

PRACTICAL CONSIDERATIONS FOR FOOTPRINTING EXPERIMENTAL SETUP

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STRUCTURAL MASS SPECTROMETRY
OCTOBER 8, 2014

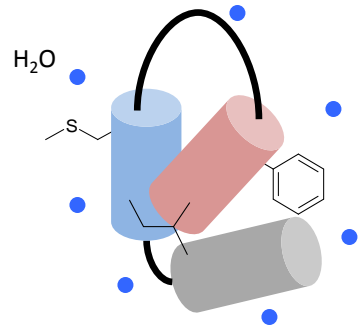
Sources: Mass Spectrometry Analysis of Protein-Protein Interactions and
Dynamics, Chance, *Wiley*, New Jersey;

Takamoto & Chance, Ann. Rev. Biophysics and Biophysical Chem. 2006

OUTLINE

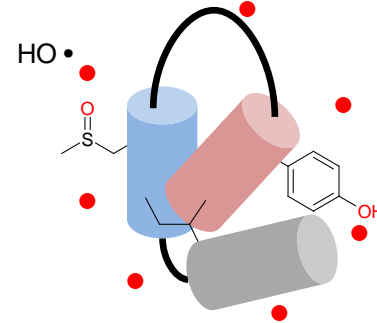
- Footprinting strategy
- Protein preparation
- Mass spectrometry detection and quantification of products
- Data analysis

FOOTPRINTING STRATEGY

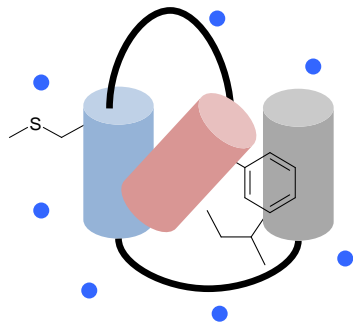


Protein
Conformation A

X-ray
→
0-20ms

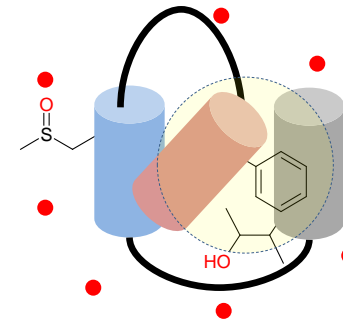


Exposed



Protein + Ligand/Protein
Conformation B

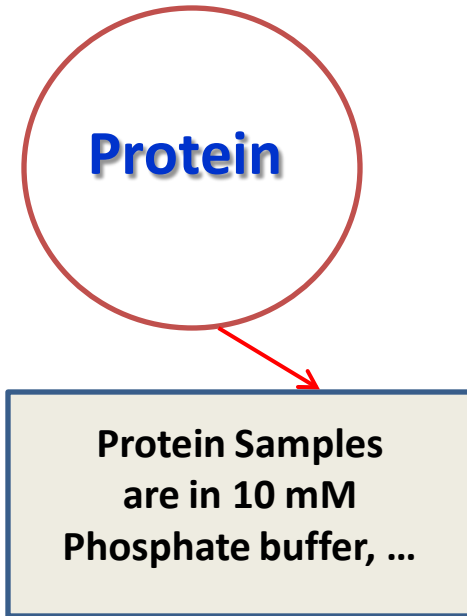
X-ray
→
0-20ms



Exposed

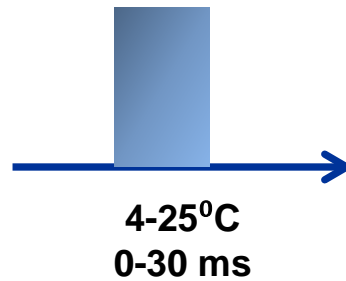
EXPERIMENTAL WORKFLOW

SAMPLE PREPARATION

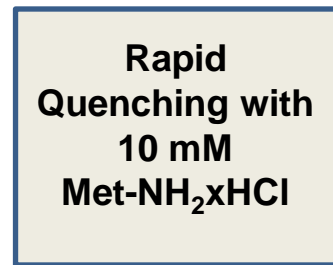


EXPOSURE

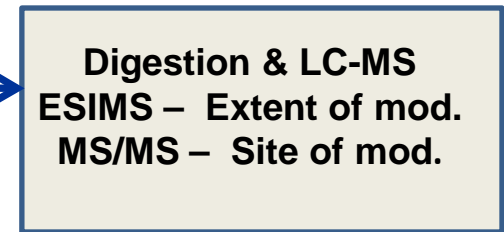
X-RAY



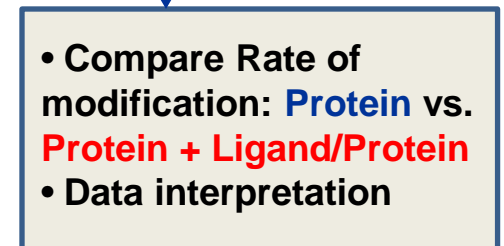
QUENCHING



DIGESTION & LC-MS



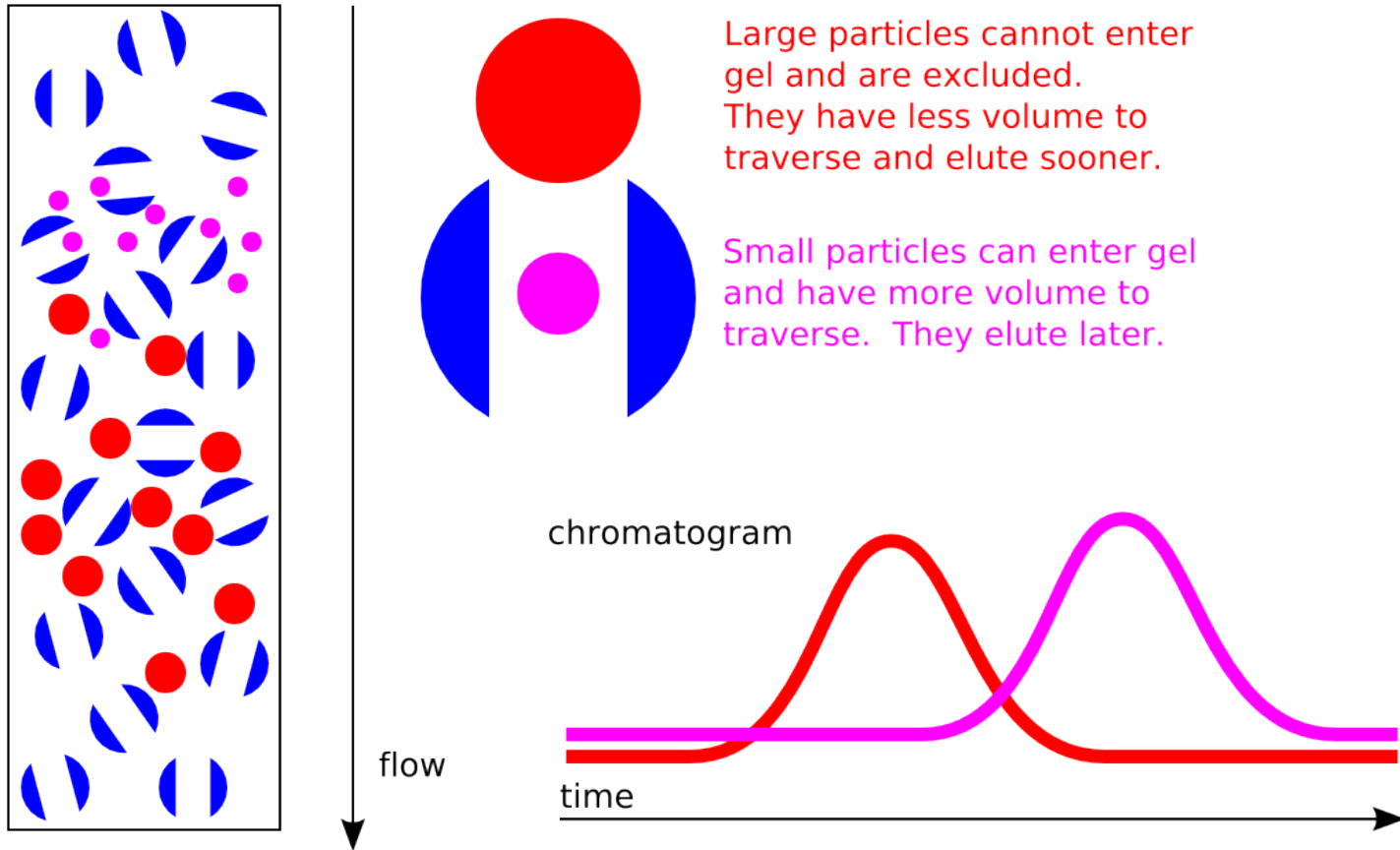
DATA ANALYSIS



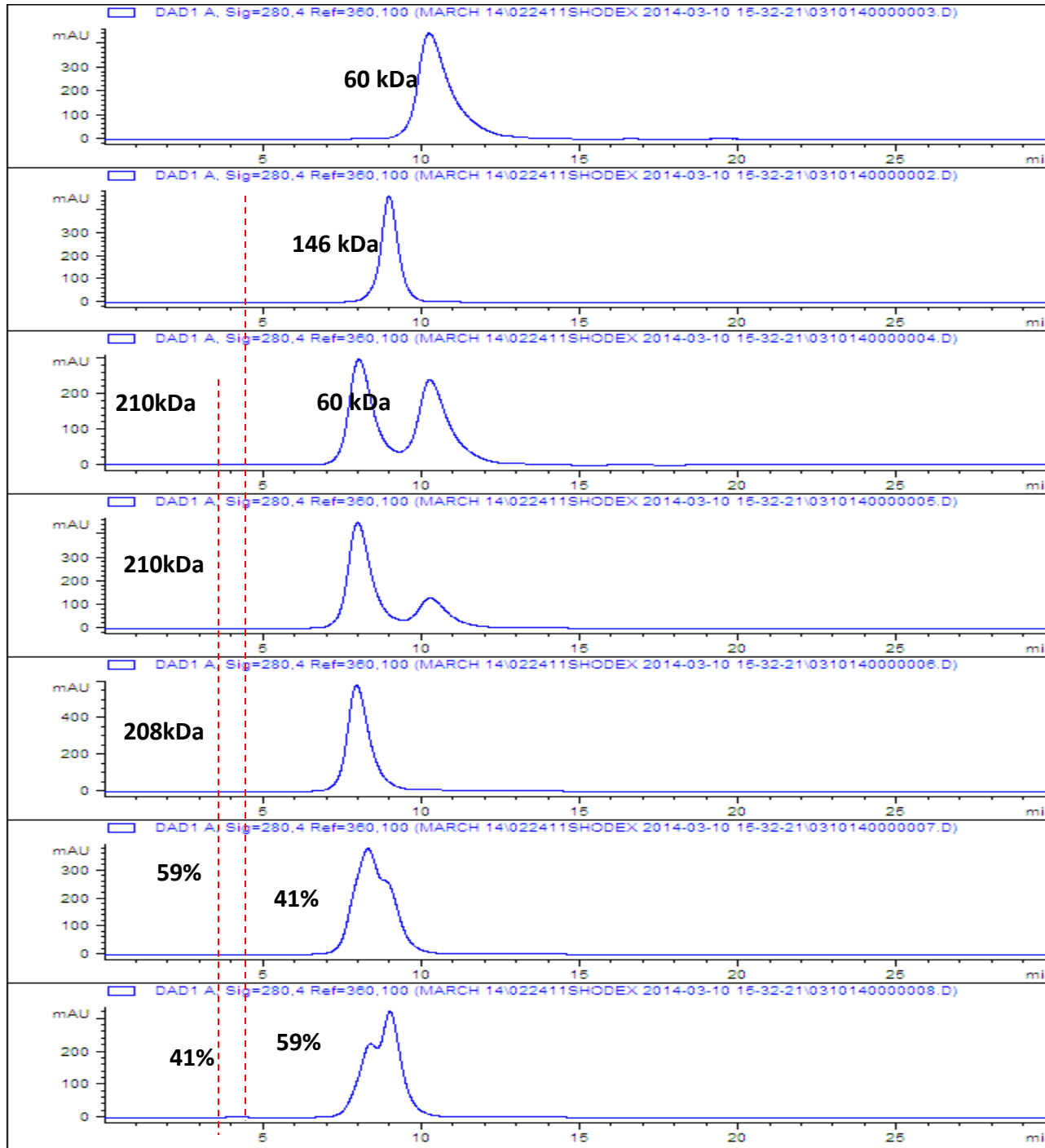
CONDITIONS TO BE OPTIMIZED PRIOR TO EXPOSURE

- Protein concentration at 1-10 μM ;
- To determine protein-protein complex ratio (size exclusion chromatography);
- To ensure that no protein aggregation (size exclusion chromatography);
- To determine optimal radical dose (Alexa experiments)
- To determine quenching effects of the protein/buffer (Alexa experiments);

HOW TO DETERMINE COMPLEX RATIO ?



Size-exclusion chromatography (SEC)



P1

P2

P1:P2
4:1

P1:P2
2:1

P1:P2
1:1

P1:P2
1:2

P1:P2
1:4

QUENCHING OF RADICAL DOSAGE BY WEIGH OF THE PROTEIN

Example: in 5 μ l

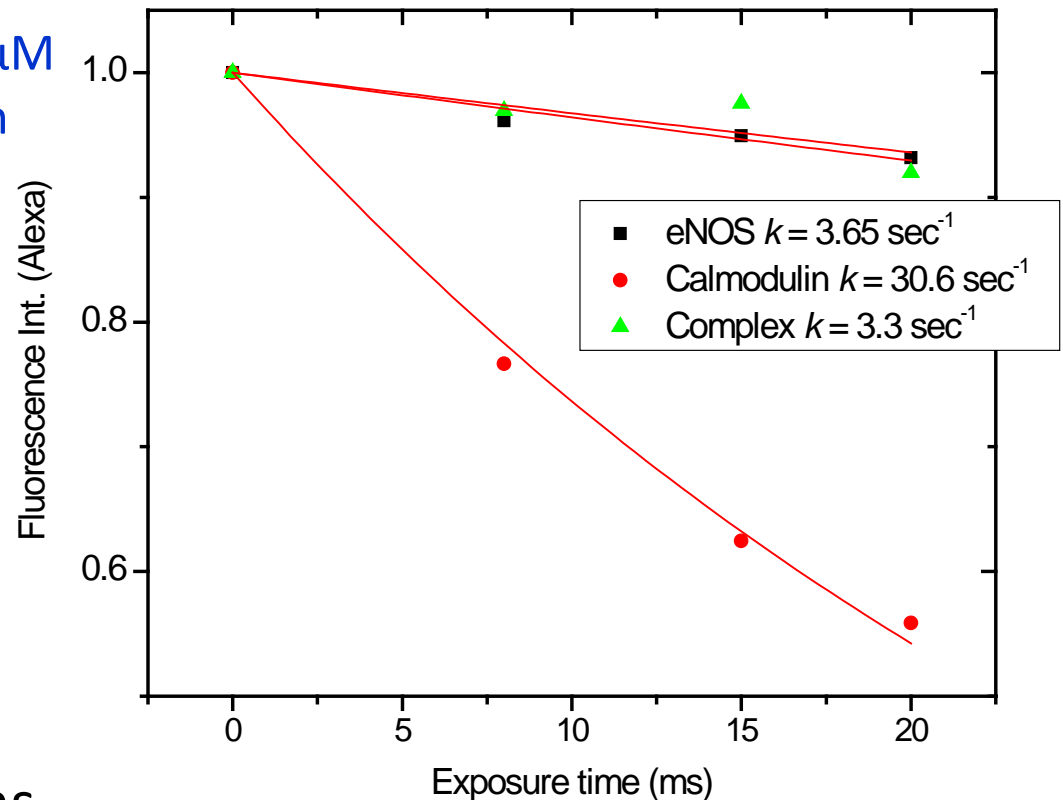
eNOS:CAM complex 6.5 μ M:6.5 μ M
Free eNOS and CAM: 6.5 μ M each

eNOS : Xg= 135KDa x 32.5 pmol

CAM: Xg= 16KDa x 32.5 pmol

Solution:

- Normalize data for CAM by 8.4 times
- Normalize beam conditions



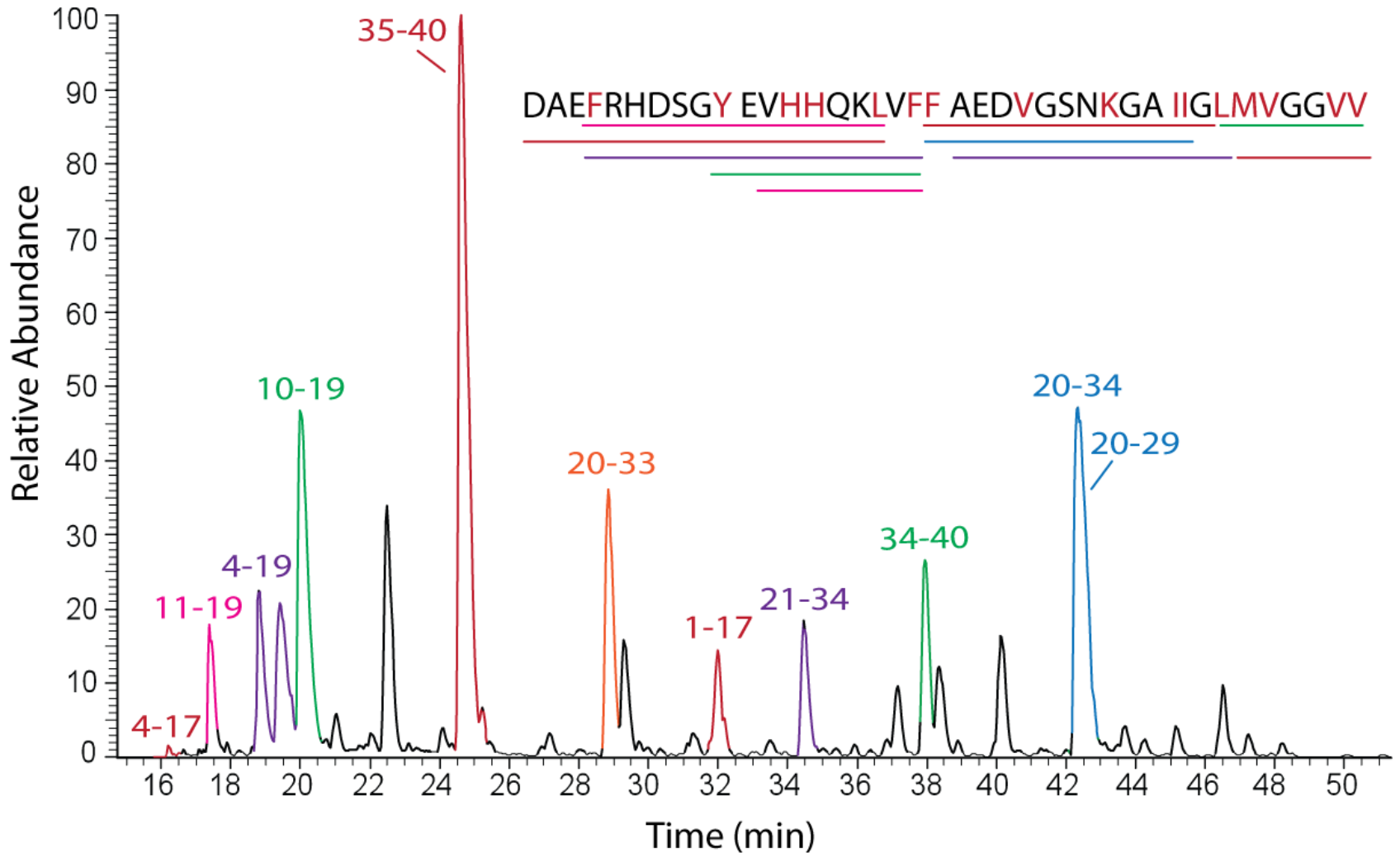
DIGESTION



- **Pepsin**
- best single amino acid resolution
- 3-6 h digestion at 37°C - best sequence coverage
- 5-15 amino acid peptide length
- many peptides have partially overlapping sequence
- pH 2-4; 1:10 (w/w) E:P ratio

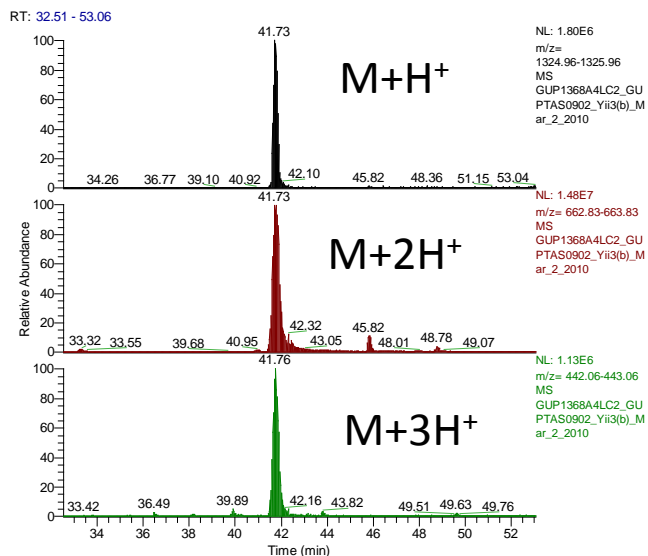
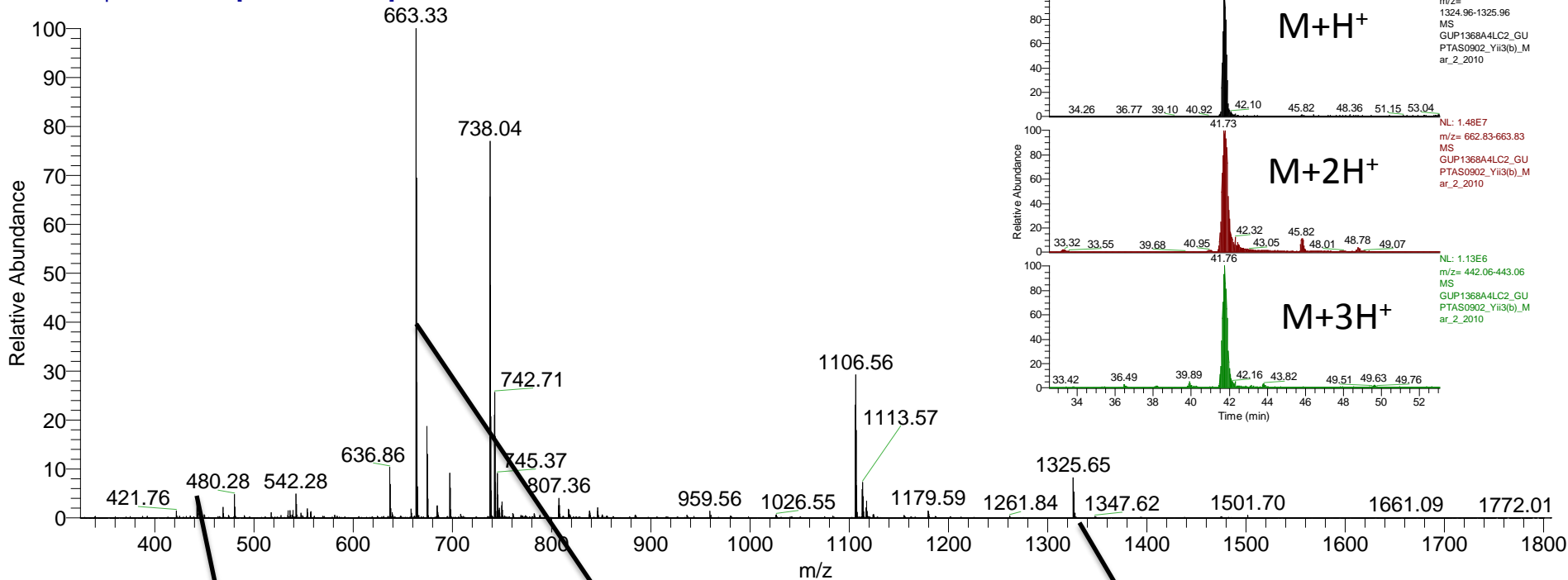
- **Trypsin**
- **Asp-N**
- **Glu-C**
- best enzyme specificity
- 12-18 h digestion at 37°C
- 8-25 amino acid peptide length
- pH 6-8; 1:40 (w/w) E:P ratio

PEPSIN DIGEST CHROMATOGRAM OF SAMPLE

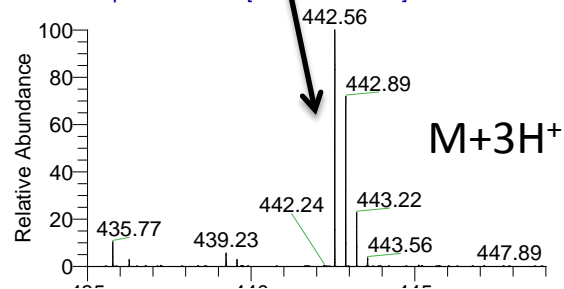


MULTIPLE CHARGE STATES

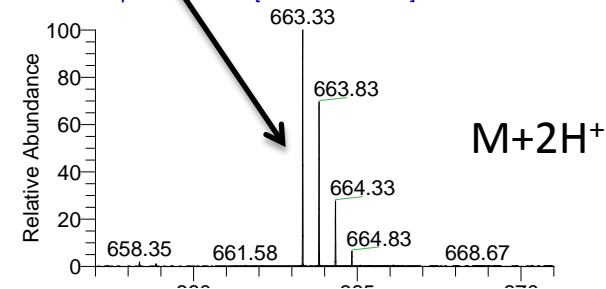
GUP1368A4LC2_GUPTAS0902_Yii3(b)_Mar_2_2010 #6898-7000 RT: 41.61-42.13 AV: 18 NL: 8.38E6
 T: FTMS + p NSI Full ms [325.00-1800.00]



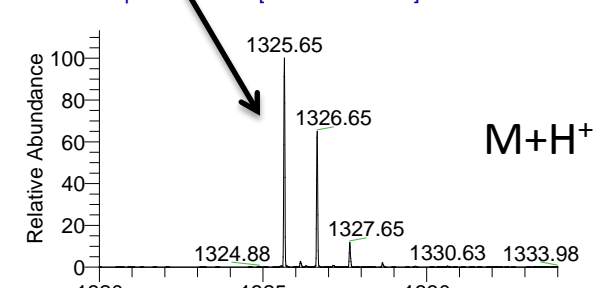
GUP1368A4LC2_GUPTAS0902_Yii3(b)_Mar_2_2010 #
 T: FTMS + p NSI Full ms [325.00-1800.00]



GUP1368A4LC2_GUPTAS0902_Yii3(b)_Mar_2_2010 #
 T: FTMS + p NSI Full ms [325.00-1800.00]

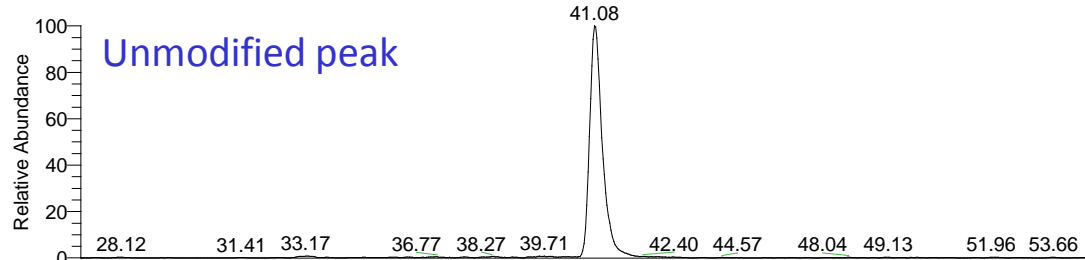


GUP1368A4LC2_GUPTAS0902_Yii3(b)_Mar_2_2010 #
 T: FTMS + p NSI Full ms [325.00-1800.00]



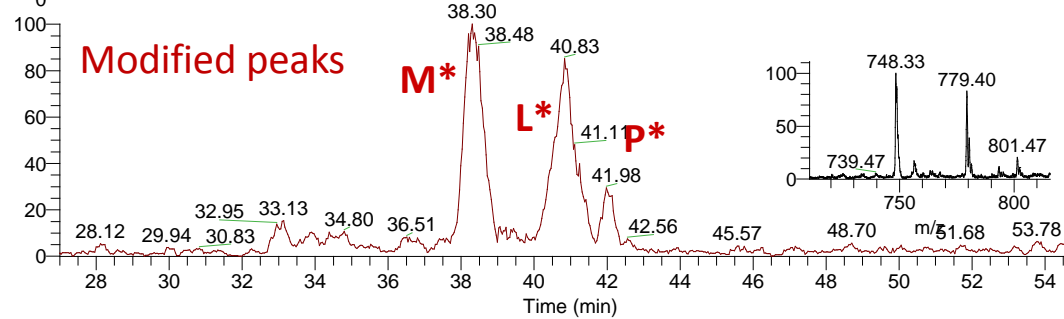
RT OF MODIFIED PRODUCTS

RT: 27.00 - 55.00 SM: 7B



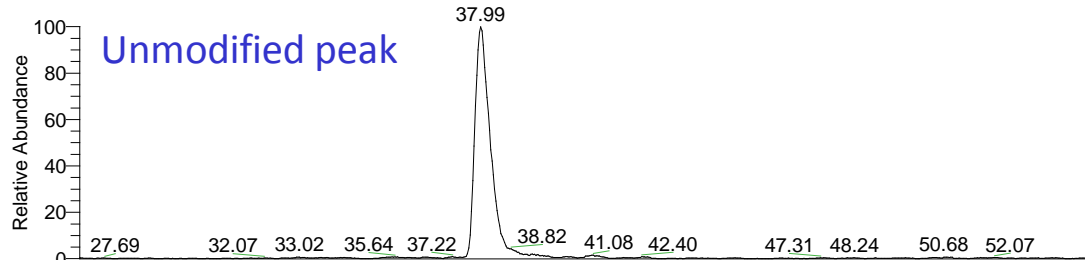
NL:
1.57E6
m/z=
747.90-
748.90 MS
Cytic_30

EETLM*EYL*ENP*K



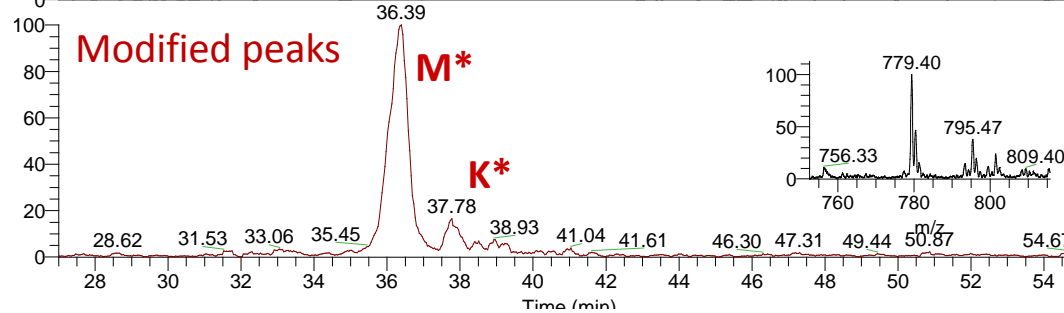
NL:
7.81E4
m/z=
755.90-
756.90 MS
Cytic_30

RT: 27.00 - 55.00 SM: 7B



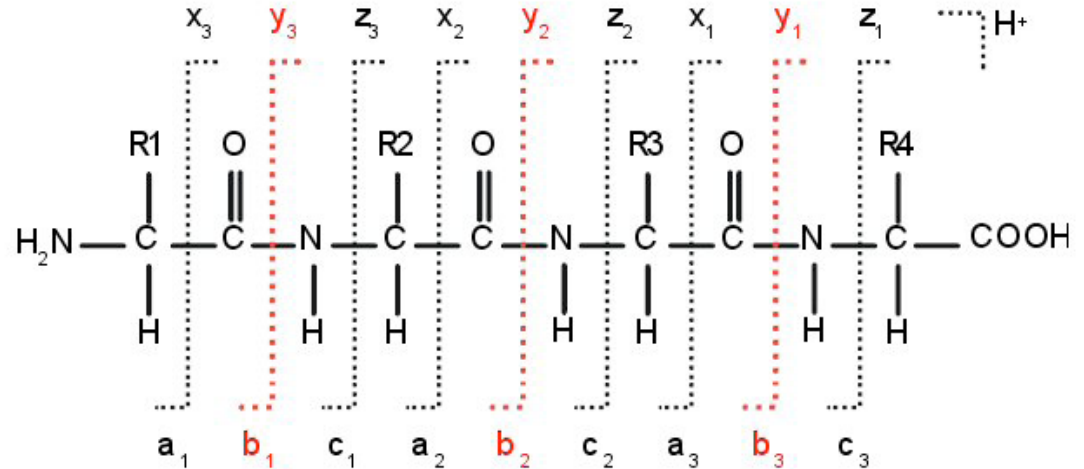
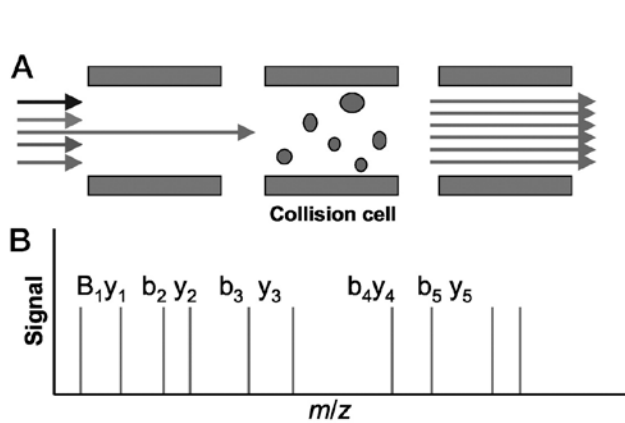
NL:
8.39E5
m/z=
778.90-
779.90 MS
Cytic_30

M*IFAGIK*

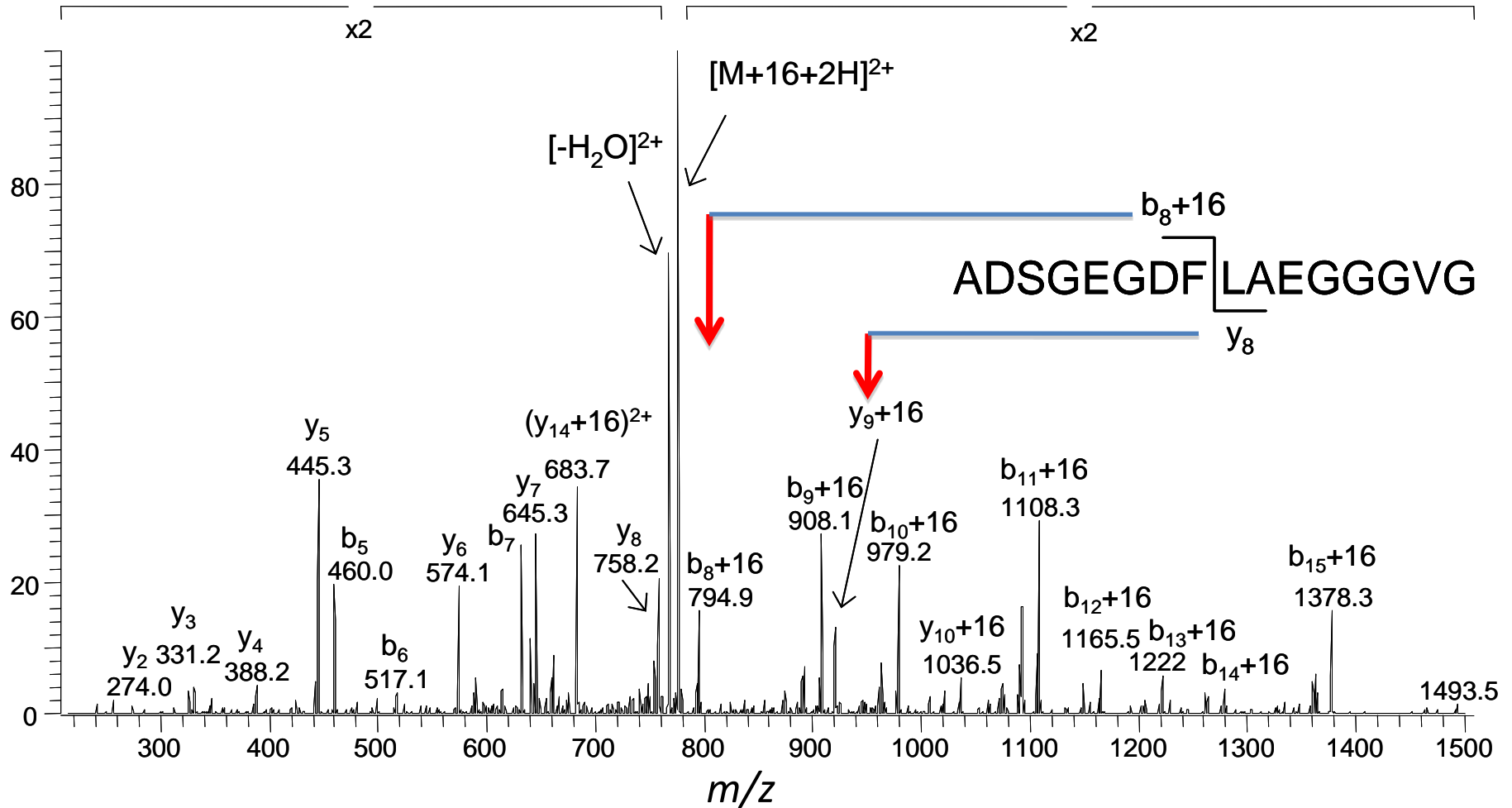


NL:
2.29E5
m/z=
794.90-
795.90 MS
Cytic_30

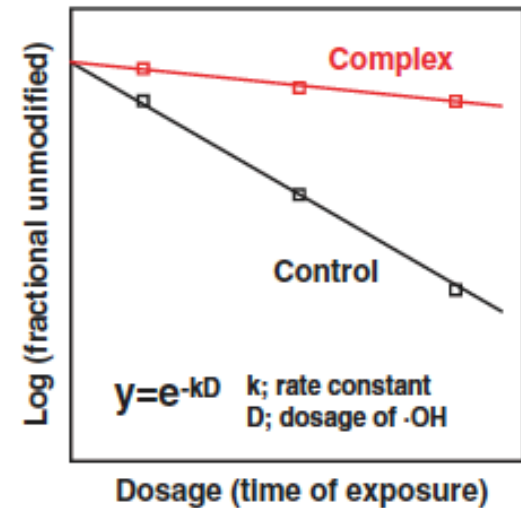
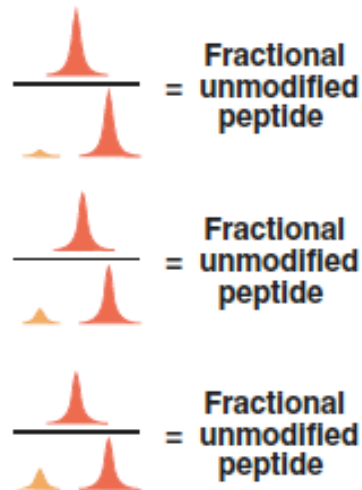
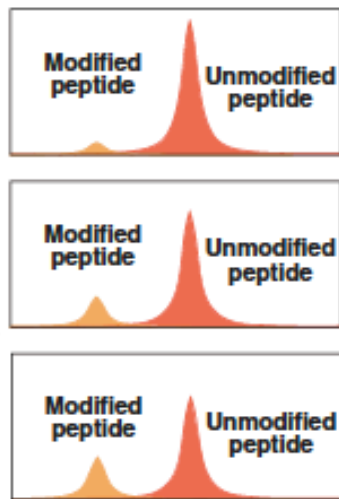
PEPTIDE FRAGMENT ION DESIGNATIONS AS PROPOSED BY ROEPSTROFF AND FOHLMAN



TANDEM MS EXAMPLE



DETERMINE RATES WITH $\cdot\text{OH}$ DOSING



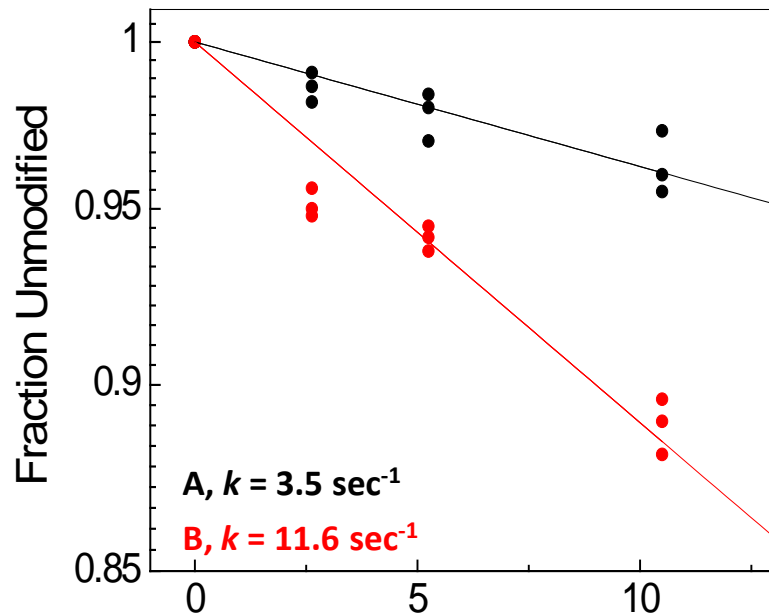
Fractional unmodified peptide determined from peak areas plotted versus increasing times of x-ray exposure

COMPARE DOSE RESPONSES: CONFORMATION A VS. B

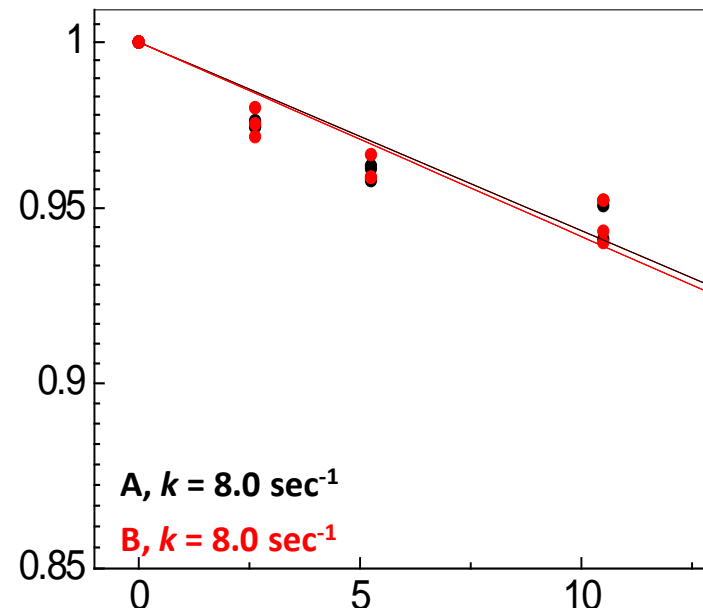
$$Y = Y_0 \exp^{-kt}$$

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

160-164 TWVM*A



333-348 ASTTVSKTETSQV*A*P*A*



Exposure Time (ms)

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

DATA ANALYSIS: SOFTWARE

- Manually – to calculate constant rates and to verify sites of modification
- MaxMatrix (http://www.massmatrix.net/mm-cgi/search_form.py) - 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, Safari 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)		
Choose Profile:	Most Recent Search (Default)	Save Form as Profile:	Don't Save
*Data files:	<input type="button" value="Browse..."/> No file selected. Janna_AB42_2_4mer_10ms_set1.mgf <input type="button" value="Delete"/>	Search data sets:	Individually
*Database:	Abeta42 <input type="button" value="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 HydroImidazolone 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
<input type="checkbox"/> Cross Link Search Options Click to expand			
<input type="checkbox"/> Quantitation Options Click to expand			
Comment:		Expert options:	
<input type="button" value="Search"/>		<input type="button" value="Reset"/>	

Fields 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** **AEDVGSNKG** **IIGLMVGGVV** **IA**

42

Sequence Coverage: **100%**

Sequence Tag Coverage: **97%**

Index	scan#	charge	score	pp	pp2	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)

REFERENCES

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