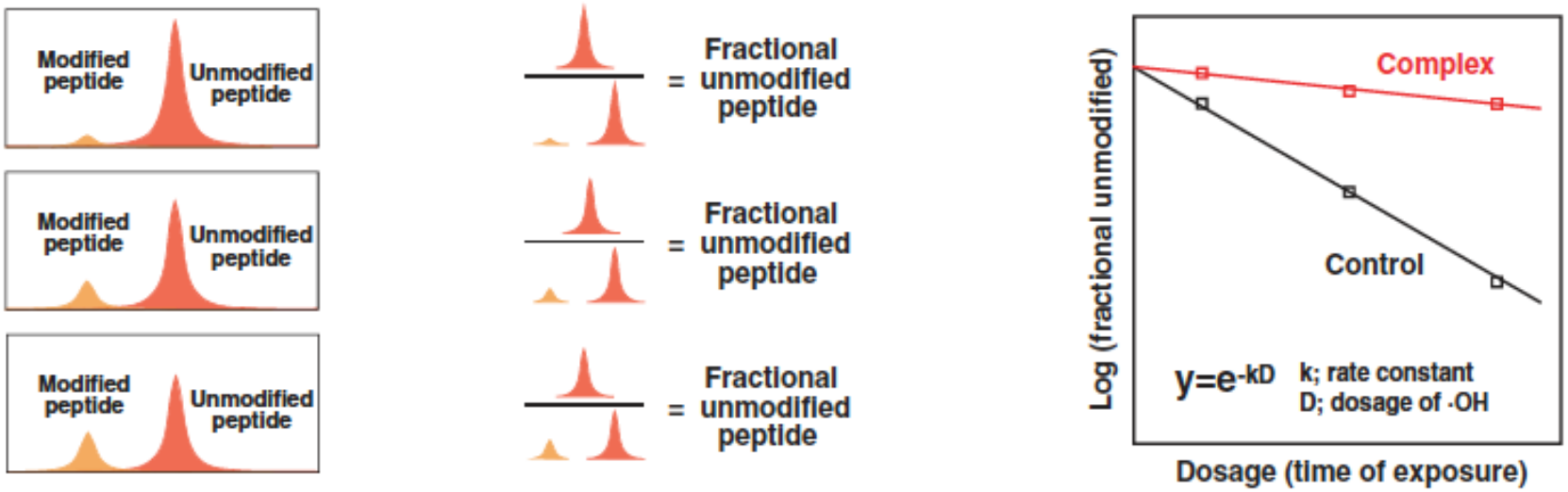
DETERMINE RATES WITH •OH DOSING



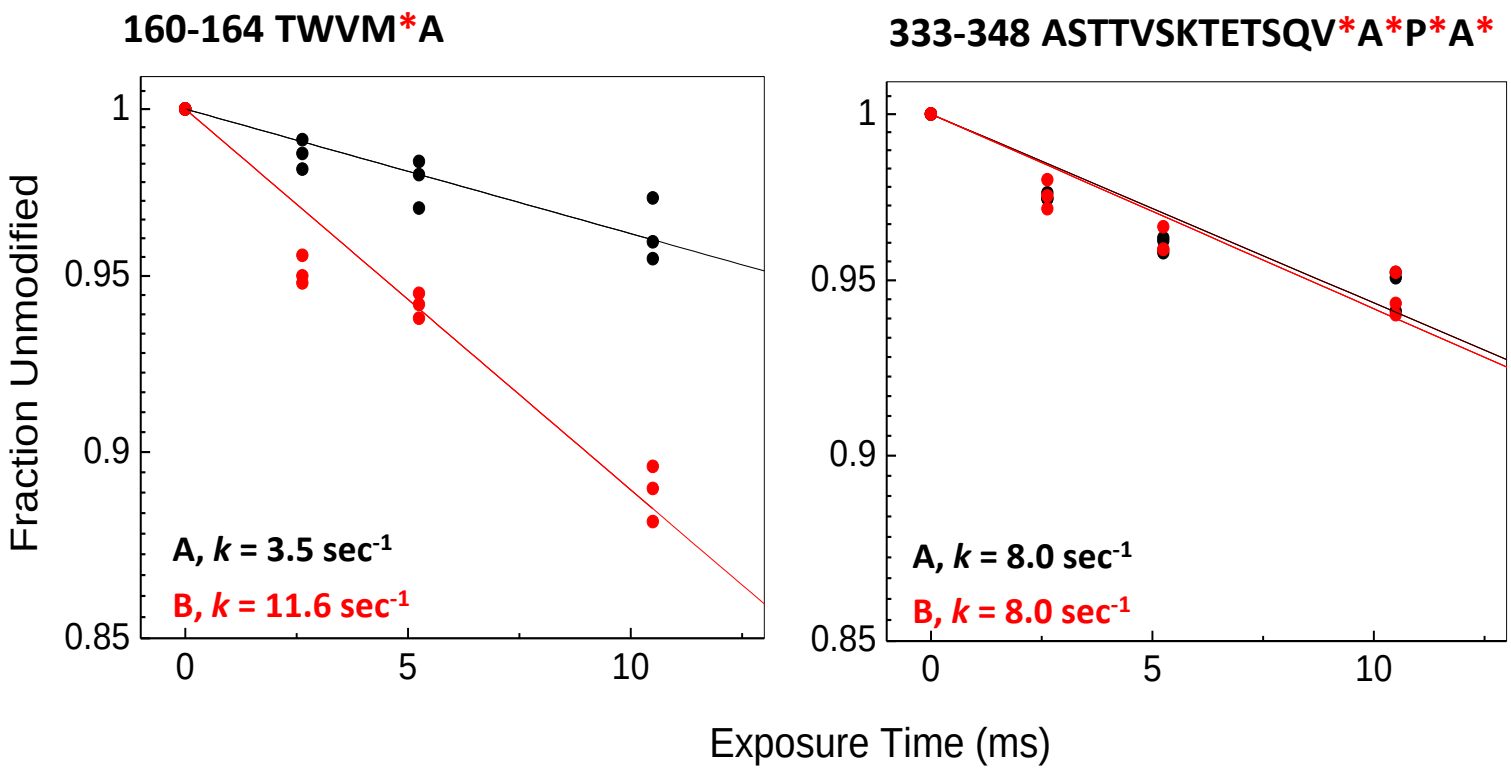
Fractional unmodified peptide determined from peak areas plotted versus increasing times of x-ray exposure

Takamoto, K. & M.R. Chance, *Annu. Rev. Biophys. Biomol Struct.* 2006. 35:251-76

COMPARE DOSE RESPONSES: CONFORMATION A VS. B

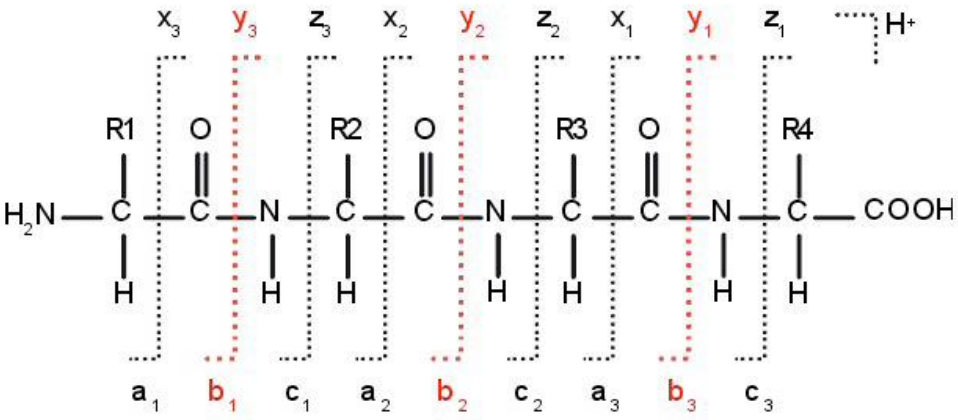
$$Y = Y_0 \exp^{-kt}$$

Y and Y0 – fraction of unmodified peptide at time t and 0



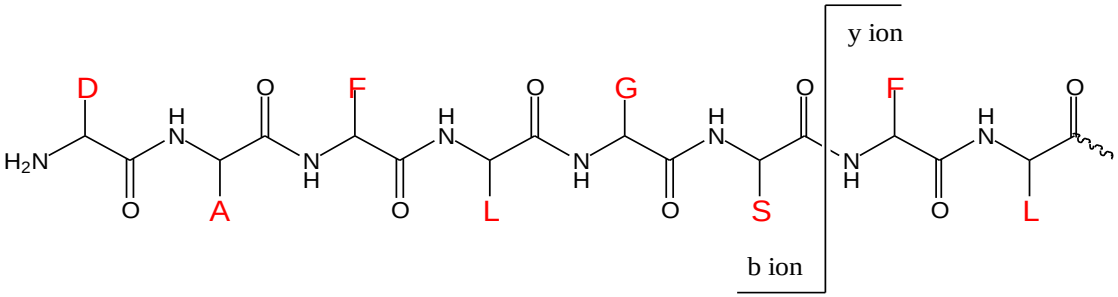
Change in **Modification Rates** = Change in the **Solvent accessibility**

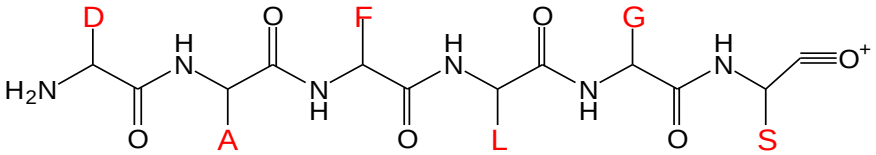
MS/MS ANALYSIS: FRAGMENTATION



N term ⟶ C term

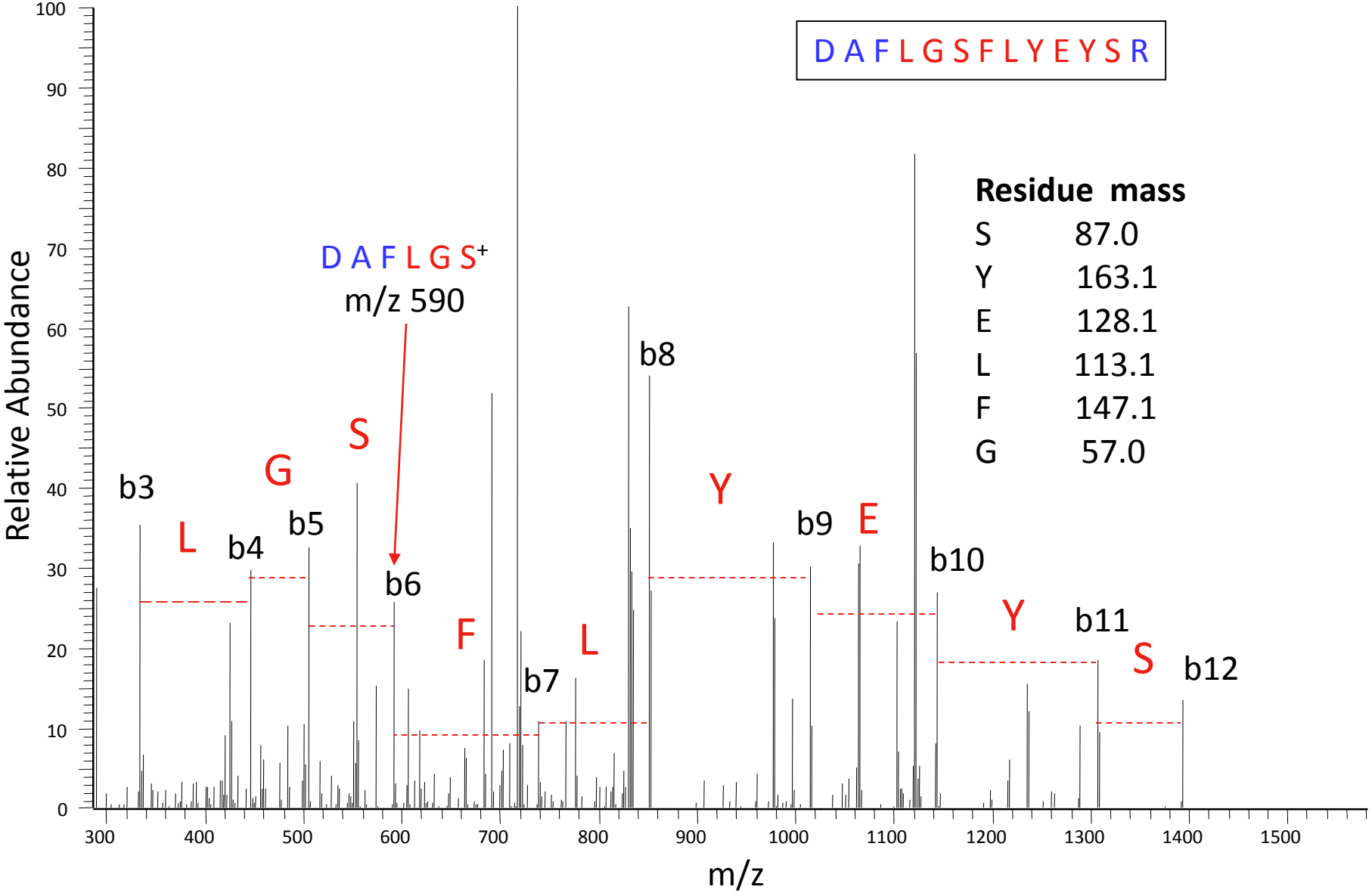
D A F L G S F L Y E Y S R



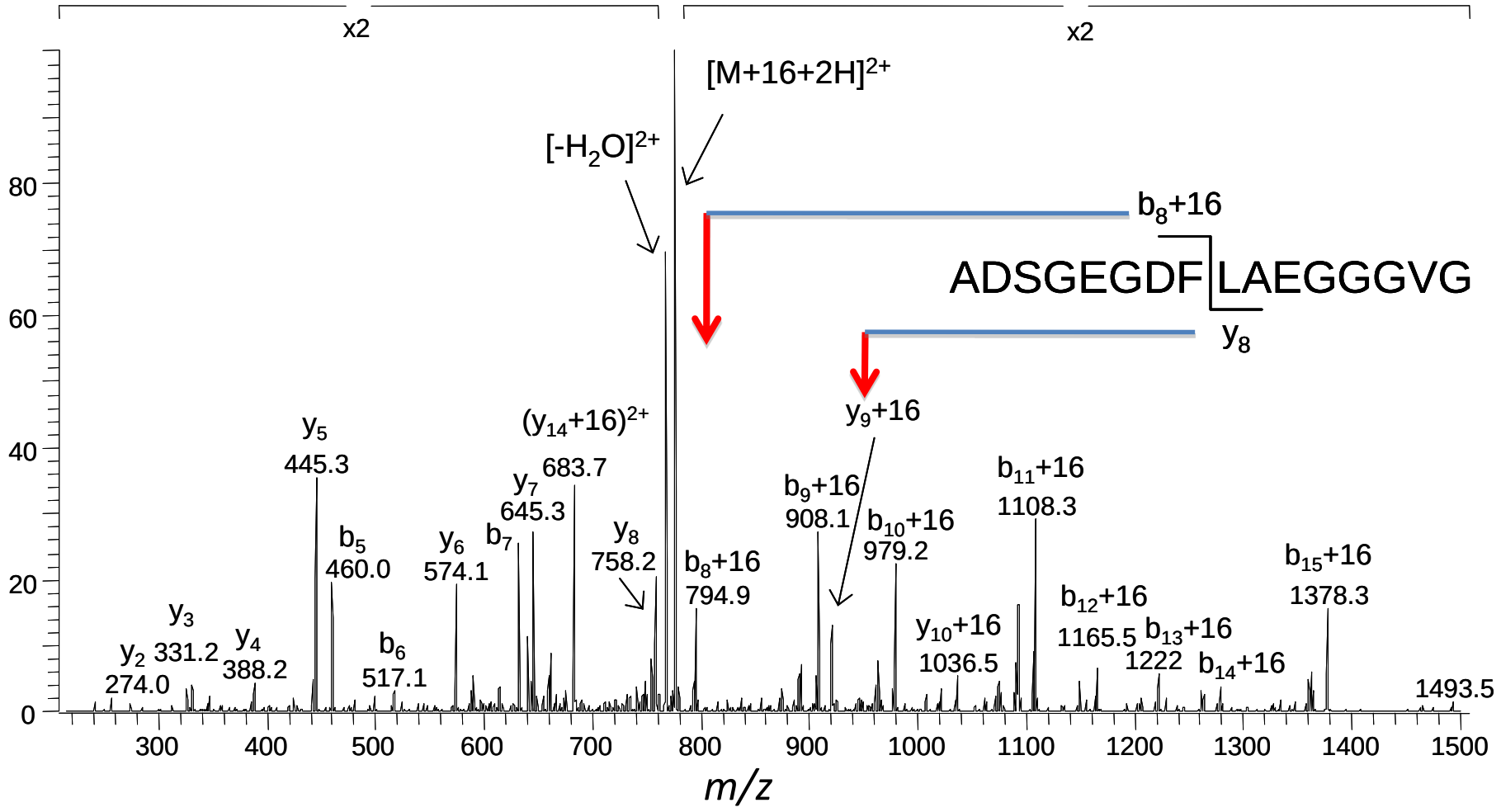


b ion
(m/z 590)

MS/MS SPECTRUM



TANDEM MS EXAMPLE OF MODIFIED PEPTIDE



DATA ANALYSIS: SOFTWARE

- Manually – to calculate constant rates and to verify sites of modification

- MaxMatrix (http://www.massmatrix.net) - to determine sites of modification

- Mascot (Matrix Science) – to determine sites of Modification

- ProtMapMS – (in-house) – all of the above

MassMatrix Database Search Engine

NEWS: MassMatrix search engine v2.4.0 has been released to support Ultraviolet Photodissociation (UVPD) and hybrid fragmentations with two or more methods, such as CID/HCD, UVPD/ETD and etc.

Basic Search | Advanced Search / Cross Link / Quantitation | Search Profile | Results | Data | Settings | Server

Tandem MS Database Search

Supported Browsers: Firefox 3.0, Google Chrome 4.0, Sarafi 4.0, IE 8.0, Opera 10.0 or any newer versions of these browsers.
Note: You may click on any field in the search form for help.
Note: Simple instructions for searching cross-links and disulfide bonds between different proteins.Click here

Search Engine:	MassMatrix Xtreme 3.0.9.7 Alpha with 4M spectra limit, Dec 8 2011 (Latest release)		
Chooase Profile:	Most Recent Search (Default)	Save Form as Profile:	Don't Save
*Data files:	Browse... No file selected. Janna_AB42_2_4mer_10ms_set1.mgf Delete	Search data sets:	Individually
*Database:	Abeta42 Configure Protein Databases	*Enzyme:	Lysine-C no P rule: K-X Lysine-C: K-X(not P) None Nonspecific/Non-restricted
Decoy database:	None	Missed cleavages:	3
Variable modifications:	Homoserine of C-term M Hydrolmidozolone ICAT(TM)-d0 of C ICAT(TM)-d8 of C Iodoacetamide/Carbamidomethyl of C	Fixed modifications:	4-hydroxynonenal (HNE) of CHK Acetylation of K Acetylation of N-term Acrylamide adduct of C Amidation of C-term
*Precursor ion tol.:	± 10 ppm	*Product ion tol.:	± 0.8 Da
Max # PTM/peptide:	4	Mass type:	Monoisotopic
Min peptide length:	5 AA	Max peptide length:	50 AA
Min pp score:	5.0	Min pp_{tag} score:	1.3
Max # match/spec:	1	Max # comb/match:	3
Fragmentation method:	CID	C13 isotope ions:	No
☐Cross Link Search Options Click to expand			
☐Quantitation Options Click to expand			
Comment:		Expert options:	
	Search		Reset

Fileds labeled by * are required!

MASS MATRIX READOUT

HIT 1

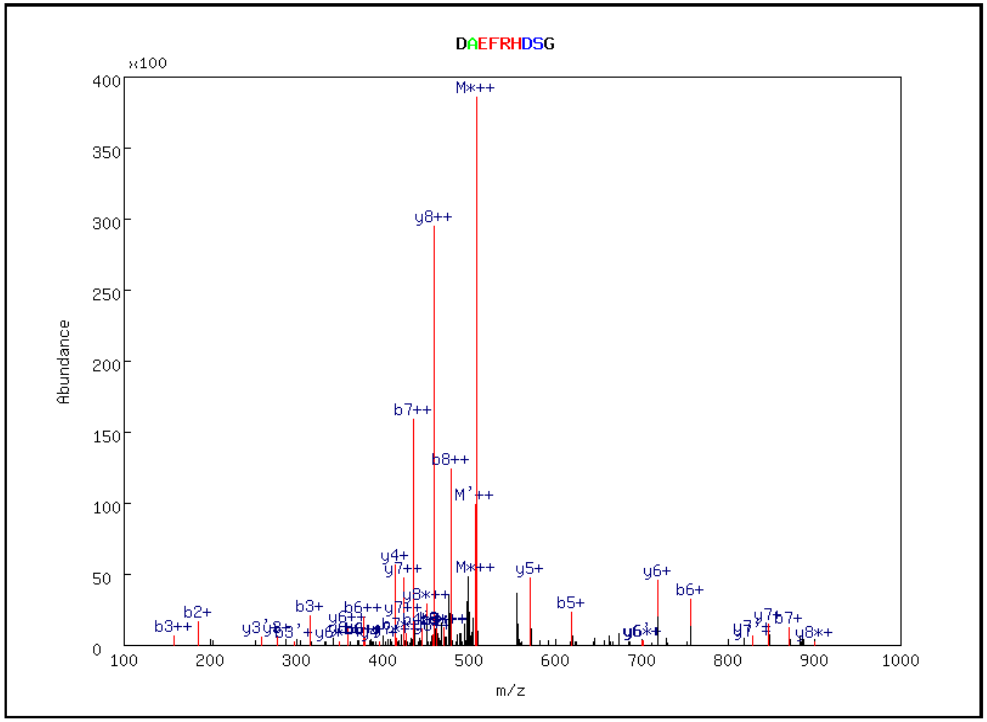
Protein Mass: 4511.270 (monoisotopic) 4514.061(average) Protein Score: **5006** Protein pp: **3672.0**
Abeta42

Sequence:
001 **DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA** 42

Sequence Coverage: **100%**
Sequence Tag Coverage: **97%**

Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modifications [start:end]
13553	4599	+2	23	11.1	11.4	4.4	517.2208	1033.4344	1033.4334	0.0010	0	√	DAEFRHDSG
13661	4545	+2	68	16.2	15.1	9.7	525.2175	1049.4278	1049.4283	-0.0005	0	√	DAEFRHDSG + ox16(4)
13662	4526	+2	72	17.7	14.9	9.7	525.2177	1049.4281	1049.4283	-0.0001	0	√	DAEFRHDSG + ox16(4)
13663	4590	+2	31	9.8	12.1	7.3	525.2185	1049.4297	1049.4283	0.0015	0	√	DAEFRHDSG + ox16(4)
13663	4590	+2	24	8.7	12.5	4.0	525.2185	1049.4297	1049.4283	0.0015	0	√	DAEFRHDSG + ox16(5) [1:9]
14470	5234	+2	58	10.9	14.4	11.4	598.7513	1196.4953	1196.4967	-0.0014	0	√	DAEFRHDSGY
14471	5375	+2	55	12.1	15.4	10.2	598.7513	1196.4953	1196.4967	-0.0014	0	√	DAEFRHDSGY
14472	5351	+2	63	14.9	16.7	14.3	598.7514	1196.4955	1196.4967	-0.0012	0	√	DAEFRHDSGY
14473	5328	+3	41	10.9	8.7	5.1	399.5034	1196.4956	1196.4967	-0.0011	0	√	DAEFRHDSGY
14474	5382	+3	34	13.5	9.8	5.1	399.5034	1196.4956	1196.4967	-0.0011	0	√	DAEFRHDSGY
14475	5325	+2	94	19.4	16.7	14.3	598.7515	1196.4958	1196.4967	-0.0009	0	√	DAEFRHDSGY
14476	5261	+2	21	9.6	8.4	9.0	598.7516	1196.4959	1196.4967	-0.0008	0	√	DAEFRHDSGY
14477	5354	+3	34	12.1	9.1	5.1	399.5035	1196.4960	1196.4967	-0.0007	0	√	DAEFRHDSGY
14478	5304	+2	93	19.4	15.8	14.3	598.7518	1196.4963	1196.4967	-0.0004	0	√	DAEFRHDSGY
14479	5305	+3	43	10.2	8.6	6.0	399.5037	1196.4965	1196.4967	-0.0002	0	√	DAEFRHDSGY
14480	5286	+2	88	18.6	15.8	14.3	598.7521	1196.4969	1196.4967	0.0002	0	√	DAEFRHDSGY
14481	5289	+3	45	12.1	9.0	6.9	399.5038	1196.4969	1196.4967	0.0002	0	√	DAEFRHDSGY
14568	5231	+2	48	14.9	14.1	11.4	606.7489	1212.4905	1212.4916	-0.0011	0	√	DAEFRHDSGY + ox16(4)
14568	5231	+2	40	14.9	14.8	15.5	606.7489	1212.4905	1212.4916	-0.0011	0	√	DAEFRHDSGY + ox16(5)
14568	5231	+2	42	15.6	13.1	11.4	606.7489	1212.4905	1212.4916	-0.0011	0	√	DAEFRHDSGY + ox16(6)
14569	5235	+3	19	10.6	8.3	8.3	404.8351	1212.4908	1212.4916	-0.0008	0	√	DAEFRHDSGY + ox16(5)
14569	5235	+3	21	9.6	7.5	6.9	404.8351	1212.4908	1212.4916	-0.0008	0	√	DAEFRHDSGY + ox16(6)
14570	5253	+3	34	12.1	7.9	6.9	404.8353	1212.4913	1212.4916	-0.0003	0	√	DAEFRHDSGY + ox16(4)
14570	5253	+3	33	14.1	9.0	8.3	404.8353	1212.4913	1212.4916	-0.0003	0	√	DAEFRHDSGY + ox16(5)
14570	5253	+3	35	12.1	8.0	6.9	404.8353	1212.4913	1212.4916	-0.0003	0	√	DAEFRHDSGY + ox16(6)

MASS MATRIX IDENTIFICATION OF PEPTIDE 1-9 OF AB42



Index	scan#	charge	score	pp	pp_2	pp_{tag}	m/z	MW(obs)	MW	delta	miss	Unique	sequence + modificati
13330	4248	+2	62	14.6	14.1	7.3	517.2202	1033.4332	1033.4334	-0.0002	0	√	DAEFRHDSG

#	b'^{++}	b^{*++}	b^{++}	b'^{+}	b^{*+}	b^{+}	seq	y'^{++}	y^{*++}	y^{++}	y'^{+}	y^{*+}	y^{+}	#
1	49.52	--	58.52	98.02	--	116.03	D	508.22	508.71	517.22	1015.42	1016.41	1033.43	M
2	85.03	--	94.04	169.06	--	187.07	A	450.70	451.19	459.71	900.40	901.38	918.41	8
3	149.56	--	158.56	298.10	--	316.11	E	415.18	415.68	424.19	829.36	830.34	847.37	7
4	223.09	--	232.09	445.17	--	463.18	F	350.66	351.15	359.67	700.32	701.30	718.33	6
5	301.14	301.63	310.15	601.27	602.26	619.28	R	277.13	277.62	286.13	553.25	554.23	571.26	5
6	369.67	370.16	378.67	738.33	739.32	756.34	H	199.08	--	208.08	397.15	--	415.16	4
7	427.18	427.68	436.19	853.36	854.34	871.37	D	130.55	--	139.55	260.09	--	278.10	3
8	470.70	471.19	479.70	940.39	941.37	958.40	S	73.03	--	82.04	145.06	--	163.07	2
	--	--	--	--	--	--	G	29.52	--	38.52	58.03	--	76.04	1

Spectral Info:

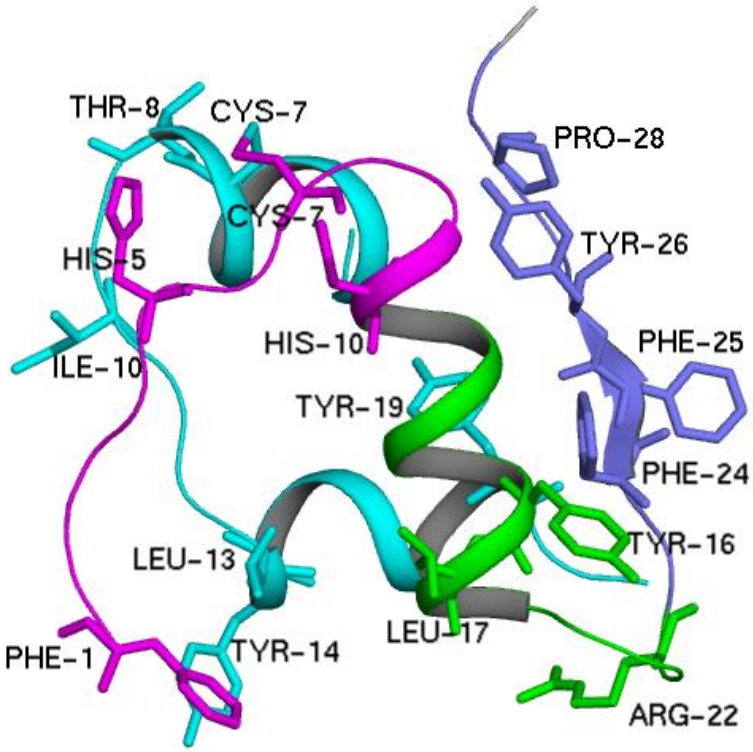
Scan#	t_R(min)	t_R(Pred)	Conf. t_R	Peak Area
4248	21.13	22.76	56.81%	N/A

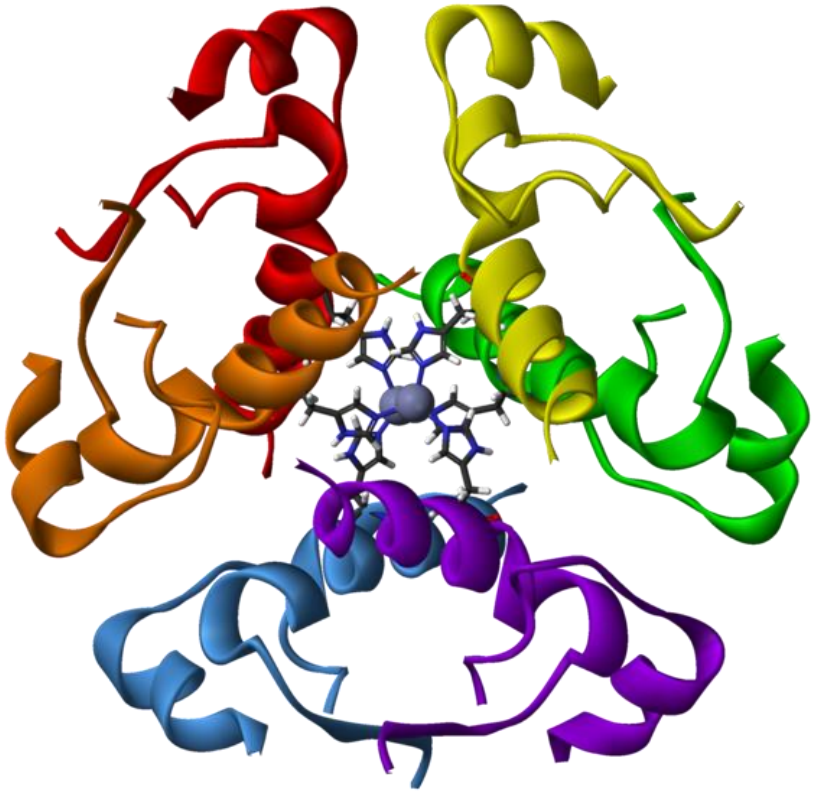
ProtMapMS: software for automation

- Removing biggest bottlenecks of data analysis and interpretation, manual identification and integration
- Identification and verification of all peptide species
- Quantification of peptide oxidation versus exposure time
- Specific, custom built for protein footprinting
- Testbed: insulin monomer/hexamer comparison

Insulin

Chain A – GIVEQCCTSICSLYQLENYCN
Chain B – FVNQHLCGSHLVEALYLVCGERGFFYTPKT





ProtMapMS
GUI

ProtMapMS

Settings

Input Files

1 Spectrum File(s) Browse...

2 FASTA Sequence File Browse...

Settings

3 Retention Time (Minutes) 10 to 30

4 Peptide Mass Range (Daltons) 500 to 3000

5 Protease Trypsin

6 Missed Cleavages 1

7 Charge State 2 to 3

8 MS1 Error Tolerance (ppm) 8

9 MS2 Error Tolerance (Da) .25

10 m/z width for SIC integration (ppm) 8

Modifications

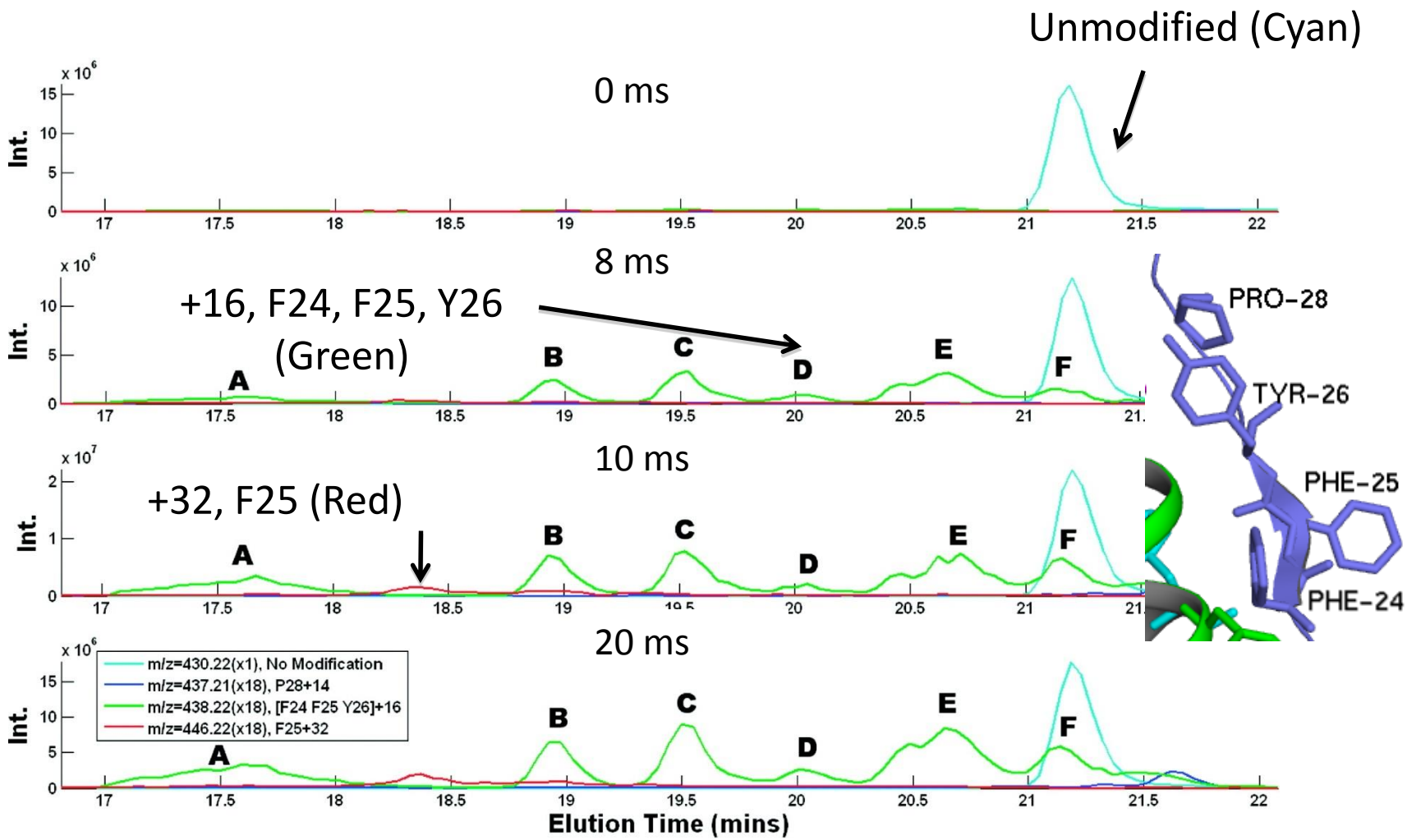
11

Residue Name(s)/Number	Mass (Da)	Modification	Notes
WMYFH	15.994915	Oxidative, Hydroxy	
PK	13.9793	Oxidative, Carbonyl	
MWYFC	31.98983	Oxidative, Double oxid	
C	57.02146	fixed	
M	-31.9898	Oxidative, aldehyde	
C	-15.9772	Oxidative, Hydroxy	
C	47.9847	Oxidative, Sulfonic Aci	
H	-22.031968	Oxidative	
H	-10.031968	Oxidative	
H	4.9789	Oxidative	
R	-43.053432	Oxidative	

12 Maximum oxidative mods per peptide 1

Analyze Data Cancel

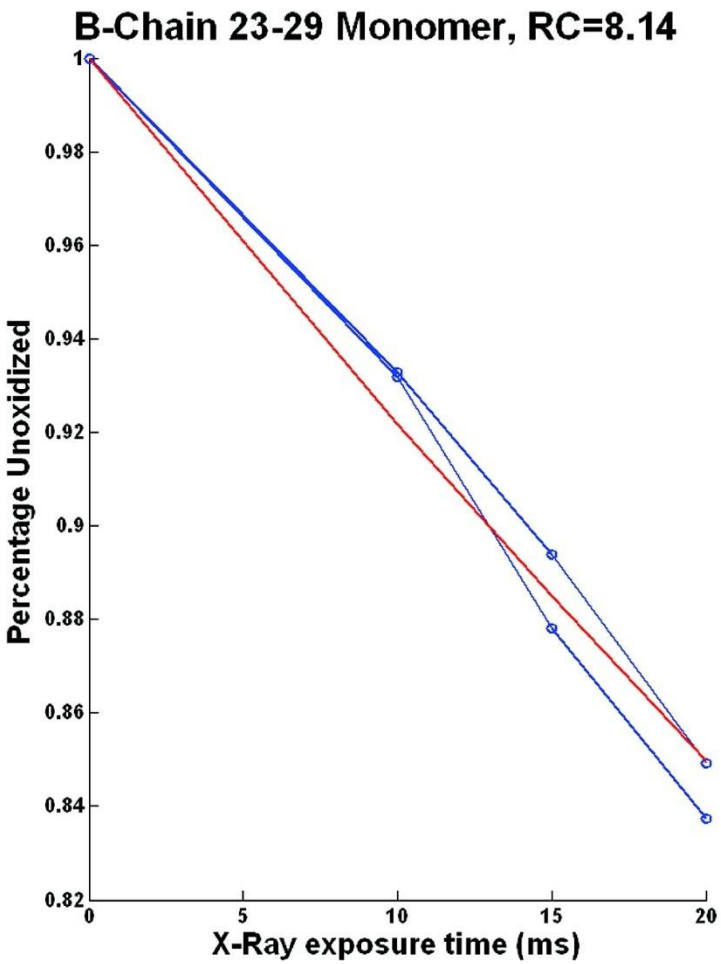
EXTRACTED ION CHROMATOGRAM: INSULIN PEPTIDE 23-29

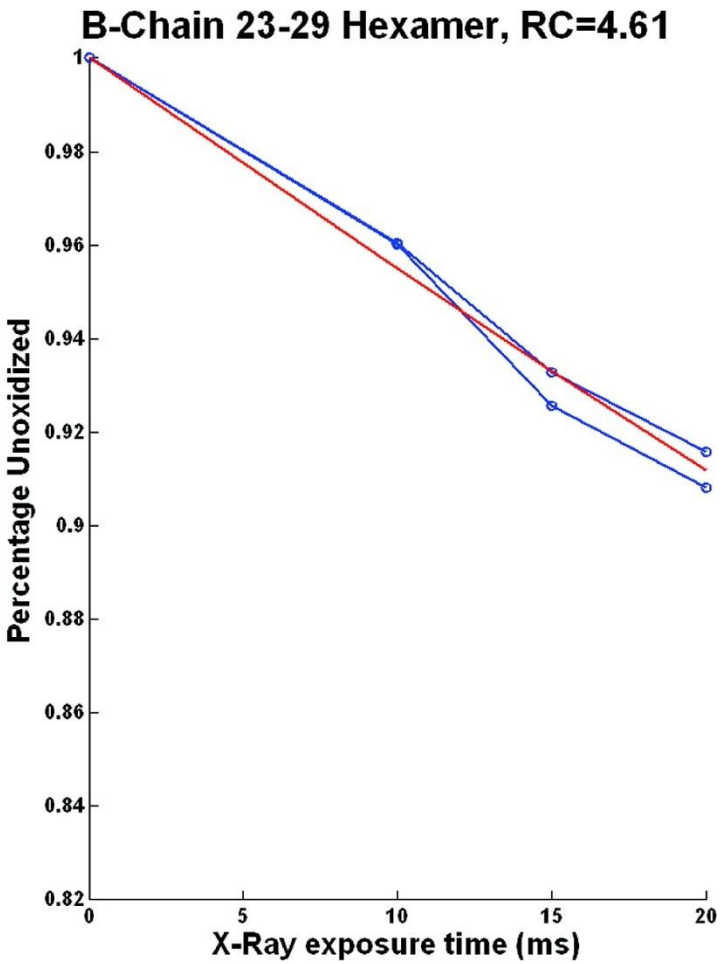


+14, P28 (Blue)

Kiselar et al., J. Biol. Chem. 2011

DR PLOT OF 23-29 INSULIN PEPTIDE





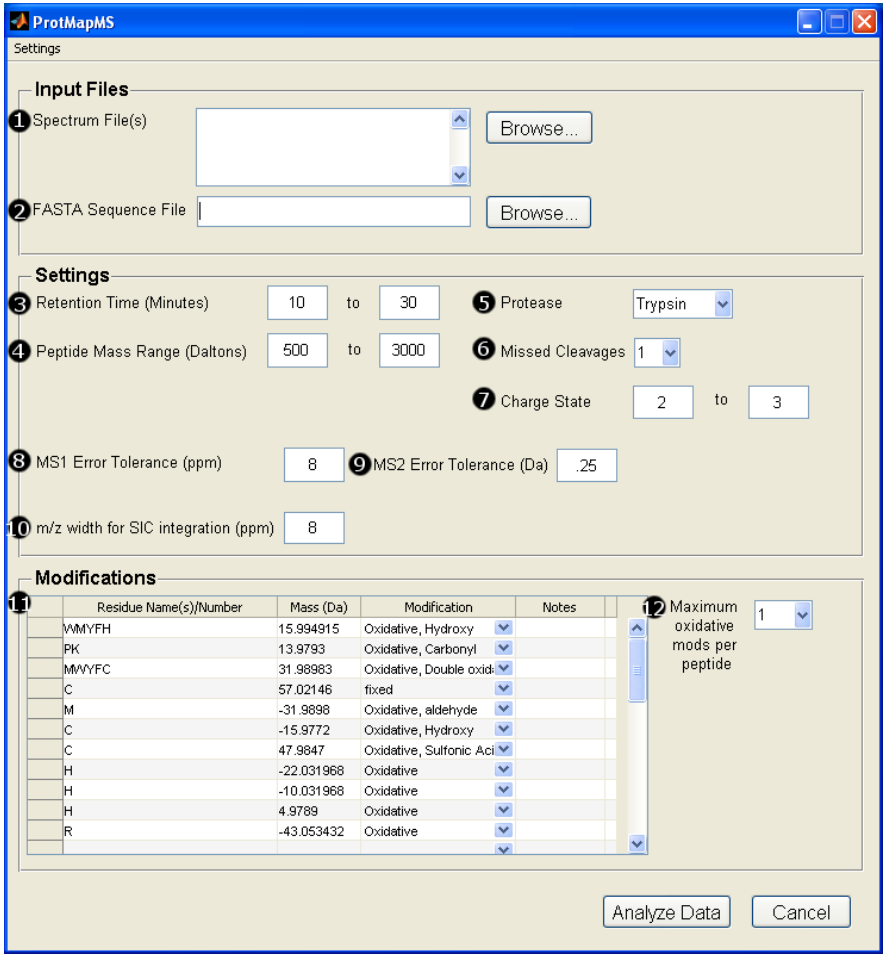
DATA SUMMARY

Peptide Sequence	Insulin Monomer				Insulin Hexamer			
	Oxid. Res.	SA (Å)	Rate Const (s^{-1}) (Manual)	Rate Const (s^{-1}) (ProtMap)	Oxid. Res.	SA (Å)	Rate Const (s^{-1}) (Manual)	Rate Const (s^{-1}) (ProtMap)
FVNQHLCGSH (1-10, B-Chain)	F1	123.6	1.41±0.14	1.36±0.16	F1	11.1	0.40±0.02	0.46±0.06
	H5	91.6	14.89±1.42	13.07±0.92	H5	92.0	7.64±0.44	6.67±0.41
	C7	44.5			C7	49.7		
	H10	119.3			H10	54.0		
LVEALYLVCGER (11-22, B-Chain)	Y16	137.3	8.59±0.57	5.64±0.55	Y16	37.1	1.81±0.11	1.53±0.24
	L17	117.2			L17	9.4		
	R22	124.1			R22	104.9		
GFFYTPK (23-29, B-Chain)	F24	30.9	9.33±0.12	8.14±0.3	F24	0.0	5.11±0.11	4.61±0.16
	F25	140.1			F25	55.8		
	Y26	66.1			Y26	9.2		
	P28	52.1			P28	15.9		

ProtMapMS: automated structural MS analysis

1. Inputs and converts mass spectrometry data
2. Using exact mass of peptides (ppm), plus known modification chemistry.
3. Extracts intensities to generate rate of modification for specific peptides; identifies sites of modification.
4. Completes task in hours, currently licensed to multiple clients (www.neoproteomics.net).

ProtMapMS 1.0 GUI



Kaur et al., *Anal. Chem*, 2009

REFERENCES

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