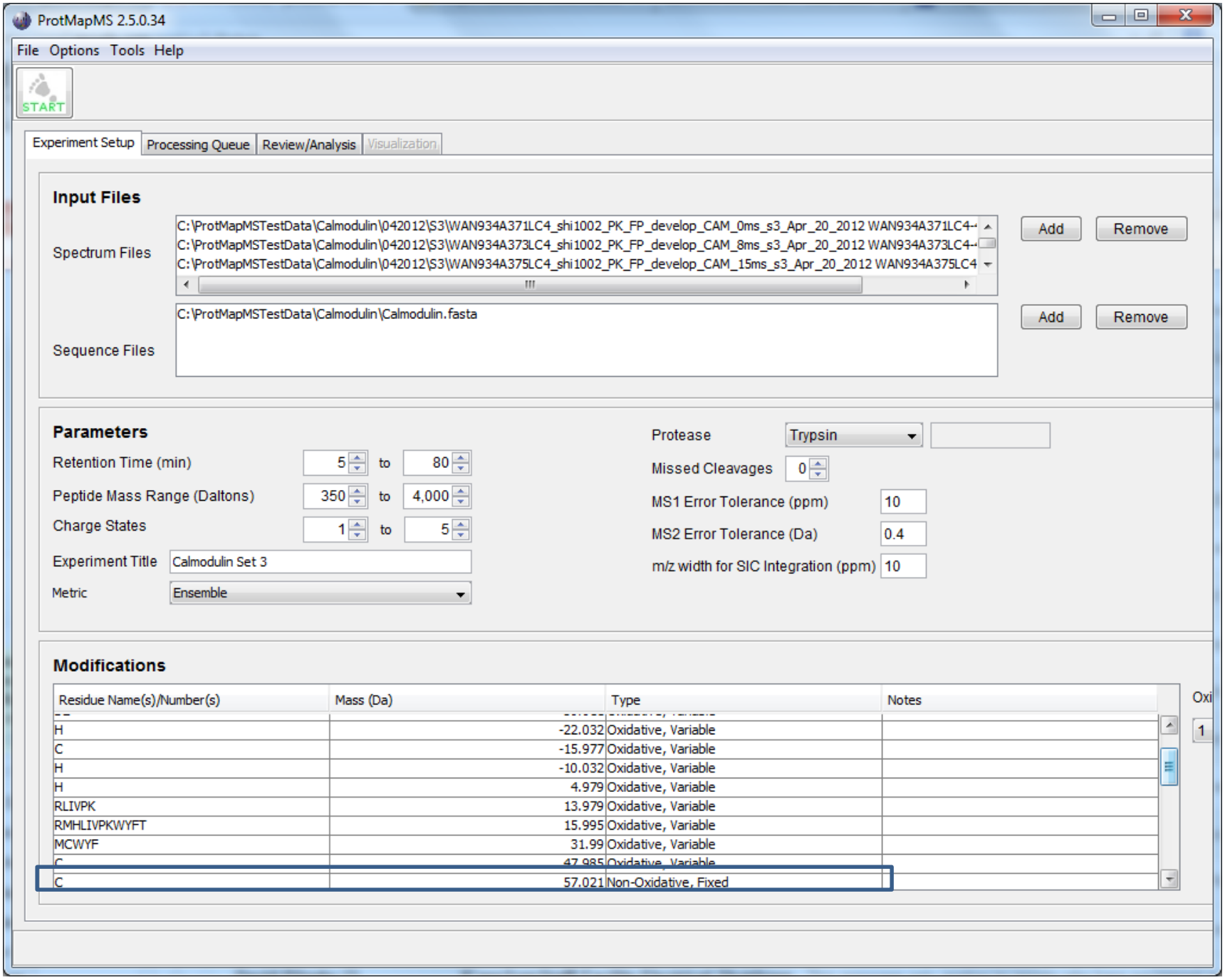
Peptide Mass Range (Daltons) 350 to 4,000
Charge States 1 to 5
Experiment Title Calmodulin Set 3
Metric Ensemble
Protease Trypsin
Missed Cleavages 0
MS1 Error Tolerance (ppm) 10
MS2 Error Tolerance (Da) 0.4
m/z width for SIC Integration (ppm) 10
Modifications
Residue Name(s)/Number(s) | Mass (Da) | Type | Notes
H | -22.032 | Oxidative, Variable
C | -15.977 | Oxidative, Variable
H | -10.032 | Oxidative, Variable
H | 4.979 | Oxidative, Variable
RLIVPK | 13.979 | Oxidative, Variable
RMHLIVPKWYFT | 15.995 | Oxidative, Variable
MCWYF | 31.99 | Oxidative, Variable
C | 47.985 | Oxidative, Variable
C | 57.021 | Non-Oxidative, Fixed
Oxi
1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Spectrum Files
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Remove
Sequence Files
C:\ProtMapMSTestData\Calmodulin\Calmodulin.fasta
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Remove
Parameters
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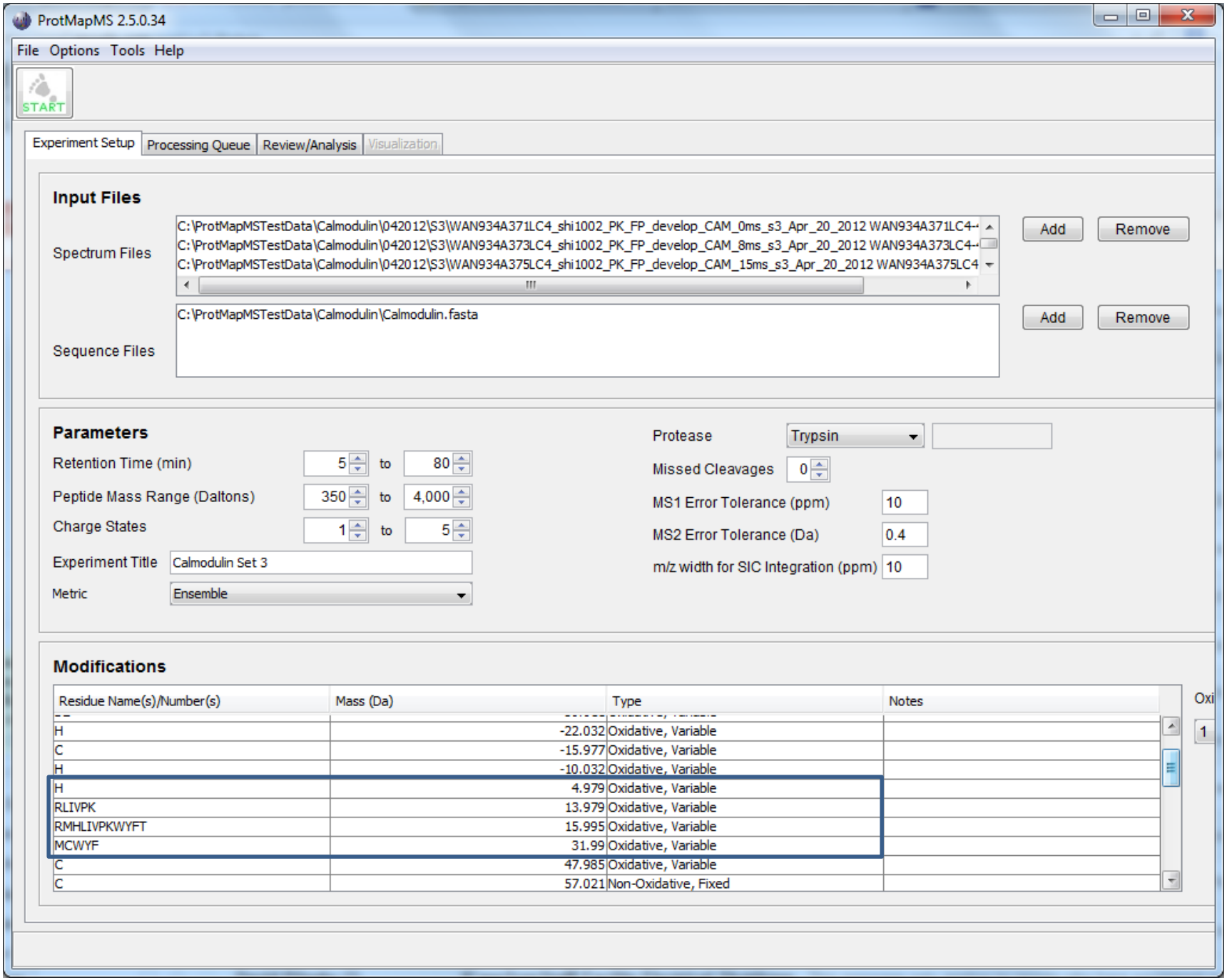
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Processing Queue
Review/Analysis
Visualization
Input Files
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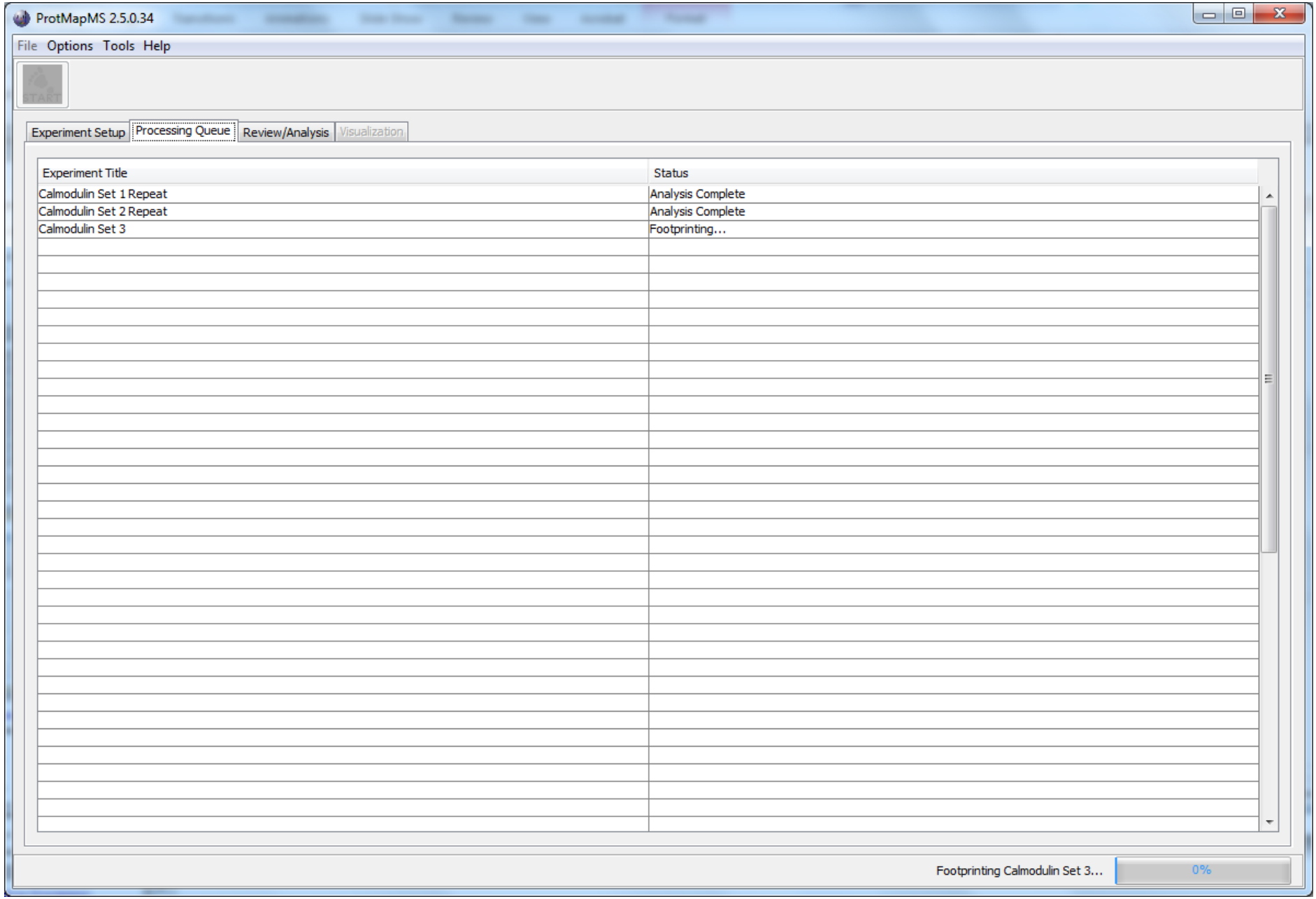
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File Options Tools Help
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Processing Queue
Review/Analysis
Visualization
Experiment Title
Status
Calmodulin Set 1 Repeat
Analysis Complete
Calmodulin Set 2 Repeat
Analysis Complete
Calmodulin Set 3
Footprinting...
Footprinting Calmodulin Set 3...
0%

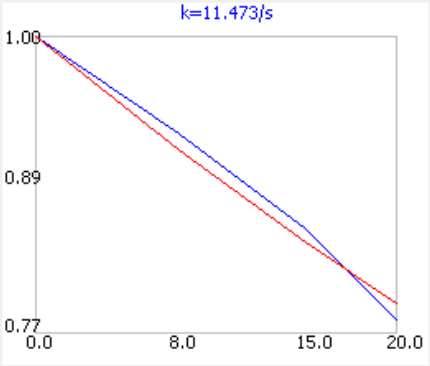
ProtMapMS 2.5.0.34

File Options Tools Help

START

Experiment Setup | Processing Queue | Review/Analysis | Visualization

DR Plot



Protein

Calmodulin

Peptide

116-126 : LTDEEVDEMIR

Species

m/z	z	t1	t2	ppm	inc?
691....	2	20.226	26.226	10	☑
660....	2	28.326	34.326	10	☑
675....	2	30.128	33.128	10	☑
682....	2	25.906	31.906	10	☑
683....	2	23.51	29.51	10	☑

Isolate | SIC/MS2 | Apply All

Charge States

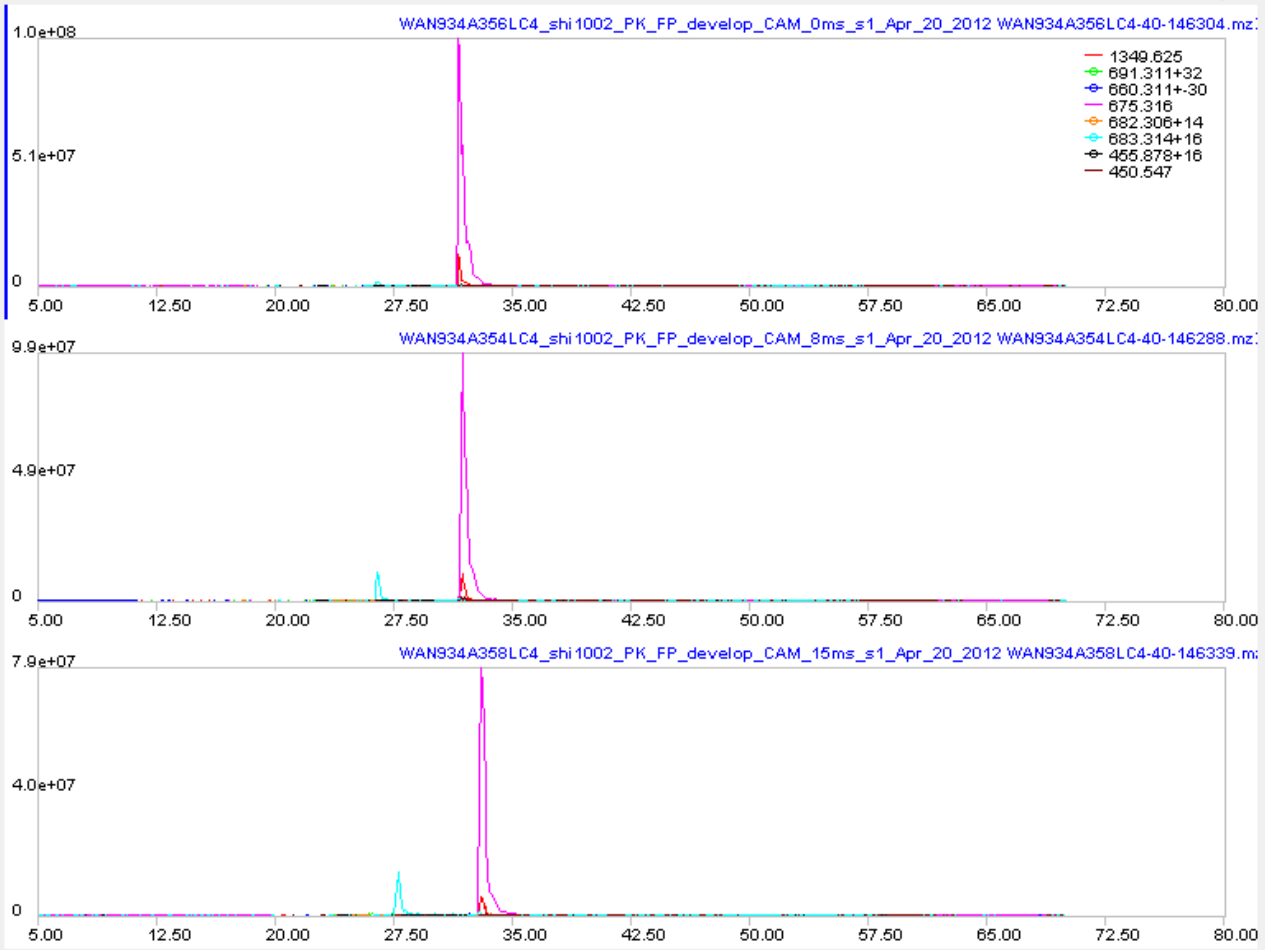
z	inc?
1	☑
2	☑

Exposure Times

ms	inc?
20	☑

SIC Plot

Calmodulin Set 1 Repeat



Analysis Com

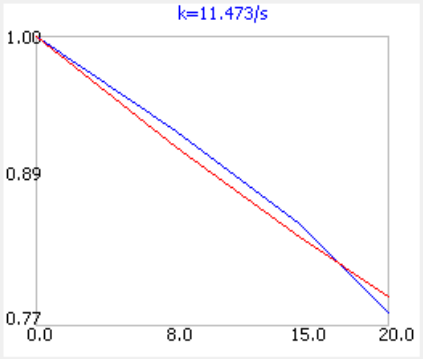
ProtMapMS 2.5.0.34

File Options Tools Help

START

Experiment Setup | Processing Queue | Review/Analysis | Visualization

DR Plot



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682....	2	25.906	31.906	10	☐
683....	2	23.51	29.51	10	☐

Isolate | SIC/MS2 | Apply All

Charge States

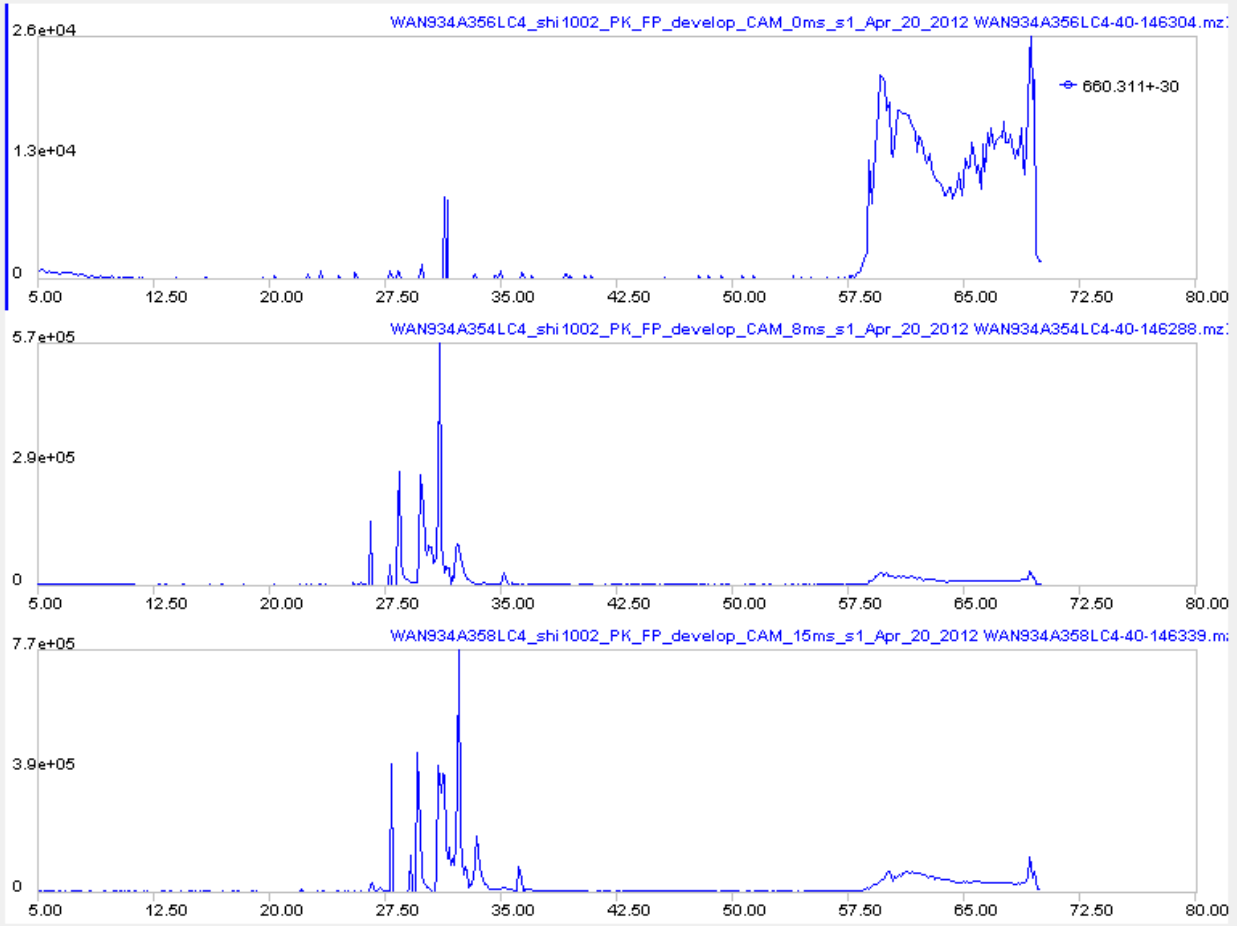
z	inc?
1	☑
2	☑

Exposure Times

ms	inc?
20	☑

SIC Plot

Calmodulin Set 1 Repeat



Analysis Com

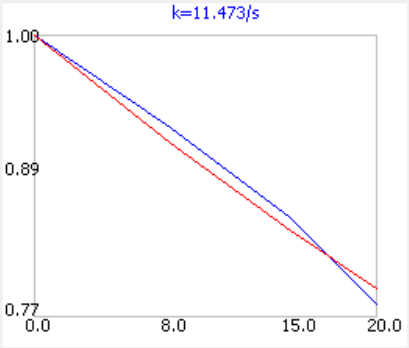
ProtMapMS 2.5.0.34

File Options Tools Help

START

Experiment Setup | Processing Queue | Review/Analysis | Visualization

DR Plot



Protein

Calmodulin

Peptide

116-126 : LTDEEVDEMIR

Species

m/z	z	t1	t2	ppm	inc?
691....	2	23.146	29.146	10	☐
660....	2	28.02	34.02	10	☑
675....	2	30.341	33.341	10	☐
682....	2	29.565	35.565	10	☐
683....	2	23.531	29.531	10	☐

Isolate | SIC/MS2 | Apply All

Charge States

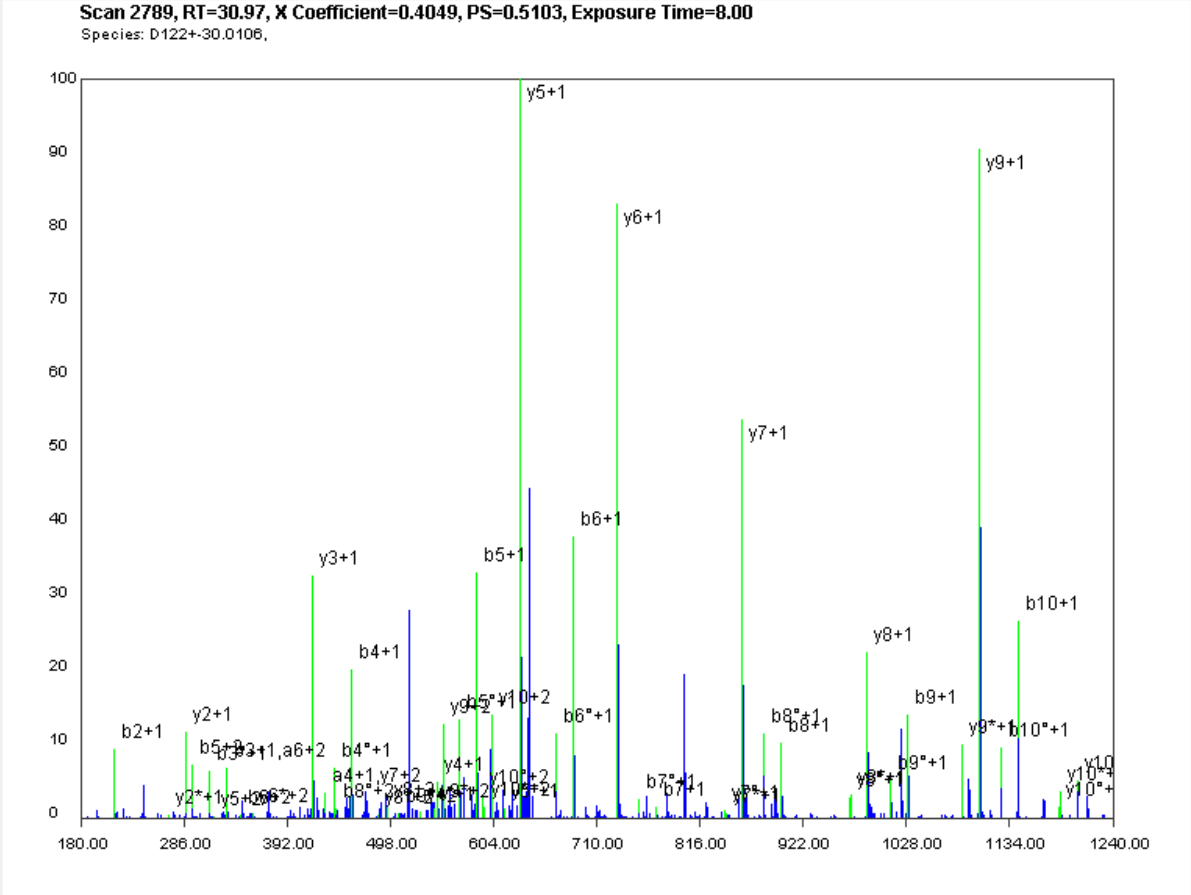
z	inc?
1	☑
2	☑

Exposure Times

ms	inc?
20	☑

SIC Plot

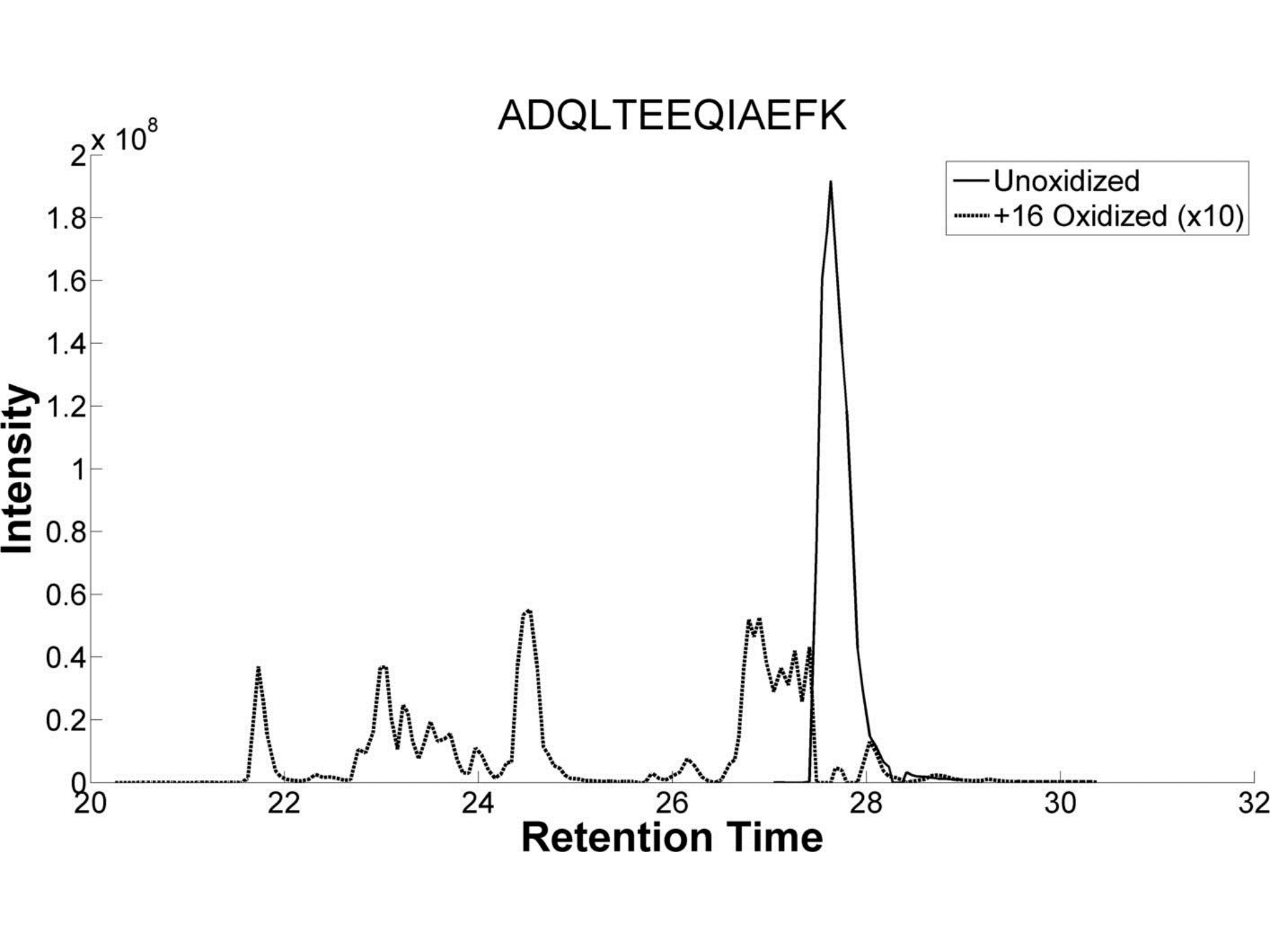
Calmodulin Set 1 Repeat

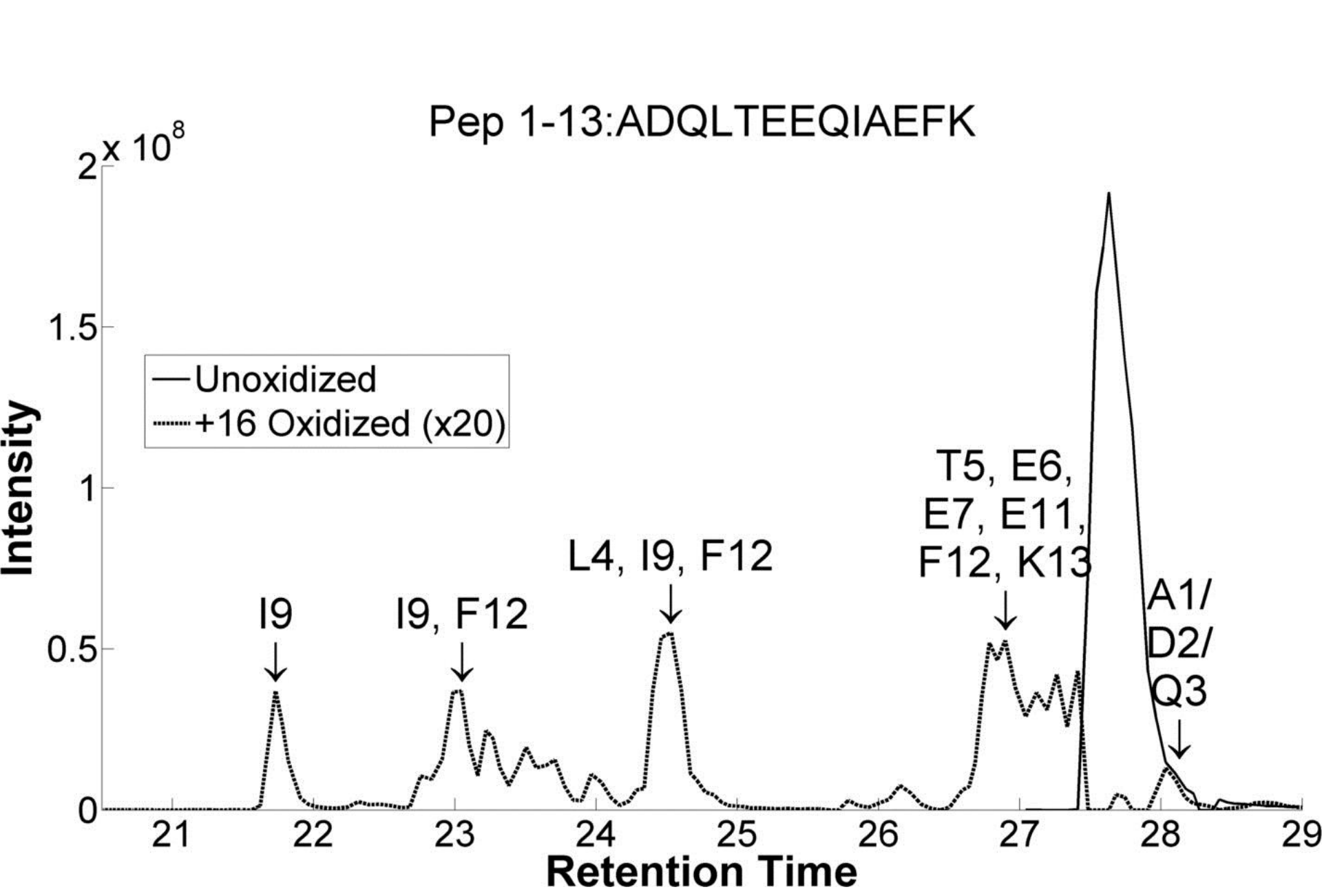


Analysis Com

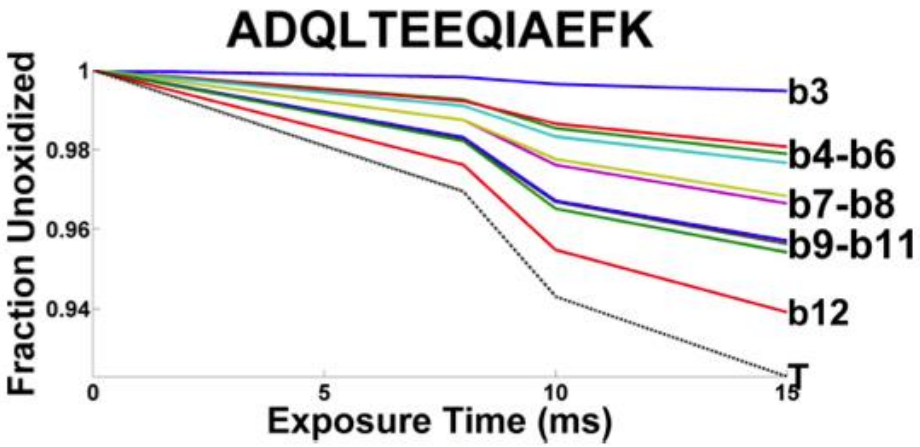
Next Steps

- What? Increase structural resolution to individual residue level
- How?
  - Targeted MS experiments
  - Quantification for a ladder of peptide fragments using the ion abundances from a combination of MS1 and MS2
  - Residue level dose response (DR) plots

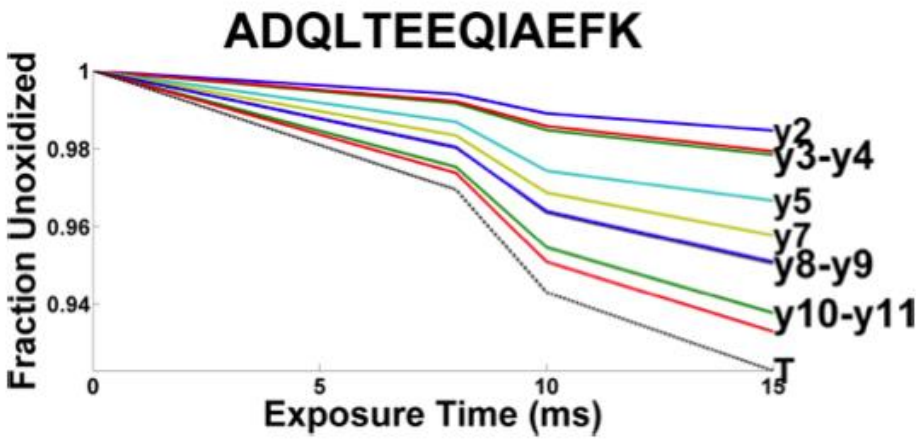

ADQLTEEQIAEFK
x 10^8
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Intensity
Unoxidized
+16 Oxidized (x10)
20
22
24
26
28
30
32
Retention Time


Pep 1-13:ADQLTEEQIAEFK
x 10^8
2
1.5
1
0.5
0
Intensity
Unoxidized
+16 Oxidized (x20)
I9
I9, F12
L4, I9, F12
T5, E6,
E7, E11,
F12, K13
A1/
D2/
Q3
21
22
23
24
25
26
27
28
29
Retention Time

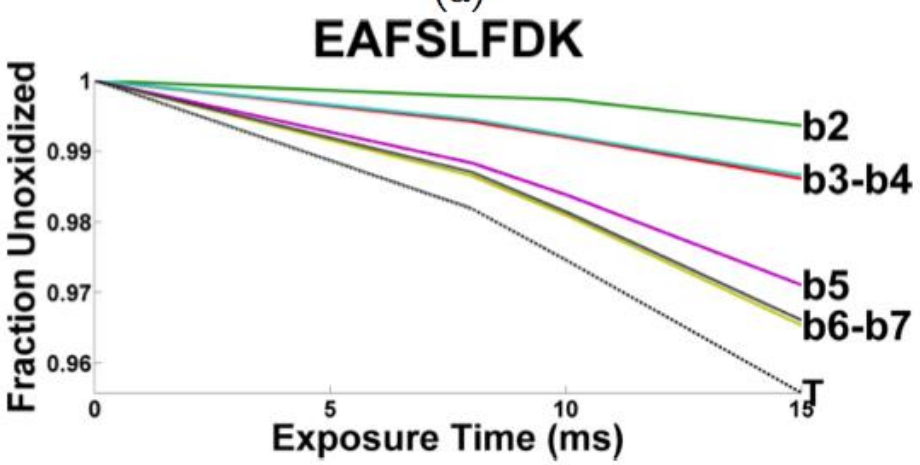
Dose Response Plots (1-13, 14-21)

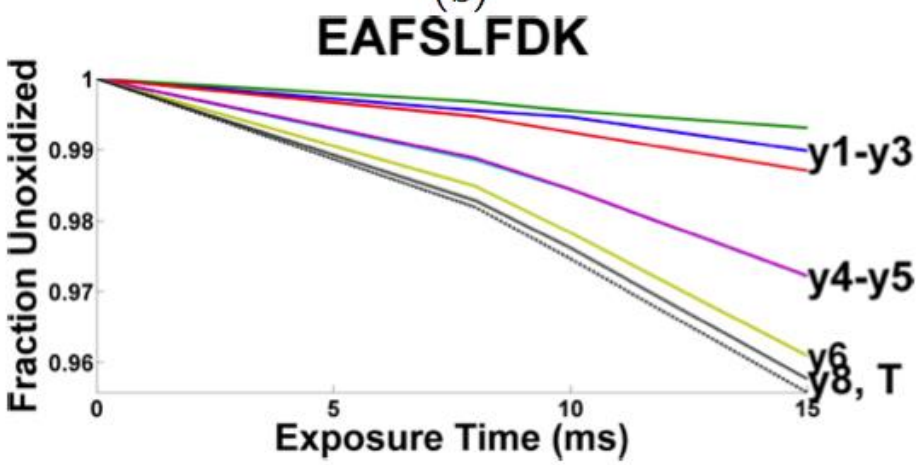


(a)



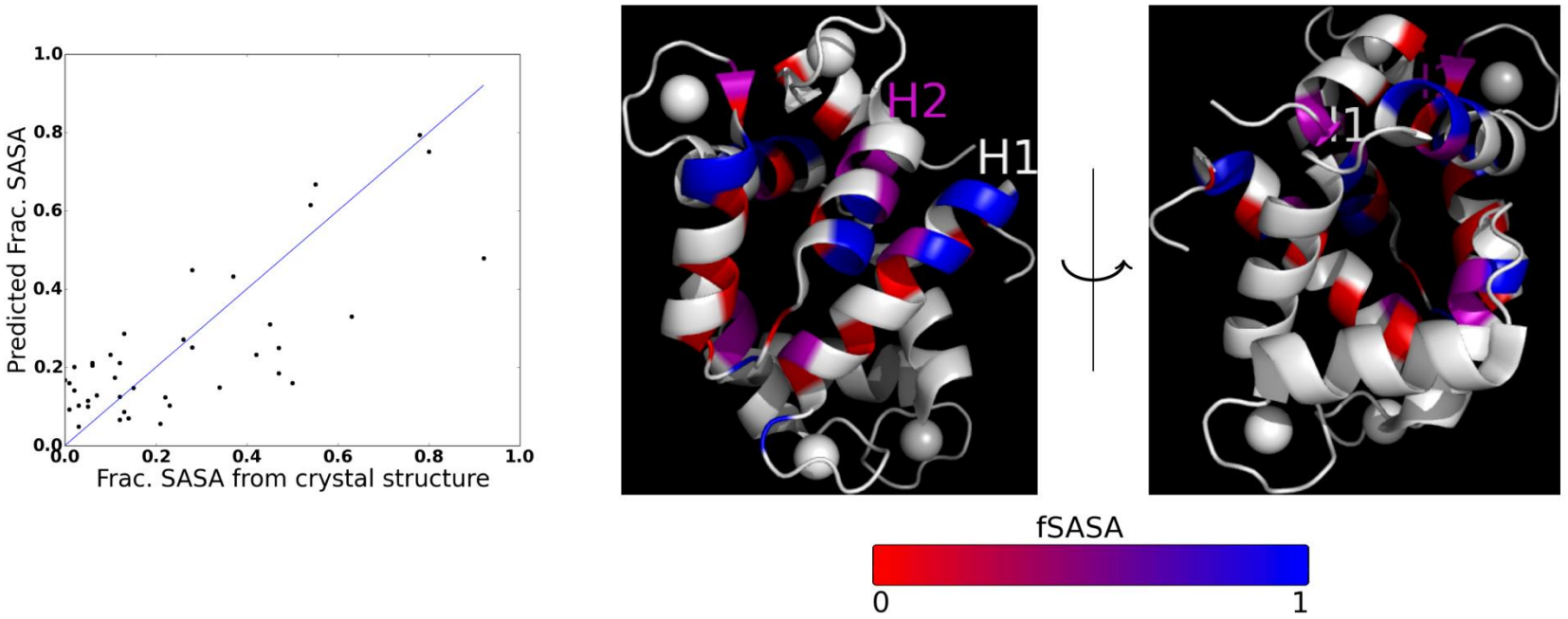
(b)





Peptide location/sequence	Residue(s)	Total SASA ($Å^2$)	b-ion RC change (s^{-1})	y-ion RC change (s^{-1})
1-13: ADQLTEEQIAEFK	A1, D2, Q3	235	0.3	NA
	L4	3	1.0	0.9
	T5	58	-0.1	0.0
	E6	143	0.3	0.5
	E7	86	0.7	0.5
	Q8	5	-0.1	-0.9
	I9	33	0.9	0.9
	A10	24	0.0	-0.1
	E11	18	0.2	0.3
	F12	5	1.0	1.0
14-21: ADQLTEEQIAEFK	E14, A15	50	0.4	0.2
	F16	22	0.5	0.7
	S17	39	0.0	0.0
	L18	8	0.9	0.9
	F19	10	0.3	0.3
	D20	6	-0.1	-0.2
	K21	83	NA	0.6
31-37: ELGTVMR	E31, L32	11	0.1	0.1
	G33, T34	58	0.2	0.2
	V35	2.5	0.1	NA
	M36	10	2.6	2.6
	R37	92	NA	0.15
78-86: DTDSEEEIR	D78, T79	160	0.2	0.2
	D80	83	0.0	0.0
	S81	20	-0.1	0.0
	E82	121	0.6	0.4
	E83	85	1.0	1.0
	E84	34	0.2	0.2
	I85	20	0.1	0.2
	R86	103	NA	0.1
95-106: DGNGYISAAELR	D95, G96, N97	181	0.3	NA
	G98	28	0.0	0.0
	Y99	85	4.0	4.7
	I100	4	NA	NA

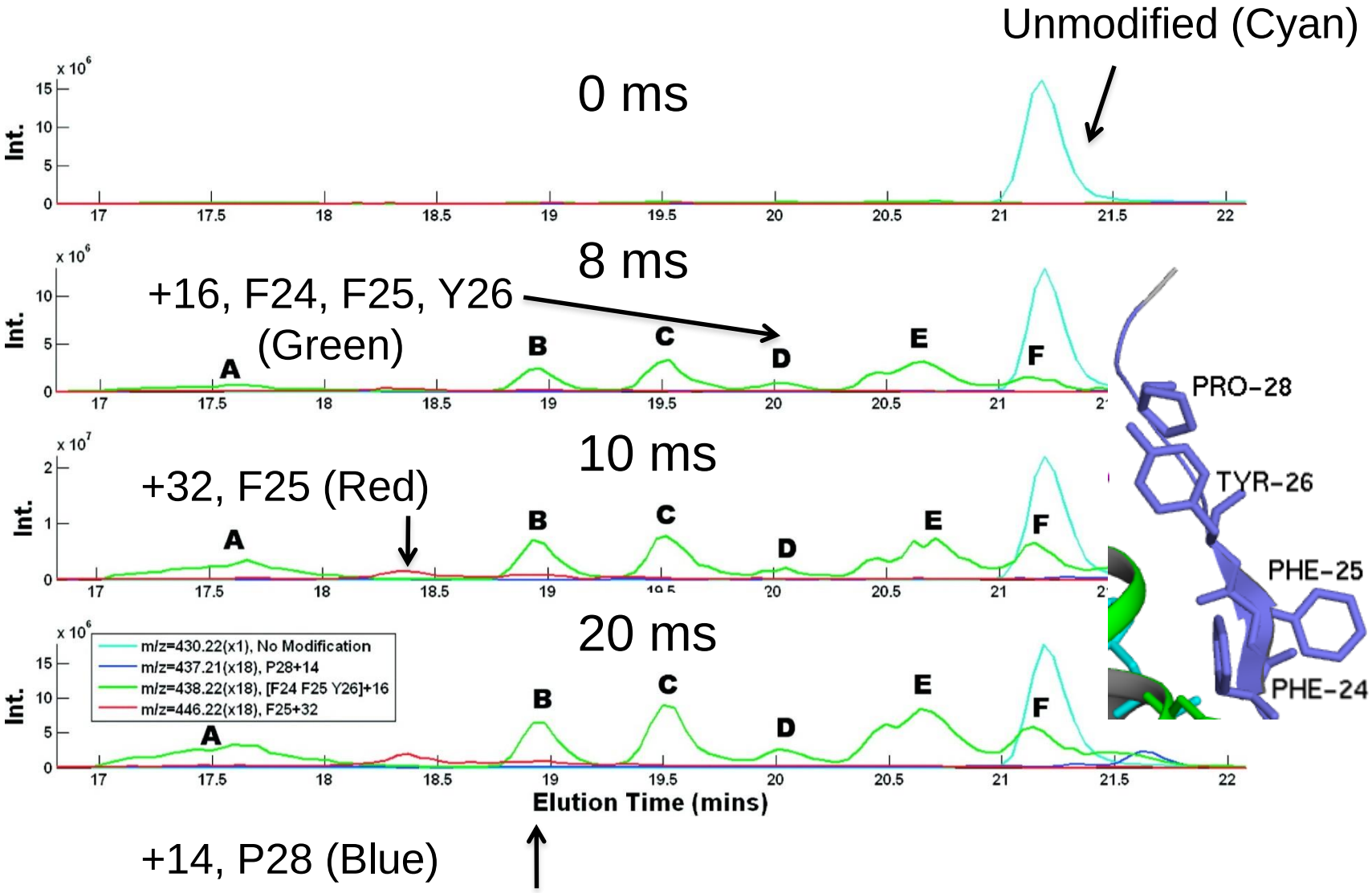
Towards absolute prediction using statistical and biophysical modeling



(a) (b)

Figure 4:

Acknowledgments

- Jen Bohon
- Mark Chance
- Rhijuta D’ Mello
- Giridharan Gokulrangan
- Sayan Gupta
- Janna Kiselar
- Sean Maxwell
- Benlian Wang
- Sichun Yang

Extracted Ion Chromatogram: Insulin peptide 23-29