



Analysis of chemically modified proteins by FT-ICR MS



PETR NOVAK

EU FT-ICR MS – End User School 1 – UEF Chemistry |
August 19-24 2018 | Joensuu, Finland

Structural Mass Spectrometry...

Disulfide bonds mapping

Limited proteolysis

H/D exchange

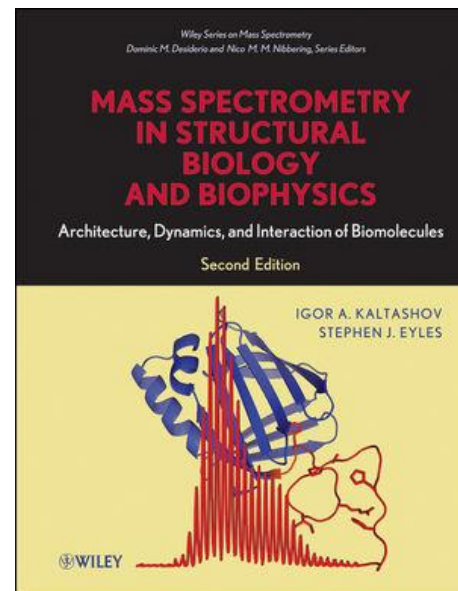
Protein covalent labeling

Phast photochemical oxidation of proteins

Chemical cross-linking

ETD/ECD fragmentation

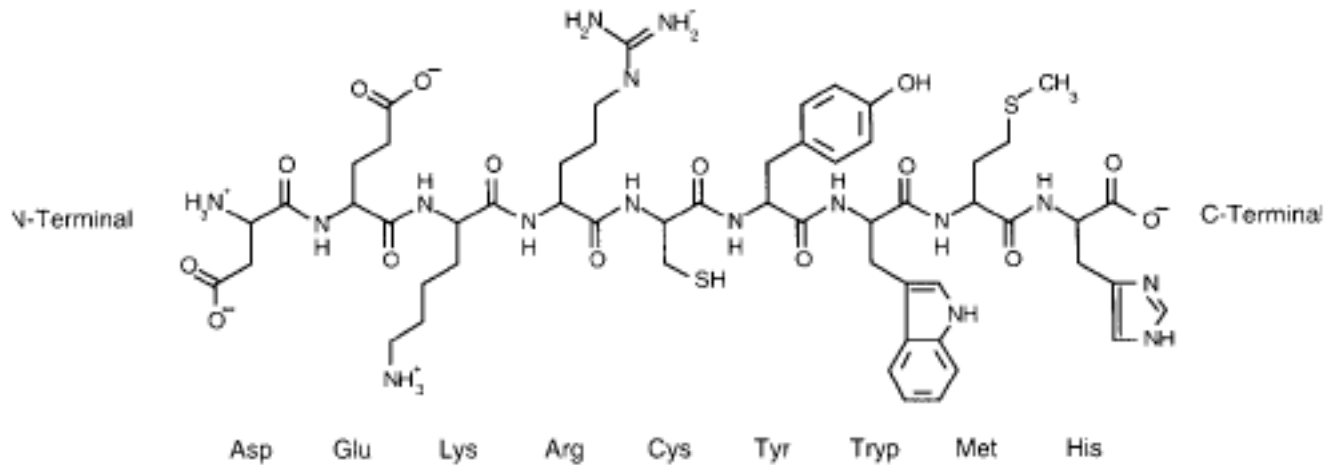
Native mass spectrometry and Ion mobility



Special Issue on Mass Spectrometry in Structural Biology (2015) Protein Science
24, 1173-1332

Protein covalent labeling and chemical cross-linking

Available amino acid sidechains for covalent modification



Carboxy groups – Asp, Glu, C-term, pK_a (3.8, 4.3, 2.3) $pH \geq 7$ » **deprotonation**

Amino groups – Lys, Arg, His, N-term, pK_a (9.4, 12, 6.8, 7.8) $7 \geq pH$ » **protonation**

Sulfhydryl groups - Cys . pK_a 8.9 $pH \geq 7$ » **-SH**

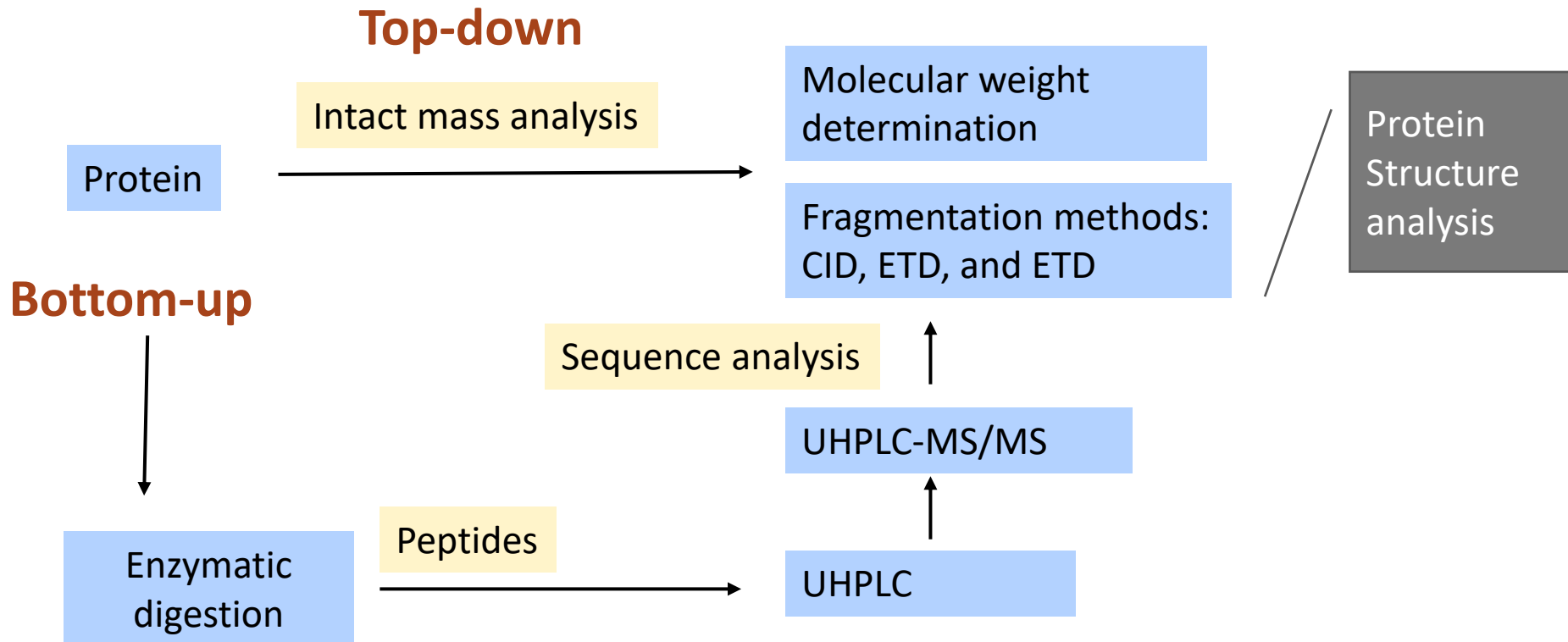
Aromatic groups– Trp (indol), Tyr (hydroxyphenyl, **pK_a 9.9**)

~ 23% of amino acid can be covalently modified

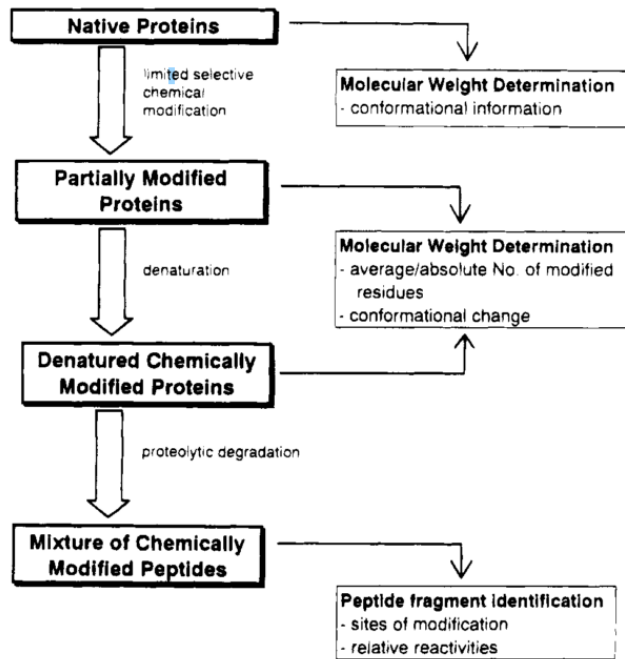
Klapper et. al. Biochem. Biophys. Res. Commun. 1977, 78, 1018.

Mass Spectrometry: Goal in Protein Structure Characterization

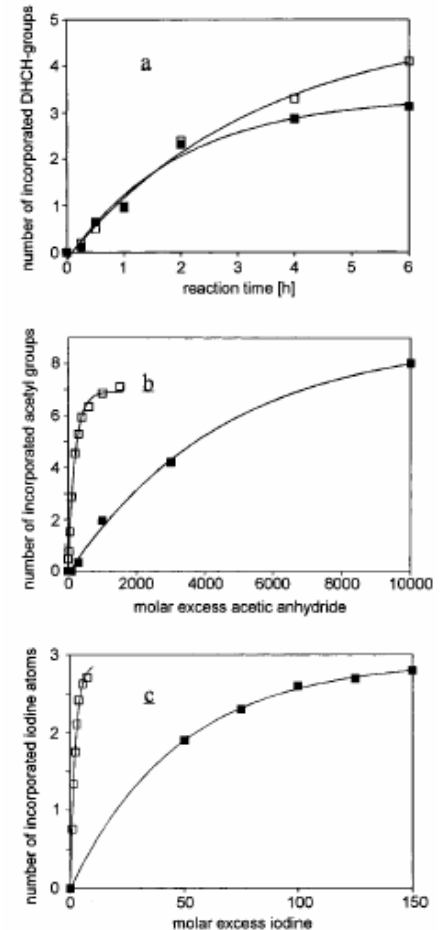
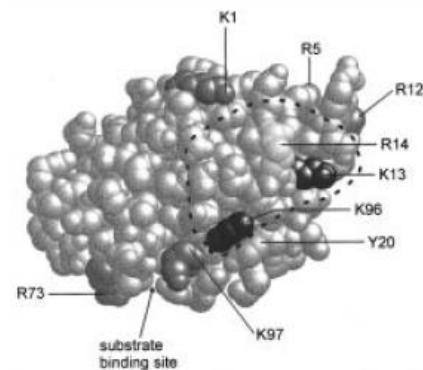
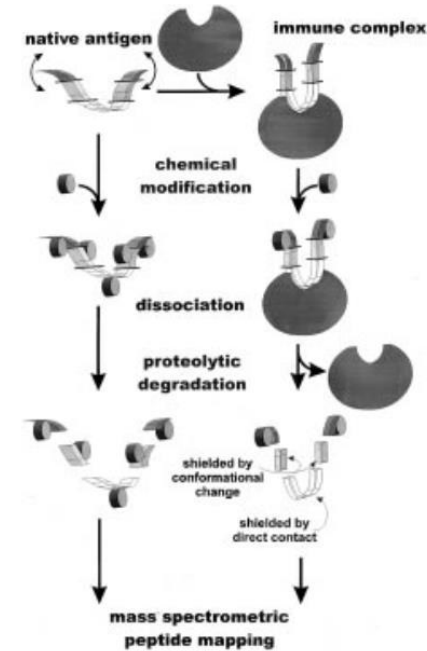
- Sensitivity
- Analysis of complex mixtures/high MW protein
- Rapid data acquisition



Protein covalent labeling and the FT-ICR bottom up experiments



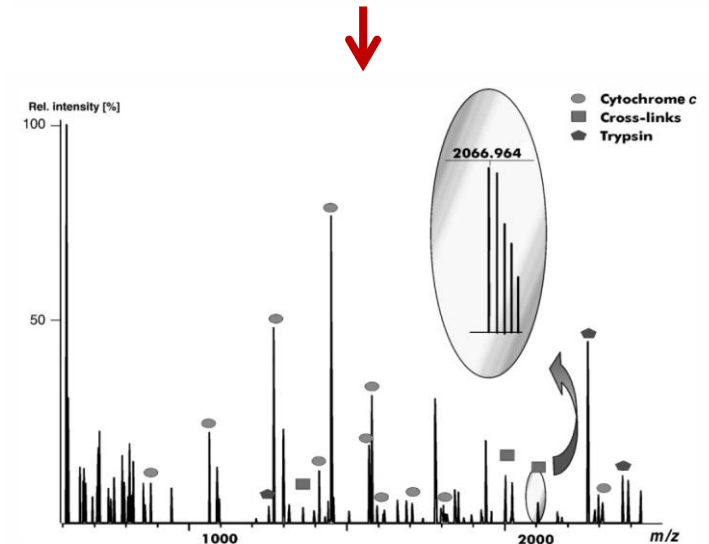
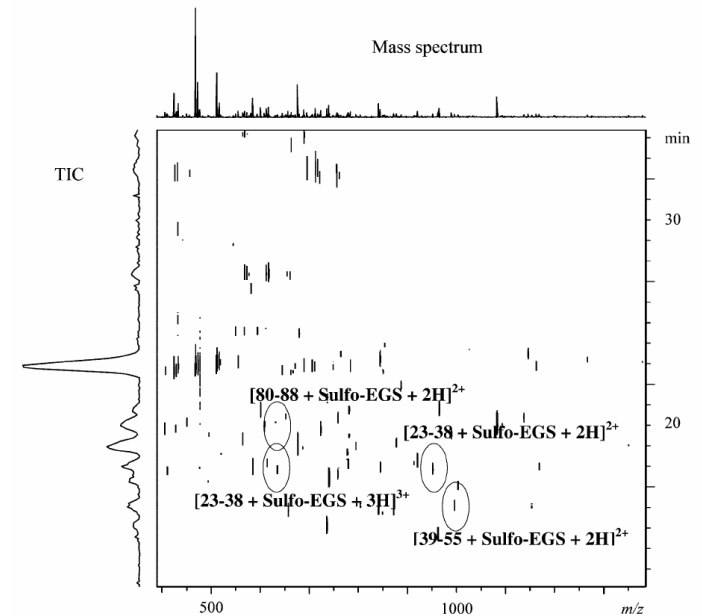
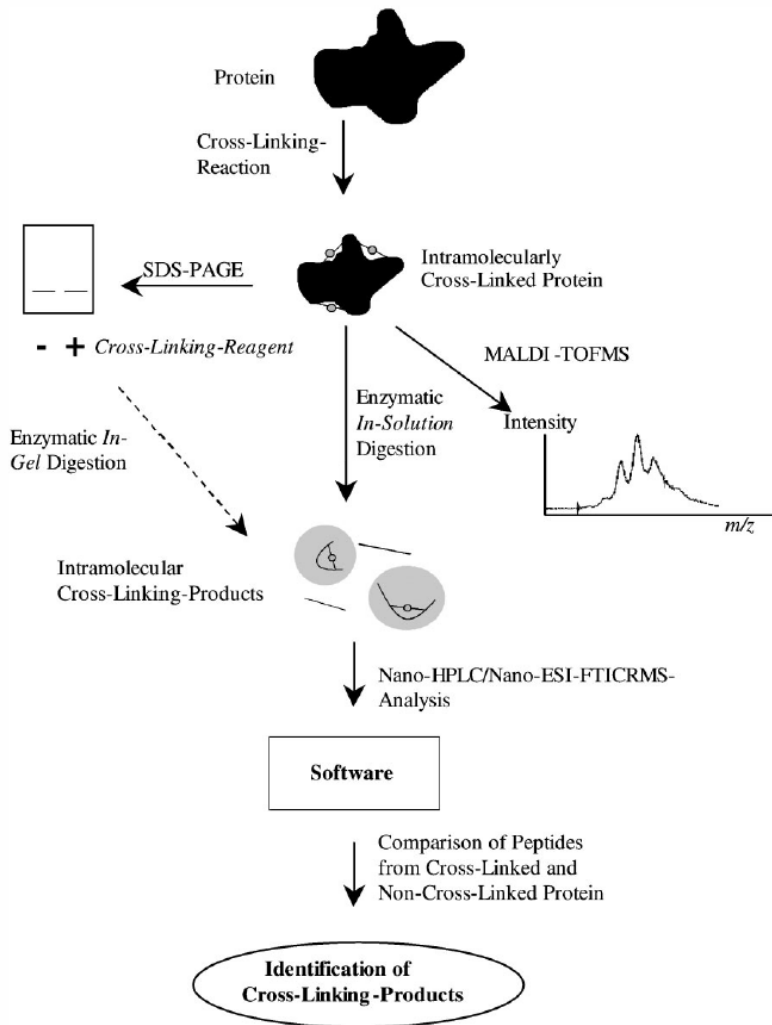
relative reactivity NH ₂ groups ^a	lysine residues, ε-amino groups		
	RNase A	HEL	myoglobin
	α-NH ₂		α-NH ₂
1	41, 104	97, 33 α-NH ₂	45, 63, 77, 79, 145, 147
2	1, 7, 37	1	16, 42, 87
3	31, 61, 91	13, 116	56, 50, 62, 78, 102
4	66, 98	96	96, 47, 87, 133, ^b 118 ^b



Suckau et. al. PNAS 1992, 89, 5630
and Glocker et. al. Bioconj. Chem. 1994, 5, 583

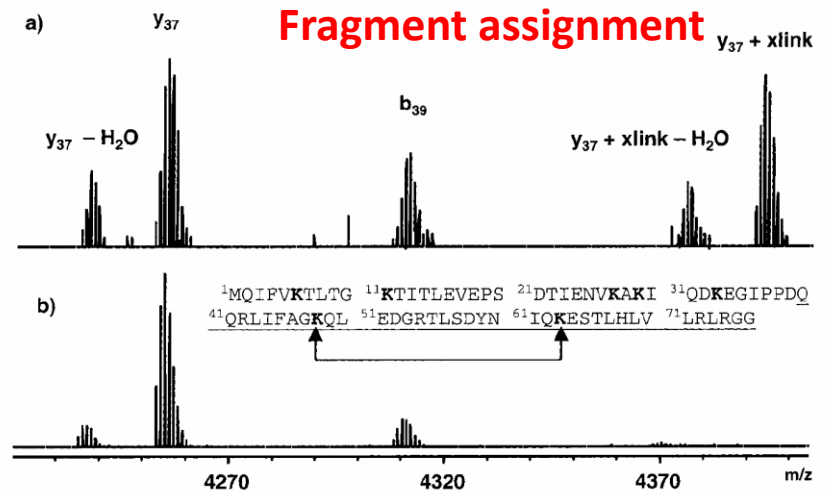
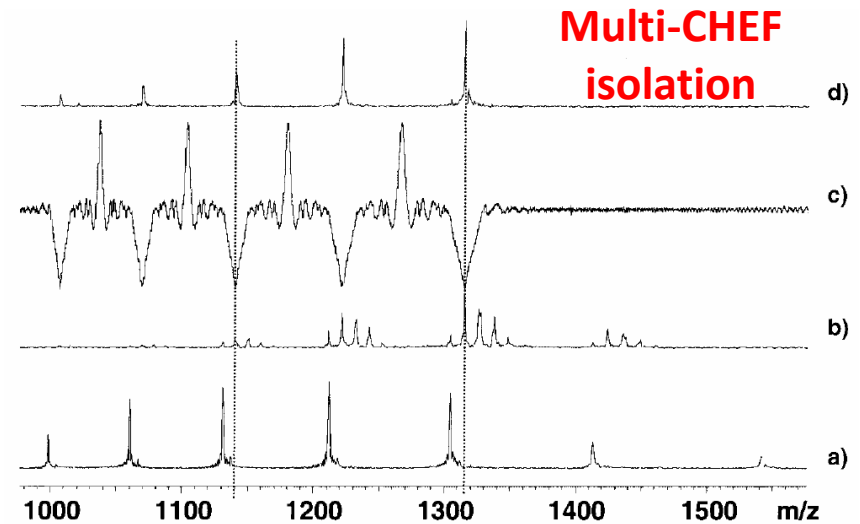
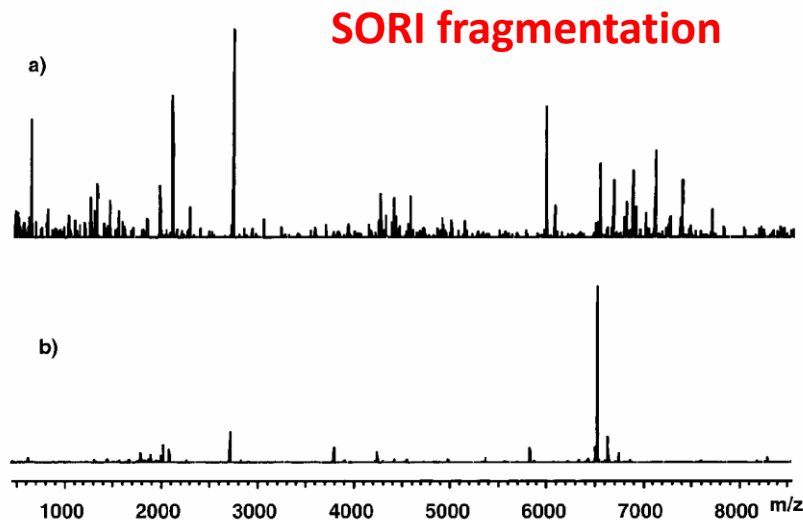
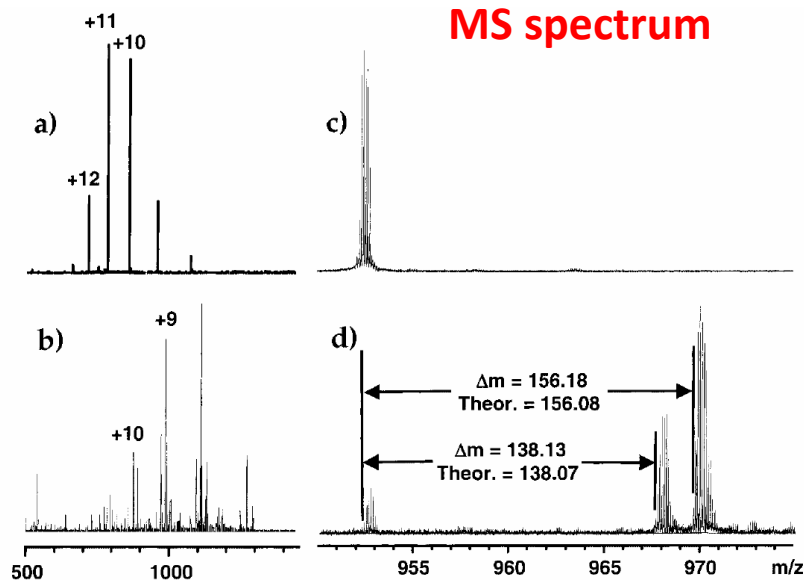
Fiedler et. al. Bioconj. Chem. 1998, 9, 236

Chemical cross-linking and the FT-ICR bottom up experiments



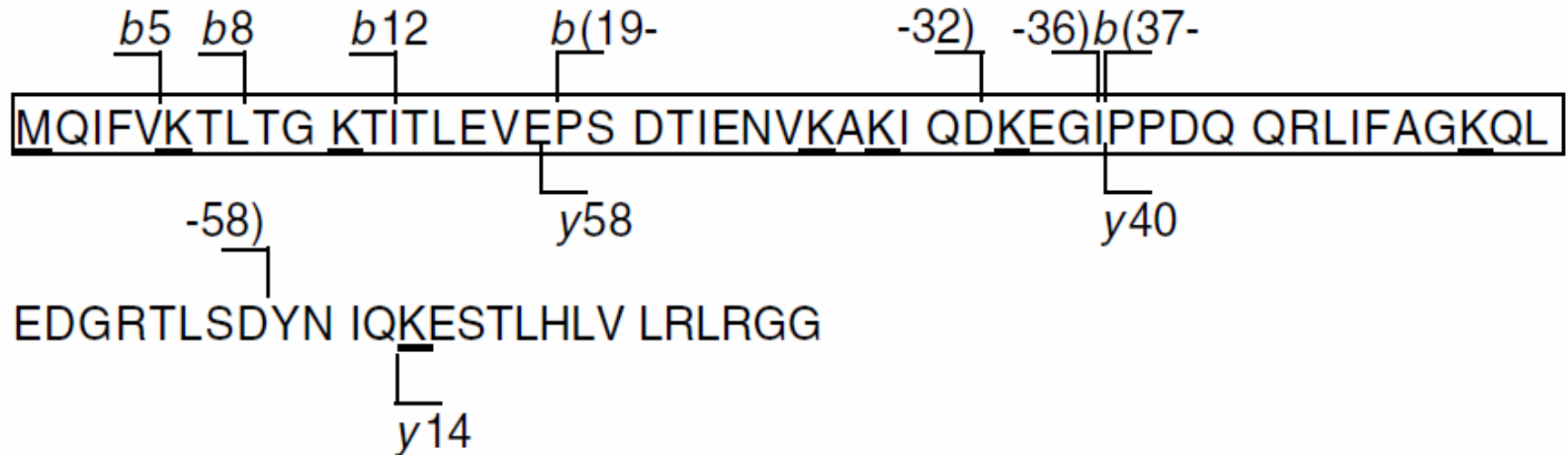
Dihazi et. al. Rapid. Commun. Mass Spectrom. 2003, 17, 2005

Chemical cross-linking and the FT-ICR Top down experiments

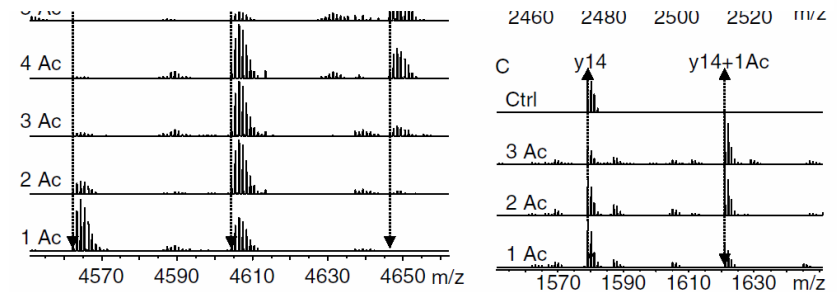
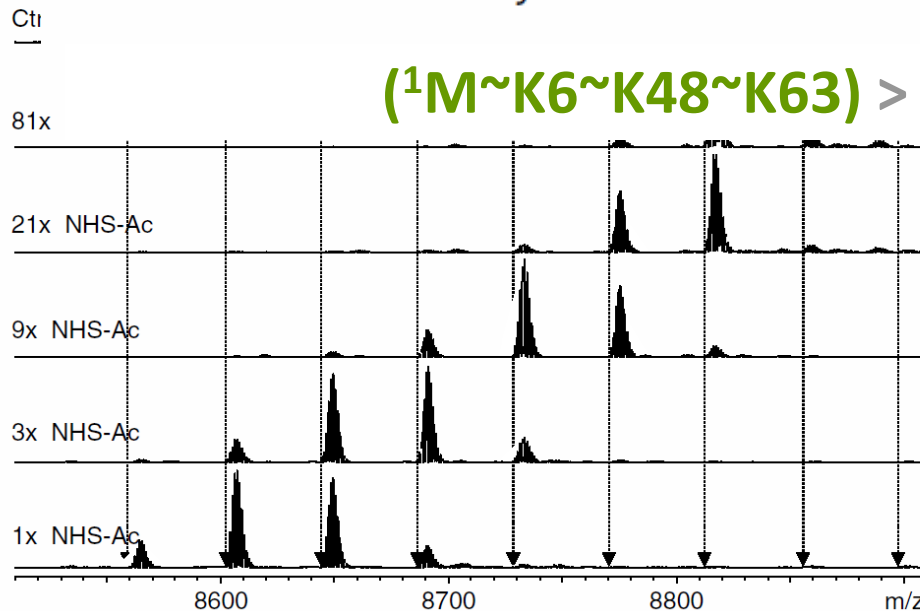


Kruppa et. al. *Rapid. Commun. Mass Spectrom.* 2003, 17, 155

Protein covalent labeling and the FT-ICR Top down experiments



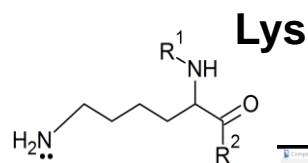
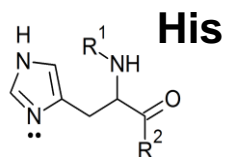
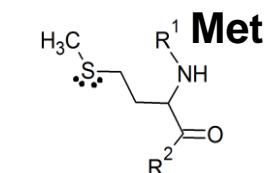
$(^1M \sim K6 \sim K48 \sim K63) > K33 > K11 > (K27, K29)$



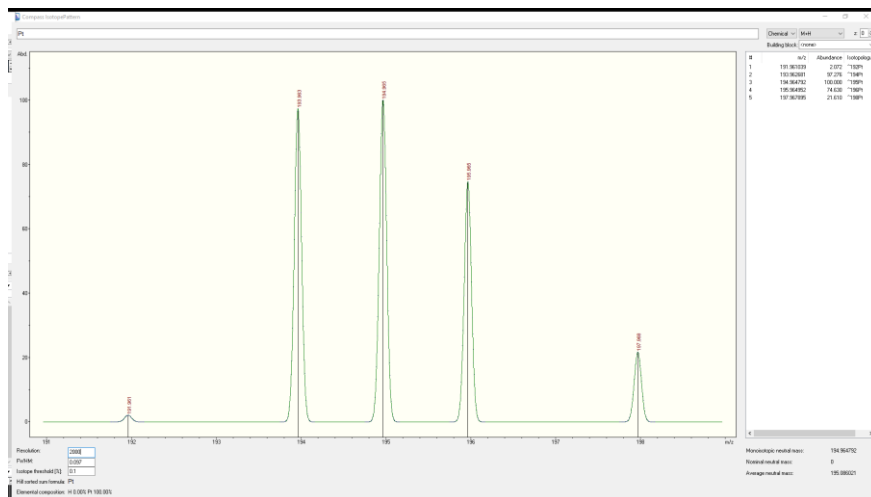
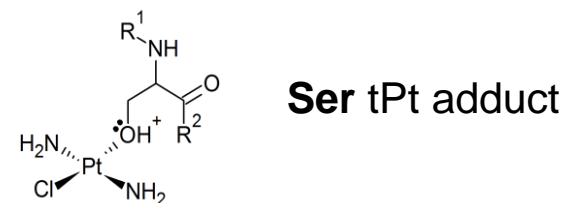
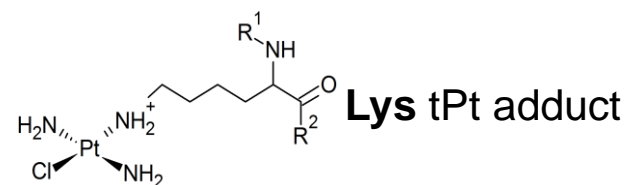
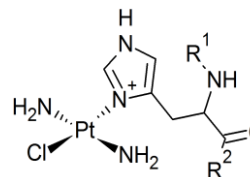
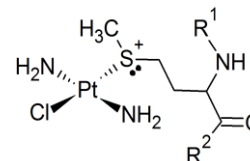
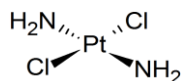
Novak et. al. J. Mass Spectrom. 2004, 39, 322

Protein labeling with noble metal

Possible amino acids susceptible to tPt modification



Transplatinum



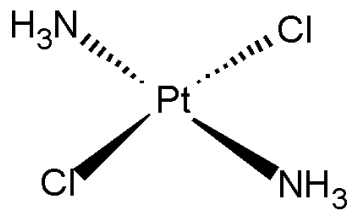
Platinum isotope pattern

Protein labeling – FT-ICR bottom up approach

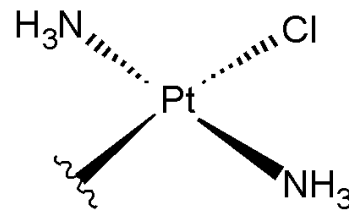
LABELING

DIGESTING

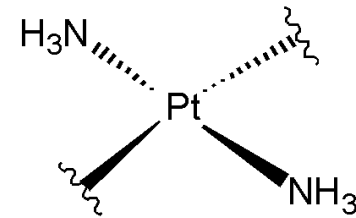
SEPARATION/ANALYSIS



Trans-platinum

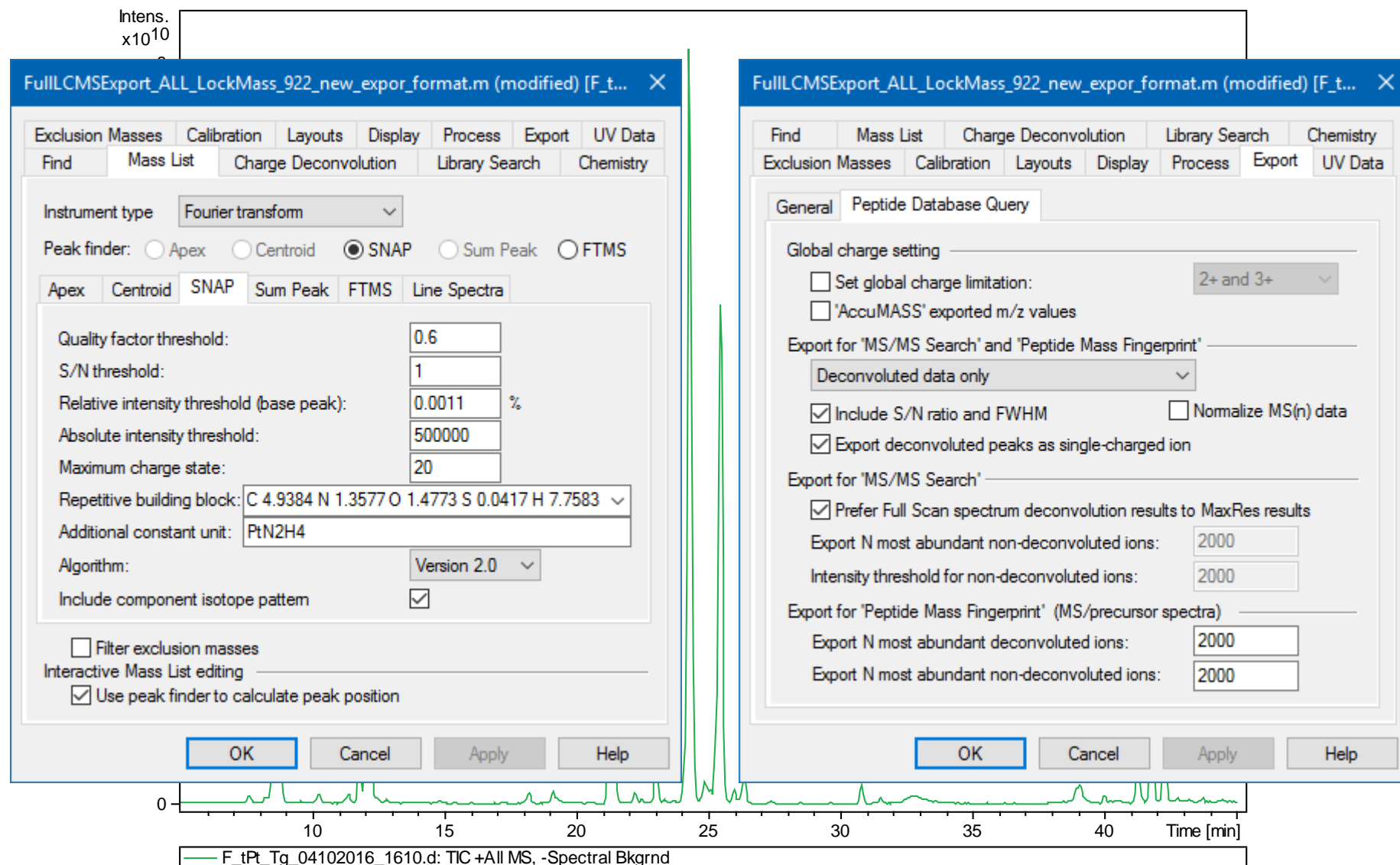


Trans-platinum
(monofunctional)



Trans-platinum
(bifunctional)

LC/FT-ICR MS and data processing



LinX algorithm offers seeing the light at the end of the tunnel.

<http://peterslab.org/downloads.php>

The screenshot displays the LinX algorithm web interface. At the top, there is a navigation bar with the following menu items: File, Properties, Help, Proteins, Proteases, Modifications, Cross-links, Measurement, Computing, and Results. The main content area is titled "Protein no. 1" and contains a form for protein input. The "Name" field is set to "FOXO4-DBD". The "Starting index" is set to "74" and "Use part from" is set to "202". To the right of these fields are buttons for "Load from file" and "Remove panel". Below the form, the protein sequence is displayed: GSHMLEDPGA VIGPRKGGSR RNAWGNQSYA ELISQAIESA PEKRLTLAQI YEMVRIYFY FKDKGDSNSS AGWKNISIRHN LSLHSKTIKV HNEATGKSSW WMLNPEGGKS GKAPRRRAAS MDSSSKLRG RSKA. At the bottom of the interface, there are buttons for "Clean form", "Add protein", "Non-specific cleavage", and "Specific cleavage".

← Links

File Properties Help

Proteins → **Proteases** → Modifications → Cross-links → Measurement → Computing → Results

Proteases

Protein No. 1

<input checked="" type="checkbox"/> Trypsin	[KR]; [^P]	
<input type="checkbox"/> Arg-C	R;	
<input type="checkbox"/> Glu-C	E;	
<input type="checkbox"/> Pro-C	P; [^P]	
<input type="checkbox"/> Chymotrypsin	[FIMGW]; [^P]	
<input type="checkbox"/> CNBr	M;	-48,00348;
<input type="checkbox"/> Lys-C	K;	
<input type="checkbox"/> TEV	E..Y.Q; [GS]	
<input type="checkbox"/> Pepsin	; [FLWY] [FLWY];	
<input type="checkbox"/> nonspecific	[A-Z];	
<input type="checkbox"/> Trypsin	[KRY]; [^P]	

Add level

Add protease

Edit proteases

Comments

Allowed missed cleavages.

☐ Don't use this protease.

Clean form

Back

Modifications

Links

File Properties Help

Proteins → Proteases → **Modifications** → Cross-links → Measurement → Computing → Results

FOXO4-DBD | Methionine (M) | All | Variable | Oxidation | ☒ Specific | OR Select template | Insert

Target	Positions	Presence
FOXO4-DBD	Methionine (M)	Variable

Links

File Properties Help

Proteins → Proteases → Modifications → **Cross-links** → Measurement → Computing → Results

FOXO4-DBD | CH3M (X) | All | PBN2H4 | ☒ Specific | OR Select template | Insert

Within one molecule | CH3M (X) | All

Target 1	Positions 1	Target 2	Types 2	Positions 2	Measurement
FOXO4-DBD	CH3M (X)	Within one molecule	CH3M (X)	All	PBN2H4 (+227.0022)

Clean form

Links

File Properties Help

Proteins → Proteases → Modifications → Cross-links → Measurement → Computing → Results

Filter peptides

Measurement: ...p-links_AU_PtN2H4\C_tPt_Tg_04102016_1614_LCMSelexport_AU_PtN2H4.txt (LCMSelexport); ...\\LCMSelexport\\Pt_p-links_AU_PtN2H4\F_tPt_Tg_04102016_1610_LCMSelexport_AU_PtN2H4.txt (LCMSelexport)

Precision: 2,00 ppm

☐ Peptide length limit: 1 (min), 1 000 000 (max).

☒ Peptide mass limit: 0 (min), 10 000 (max).

Find

Links

File Properties Help

Proteins → Proteases → Modifications → Cross-links → Measurement → Computing → Results

Task: 3 of 8

63%

Initialization.

Testing single peptides: 78 candidates (peptides)...

found 1738 single matching peptides.

Testing cross-links between different peptides: 361 candidates (bonds)...

- yet found 1831 hits.

Cancel

Stop

LinX output

← Links

File Properties Help

Proteins → Proteases → Modifications → Cross-links → Measurement → Computing → **Results**

Proteins: FOXO4-DBD

Modifications: All proteins → M → All → Var - Oxidation

Bonds: FOXO4-DBD → X → All, Within one molecule → X → All - PIN2H4

Specific digest: Trypsin

Settings: Maximal allowed number of missed-cleavages is 3. Cleave on modified aminoacids. Tolerance is 2,00ppm. Peptides must weigh at least 100Da and no more than 10 000Da.

... \.. \CMSExport\IPT_p-pXlinks_AU_PIN2H4\IC_IPT_Tg_04102016_1610_LCMSExport.txt (LCMSExport) .. \.. \CMSExport\IPT_p-pXlinks_AU_PIN2H4\IC_IPT_Tg_04102016_1610_LCMSExport.txt (LCMSExport)

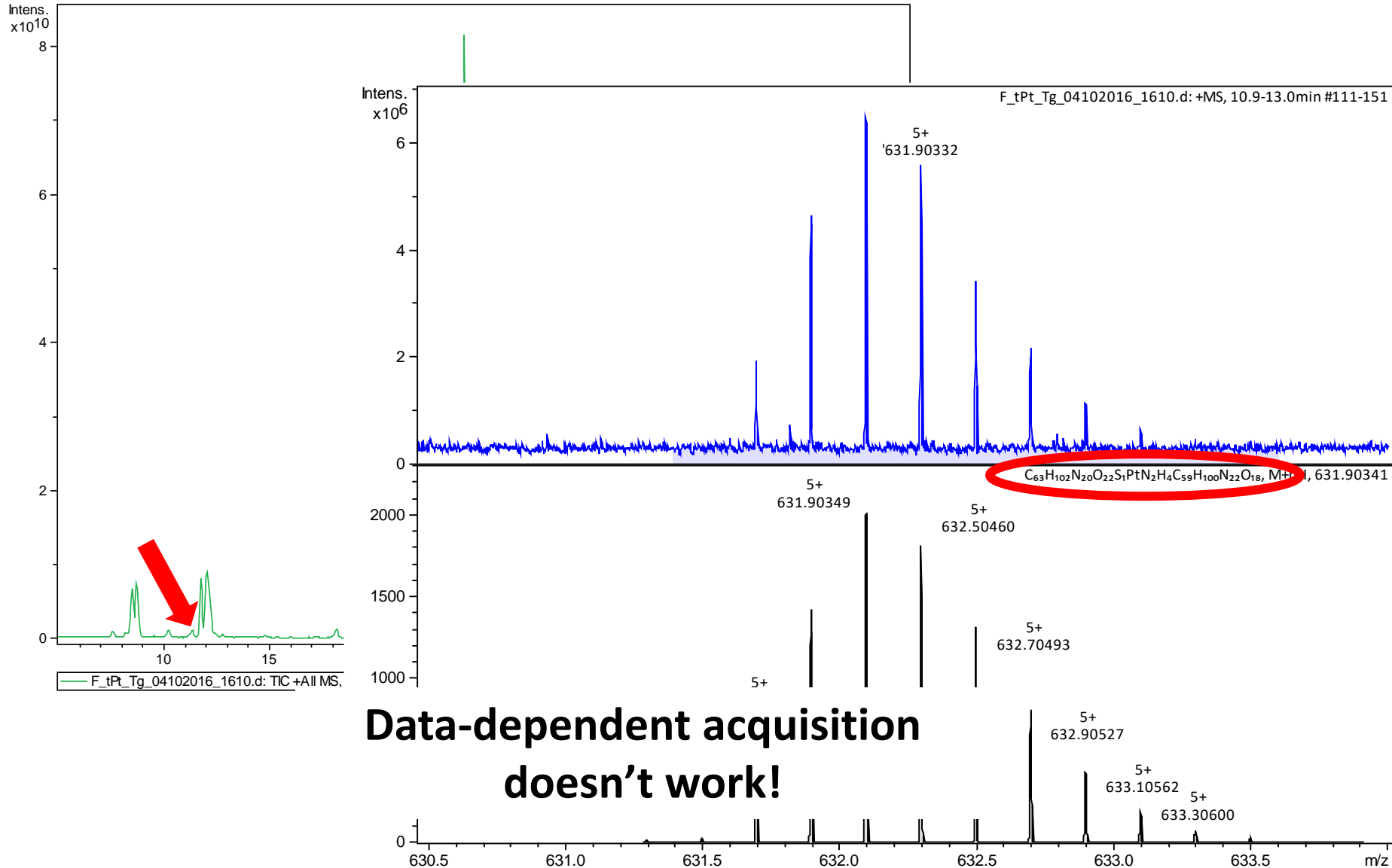
2195 hits, matched 650 of 2960 entries.

Exp. Mass	Thr. Mass	Error	Protein (from, to)	Chain	Modifications	Bonds	Intensity	Retention time	Other
2 517,1885	2 517,1896	-0,42	FOXO4-DBD (89, 89) [A] - FOXO4-DBD (171, 189) [B]	K - SSWVMNLNPEGKSGKAPRR		PIN2H4 (A.89; B.182) PIN2H4 (A.89; B.185) PIN2H4 (A.89; B.175)	1,356	416	3 759,734358222611
2 617,1615	2 617,1580	+1,32	FOXO4-DBD (89, 89) [A] - FOXO4-DBD (163, 182) [B]	K - VHNATGKSSWVMNLNPEGK	Oxidation (B.175)	PIN2H4 (A.89; B.164) PIN2H4 (A.89; B.170) PIN2H4 (A.89; B.182)	2,796	383	4 655,045821173428
2 617,1612	2 617,1580	+1,23	FOXO4-DBD (89, 89) [A] - FOXO4-DBD (163, 182) [B]	K - VHNATGKSSWVMNLNPEGK	Oxidation (B.175)	PIN2H4 (A.89; B.164) PIN2H4 (A.89; B.170) PIN2H4 (A.89; B.182)	1,426	384	3 873,058588314647
2 617,1612	2 617,1580	+1,21	FOXO4-DBD (89, 89) [A] - FOXO4-DBD (163, 182) [B]	K - VHNATGKSSWVMNLNPEGK	Oxidation (B.175)	PIN2H4 (A.89; B.164) PIN2H4 (A.89; B.170) PIN2H4 (A.89; B.182)	4,296	385	4 655,045747891466
2 608,2094	2 608,2051	+1,66	FOXO4-DBD (89, 94) [A] - FOXO4-DBD (136, 151) [B]	KGGRR - DKGDSNSSAGWKNIR		PIN2H4 (A.89; B.137) PIN2H4 (A.89; B.147)	2,116	383	3 870,074656374171
2 605,1545	2 605,1540	+0,22	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	3,306	88	4 652,044091021485
2 605,1537	2 605,1540	-0,10	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	6,586	89	4 652,043886409786
2 605,1541	2 605,1540	+0,04	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	7,906	90	4 652,043951138806 3 869,05601183653...
2 605,1543	2 605,1540	+0,12	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	4,056	91	4 652,044029724854
2 605,1533	2 605,1540	+1,21	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	1,876	92	4 652,043789389454
2 605,1537	2 605,1540	-0,11	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	2,836	93	4 652,043879020294
2 605,1543	2 605,1540	+0,11	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	4,136	94	4 652,044024101496
2 605,1538	2 605,1540	-0,06	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	3,146	95	4 652,043907749459
2 605,1532	2 605,1540	-0,30	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (163, 170) [B]	GSHMLEDPGAVTGPR - VHNATGK		PIN2H4 (A.77; B.164) PIN2H4 (A.77; B.170) PIN2H4 (A.76; B.164) PIN2H4...	1,456	96	4 652,043751353846
2 912,2293	2 912,2300	-0,24	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	2,056	118	4 728,812792842057
2 912,2291	2 912,2300	-0,34	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	1,976	120	4 728,812721065697
2 912,2286	2 912,2300	-0,50	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	2,006	127	4 728,81260450156
2 912,2272	2 912,2300	-0,97	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	2,536	128	4 728,812264500751
2 912,2299	2 912,2300	-0,05	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	2,996	129	4 728,812933555396
2 912,2287	2 912,2300	-0,45	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	2,686	130	4 728,812642880646
2 912,2289	2 912,2300	-0,39	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	1,806	132	4 728,812686099439
3 382,4899	3 382,4902	-0,07	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	1,286	134	5 677,303807216042
2 912,2288	2 912,2300	-0,43	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	1,416	137	4 728,812653411851
2 912,2276	2 912,2300	-0,83	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	1,306	138	4 728,812367098592
2 912,2270	2 912,2300	-1,04	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.159) PIN2H4 (A.77; B.157) PIN2H4...	1,606	81	4 728,8122088296
2 685,2287	2 685,2278	+0,32	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	1,766	118	5 537,851555665349
2 685,2279	2 685,2278	+0,03	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	2,037	119	4 672,062429719762 3 895,74658888409...
2 685,2277	2 685,2278	-0,05	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	5,417	120	4 672,062357914325 3 895,74756776066...
2 685,2277	2 685,2278	-0,05	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	5,637	121	4 672,062338523669 3 895,74763453628...
2 685,2276	2 685,2278	-0,09	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	3,267	122	4 672,062343883146 3 895,74773854373...
2 685,2275	2 685,2278	-0,10	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	1,697	123	4 672,062407640042 5 537,851237988471
2 685,2272	2 685,2278	-0,22	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	4,785	124	4 672,062393150902 5 537,851042627119
3 155,4878	3 155,4879	-0,03	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	8,566	125	6 311,903364702254 4 789,62756451144...
2 685,2277	2 685,2278	-0,06	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	4,556	124	5 537,8512371054392
3 155,4878	3 155,4879	-0,03	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	9,596	125	6 311,903415730287 4 789,62733805432...
2 685,2276	2 685,2278	-0,06	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (152, 159) [B]	GSHMLEDPGAVTGPR - HNLSLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	2,786	126	4 672,062329733536 5 537,851399882294
3 155,4873	3 155,4879	-0,21	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	8,956	126	6 311,903308573633 4 789,6272778641 ...
3 155,4866	3 155,4879	-0,42	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	7,666	127	6 311,9032168353 4 789,626788771234
3 155,4881	3 155,4879	+0,04	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	6,236	128	6 311,903410766053 4 789,627540767232
3 155,4884	3 155,4879	+0,14	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	6,906	129	6 311,903493400345 4 789,627572549902
3 155,4867	3 155,4879	-0,38	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	4,186	130	6 311,903199345692 4 789,627027574649
3 155,4887	3 155,4879	+0,26	FOXO4-DBD (74, 88) [A] - FOXO4-DBD (148, 159) [B]	GSHMLEDPGAVTGPR - NSIRHNLHSHK		PIN2H4 (A.77; B.152) PIN2H4 (A.77; B.157) PIN2H4 (A.77; B.159) PIN2H4...	3,106	131	5 537,8513956983016
1 750,7300	1 750,7293	+0,35	FOXO4-DBD (74, 88)	GSHMLEDPGAVTGPR		PIN2H4 (76; 77)	5,156	100	3 584,248170205136
1 750,7293	1 750,7293	-0,01	FOXO4-DBD (74, 88)	GSHMLEDPGAVTGPR		PIN2H4 (76; 77)	5,426	101	3 584,24795907777
1 750,7290	1 750,7293	-0,21	FOXO4-DBD (74, 88)	GSHMLEDPGAVTGPR		PIN2H4 (76; 77)	4,536	102	3 584,247840569531
1 750,7295	1 750,7293	+0,10	FOXO4-DBD (74, 88)	GSHMLEDPGAVTGPR		PIN2H4 (76; 77)	4,856	103	3 584,248021090356
1 750,7288	1 750,7293	-0,31	FOXO4-DBD (74, 88)	GSHMLEDPGAVTGPR		PIN2H4 (76; 77)	3,386	104	3 584,2478444463

Save all Save current Draw Grouping ▲ Filtering ▲

Back New

Validation of assigned signals – isotopic signature



Stable covalent labeling

- Radical footprinting ($\cdot\text{OH}$, $\cdot\text{I}$, $\cdot\text{CF}_3$)
- Chemical footprinting and cross-linking

- Hydroxyl radicals can be generated by various means:

- Irradiation of water by x-rays or electron beams
- Fenton reaction
- Photolysis of hydrogen peroxide
FPOP (fast photochemical oxidation of proteins)

The relative reactivity of the amino acid side chains

Cysteine, Methionine,
Tryptophan

Tyr > Phe > His
> Leu ~ Ile >
Arg ~ Lys ~ Val
> Ser ~ Thr ~
Pro > Gln ~ Glu
> Asp

Alanine,
Glycine

Takamoto K . et al. Annu Rev Biophys Biomol Struct. 2006, 35, 251-276

Methods and Simulations

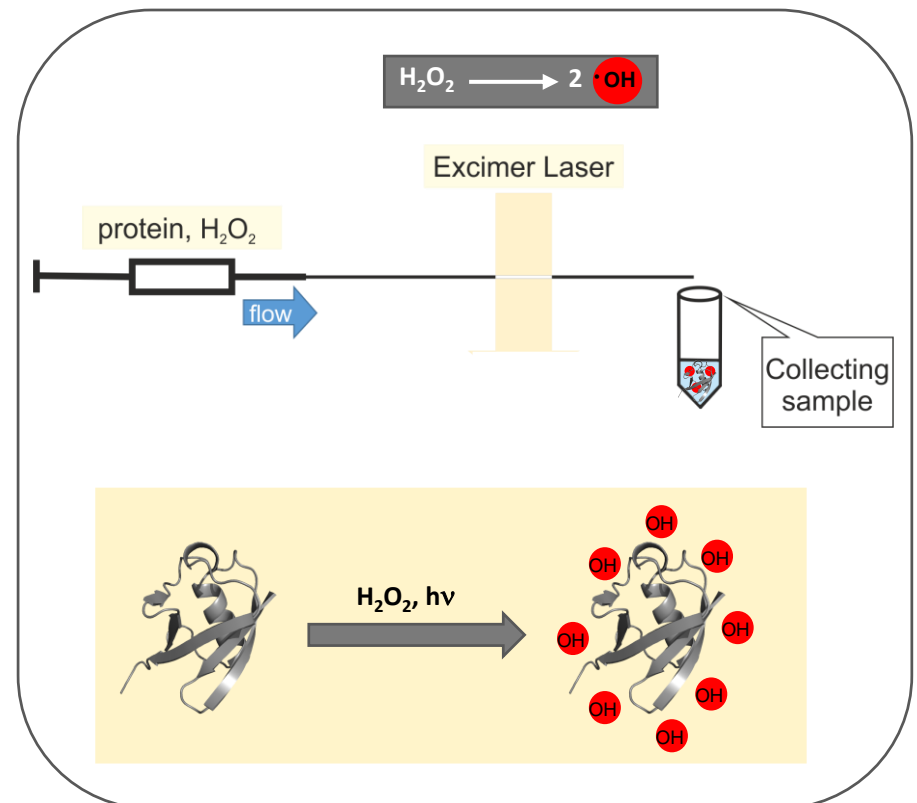
FPOP \Rightarrow a pulsed laser to photolyze hydrogen peroxide \Rightarrow generate OH radicals and modify proteins in a flow system

- Advantageous

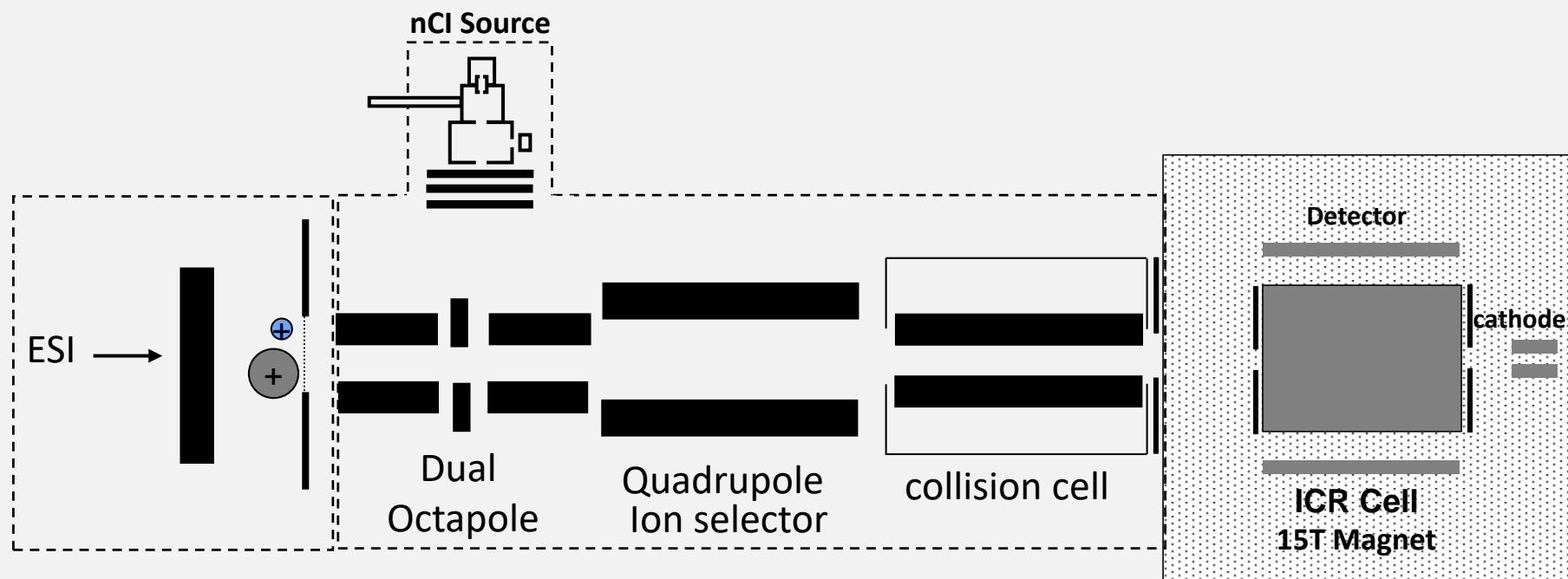
- Covalent modification \Rightarrow preserves the primary sequence of modified residues
- High reactivity of $\bullet\text{OH}$ \Rightarrow the modifications of more than half of amino acid side-chains, providing a higher coverage
- $\bullet\text{OH}$ size \Rightarrow comparable to a water molecule that able to probe the solvent accessibility of a protein of interest
- Fragmentation techniques
 - collision induced dissociation (CID)
 - Electron capture dissociation (ECD)
 - electron transfer dissociation (ETD)

- Data analysis

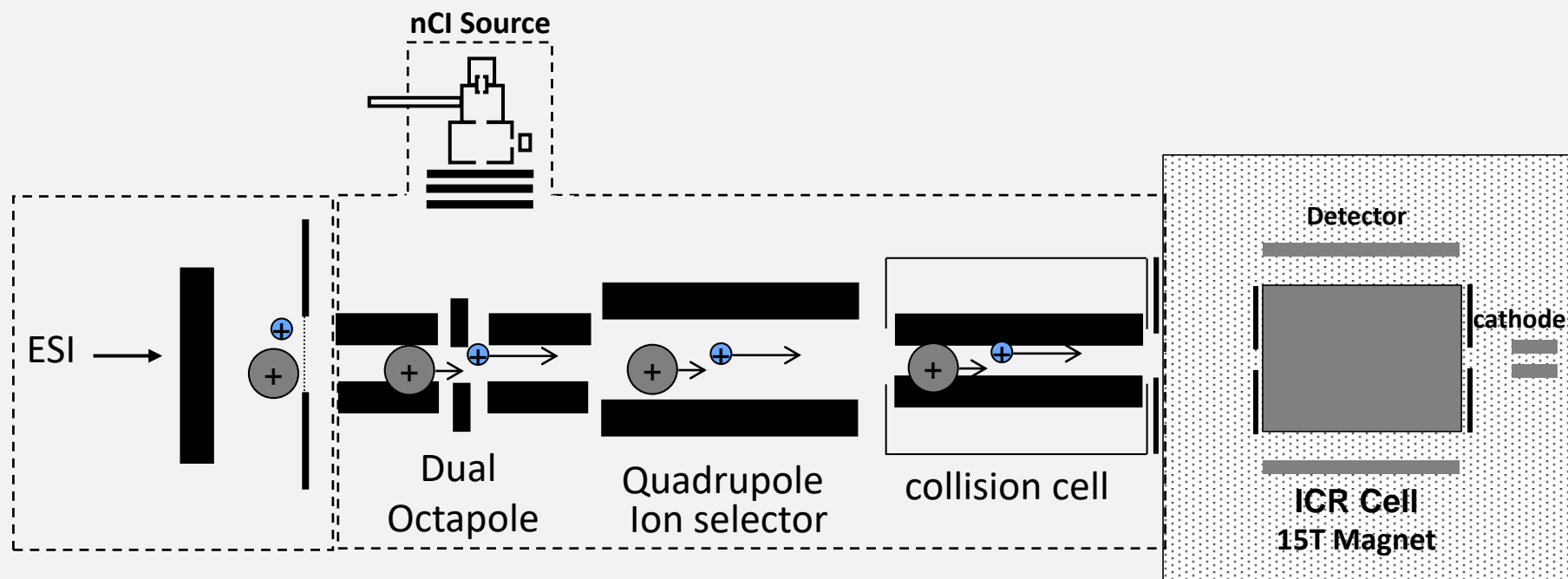
Fast Photochemical Oxidation of Proteins (FPOP)



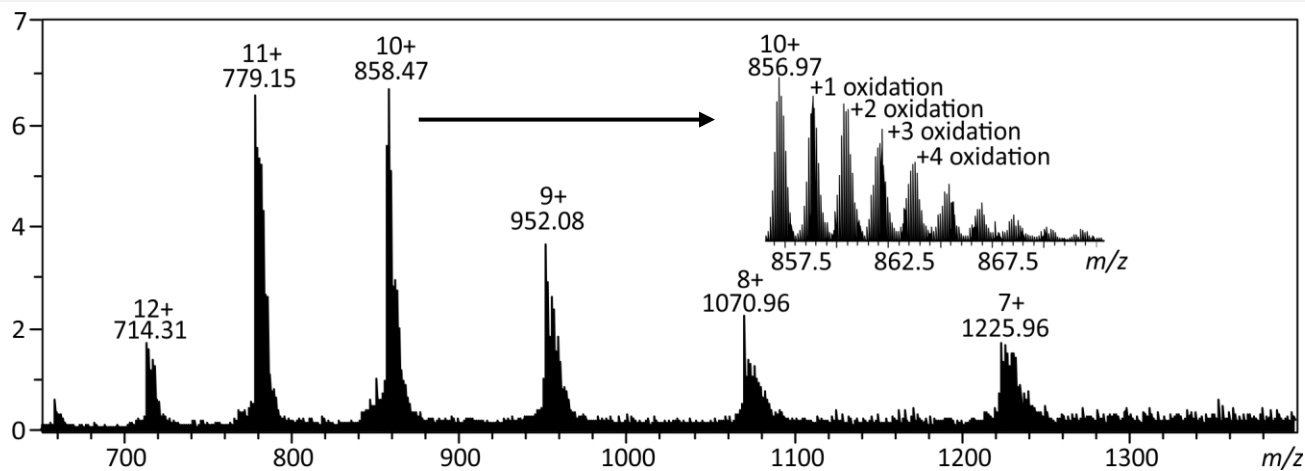
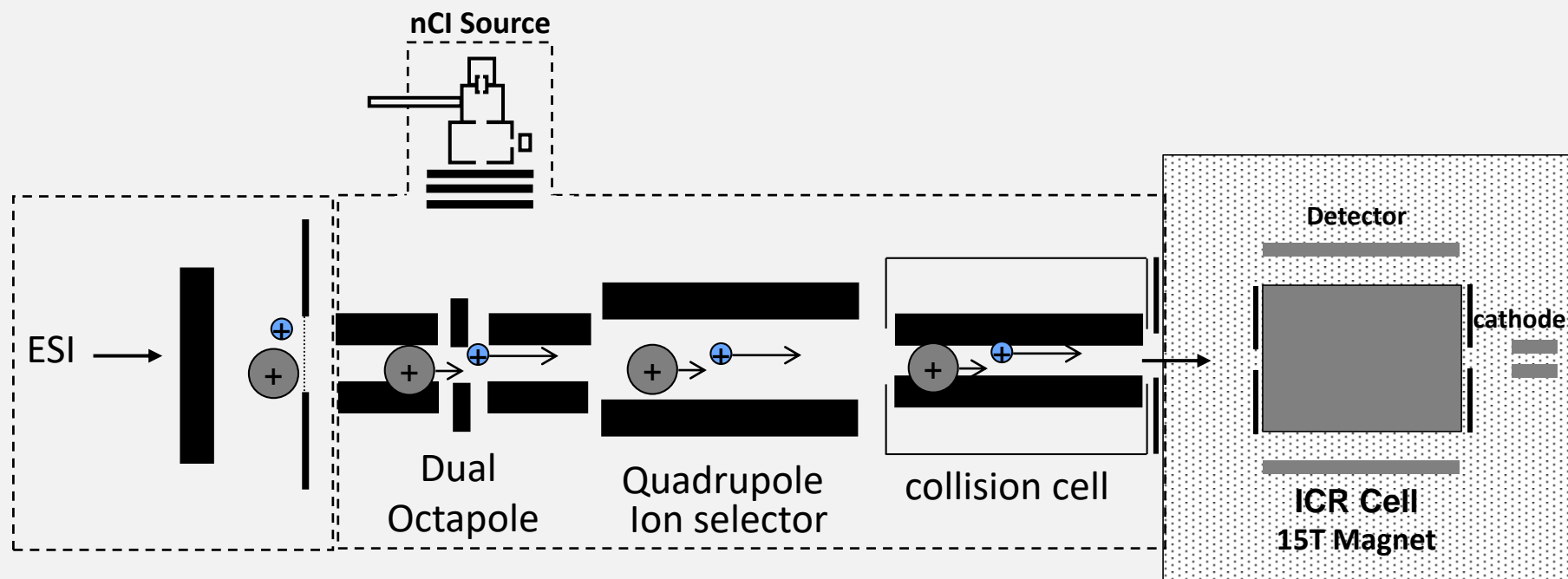
Experimental part



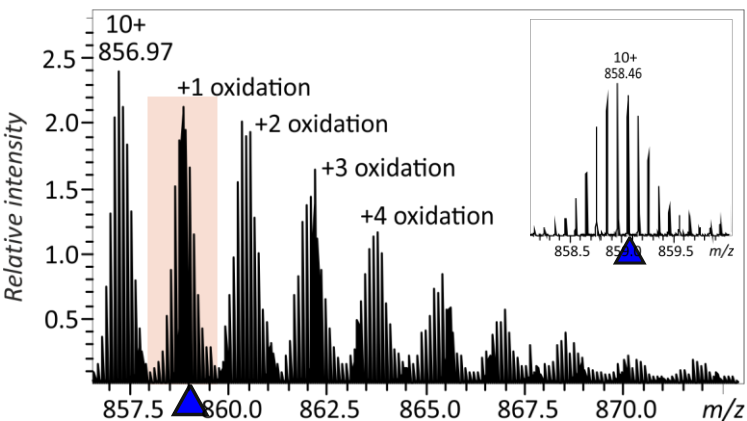
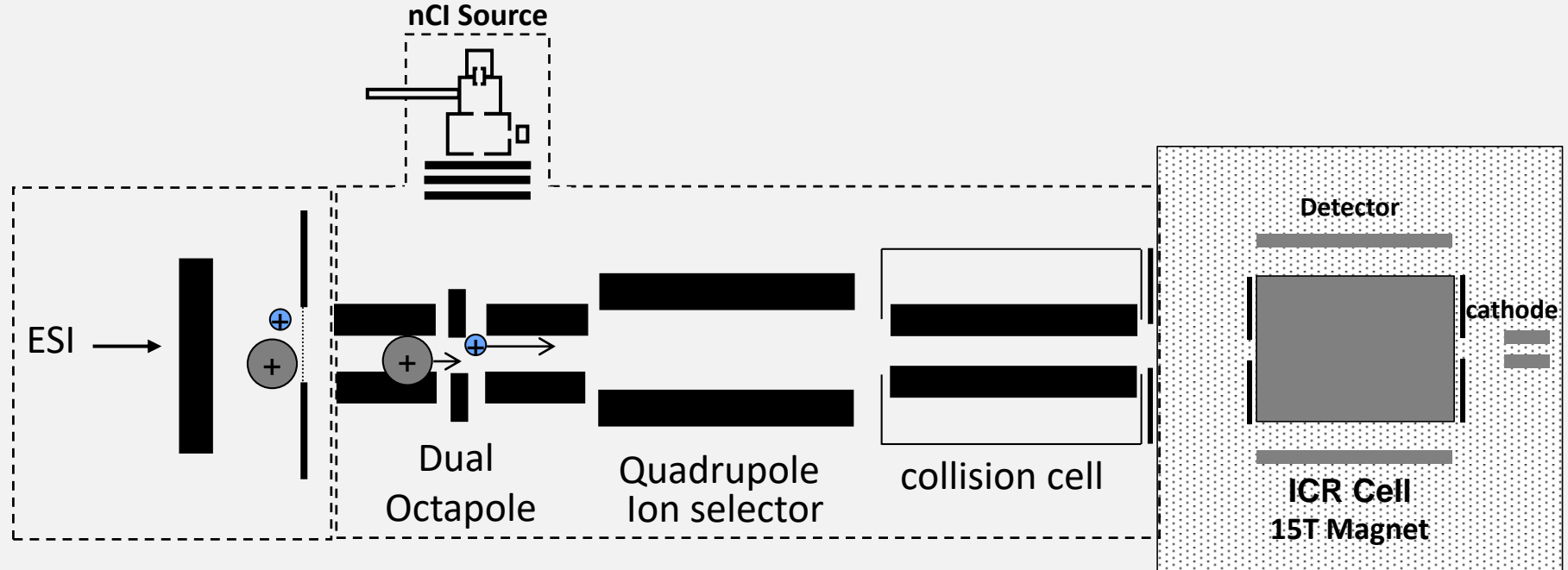
Experimental part



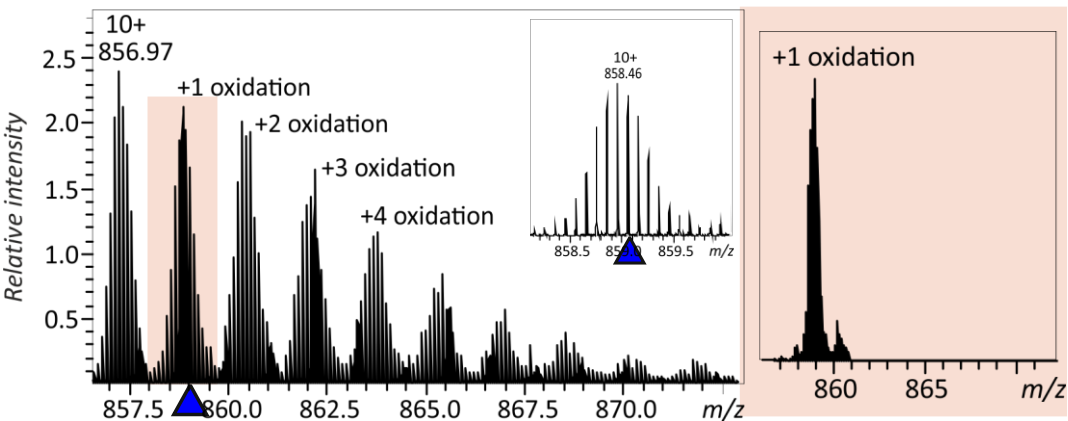
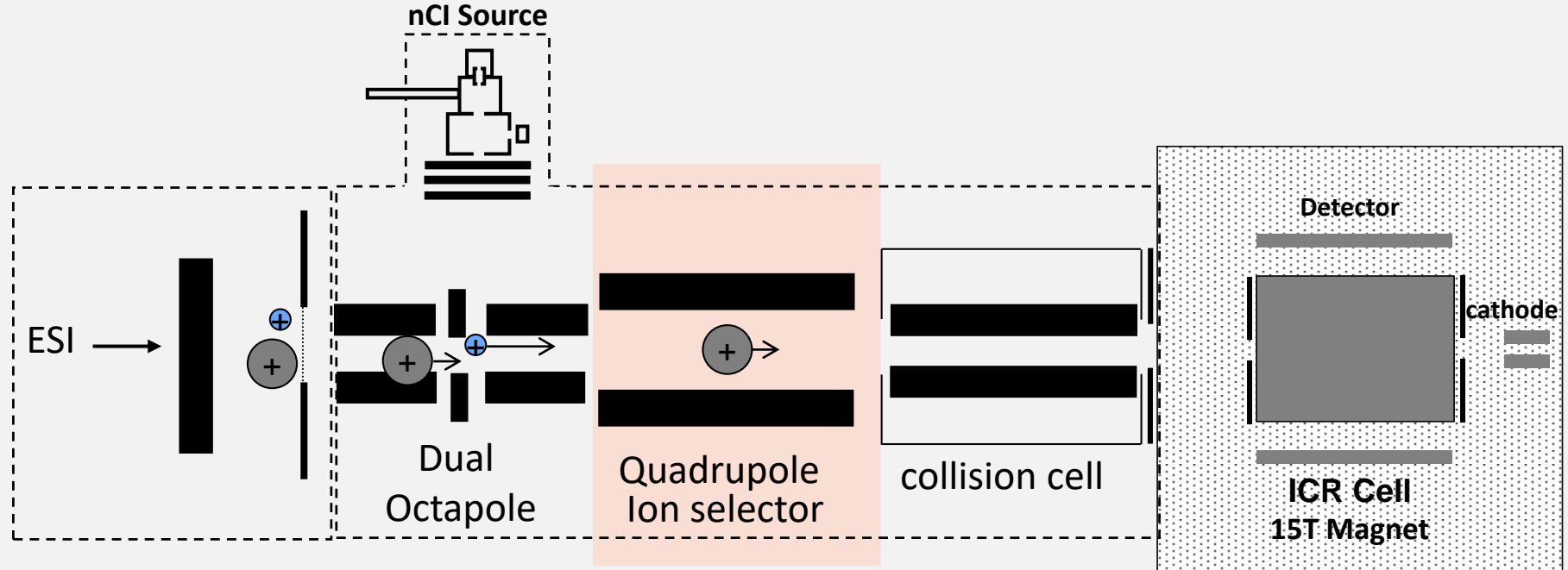
Experimental part



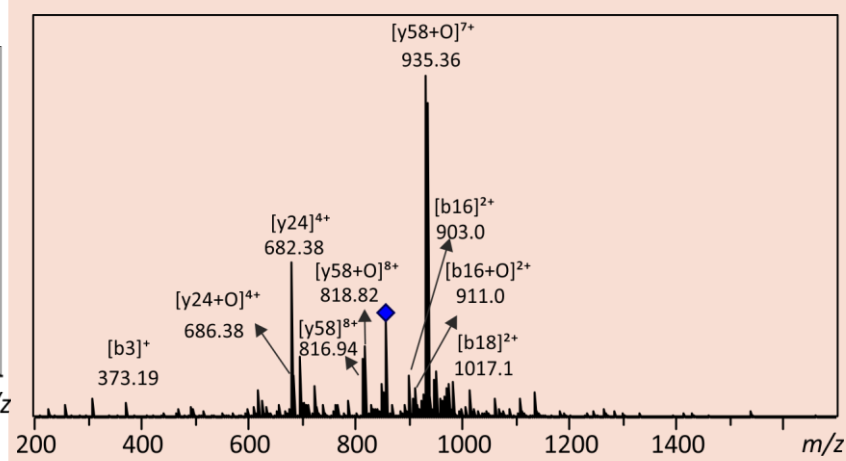
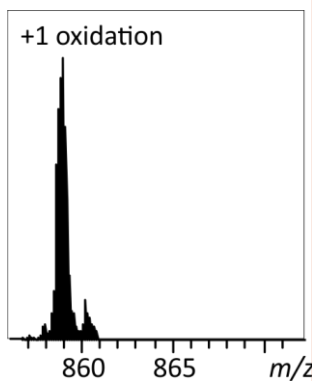
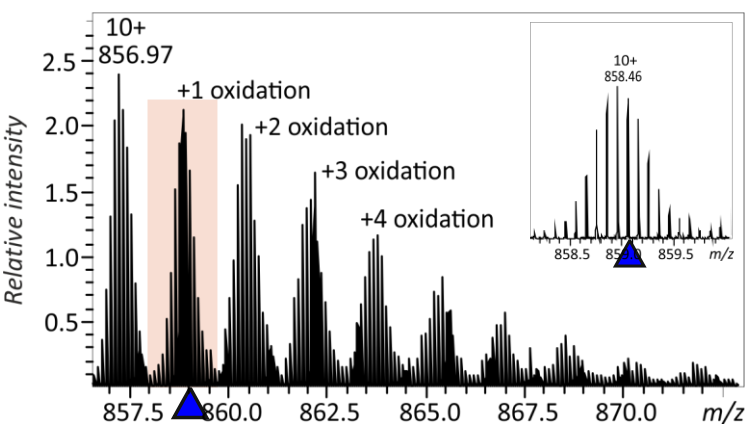
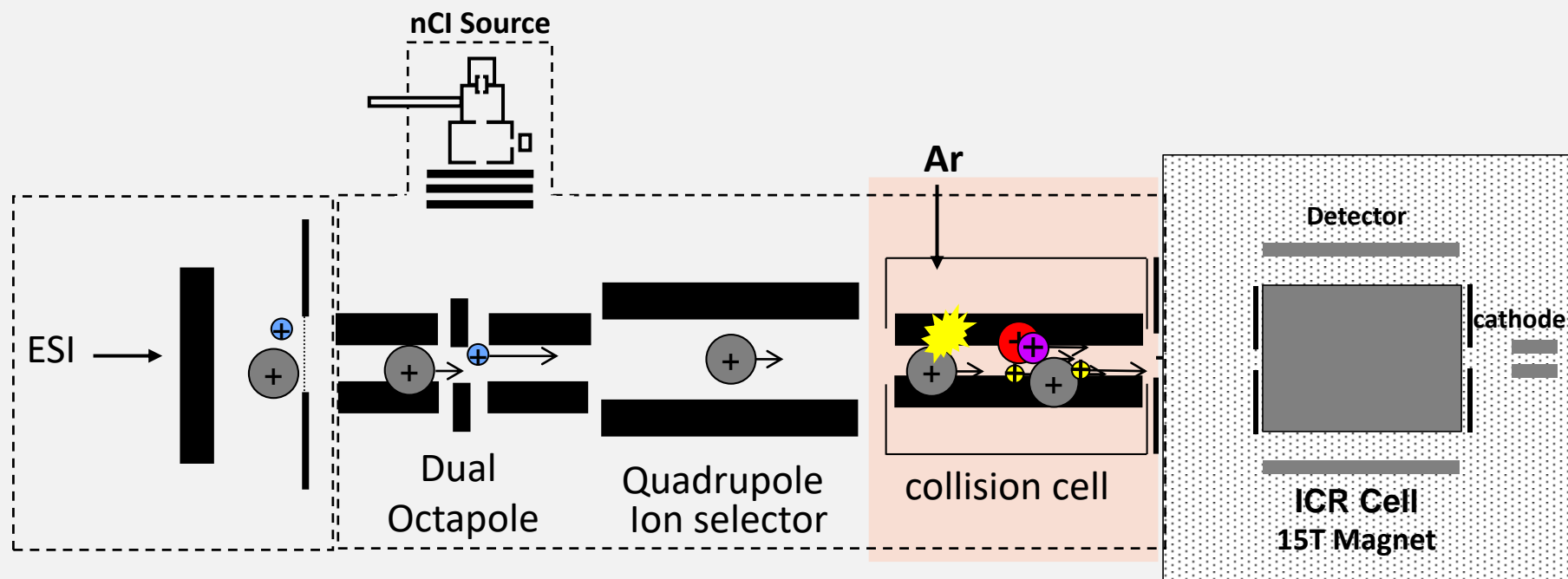
Collision-induced dissociation (CID)



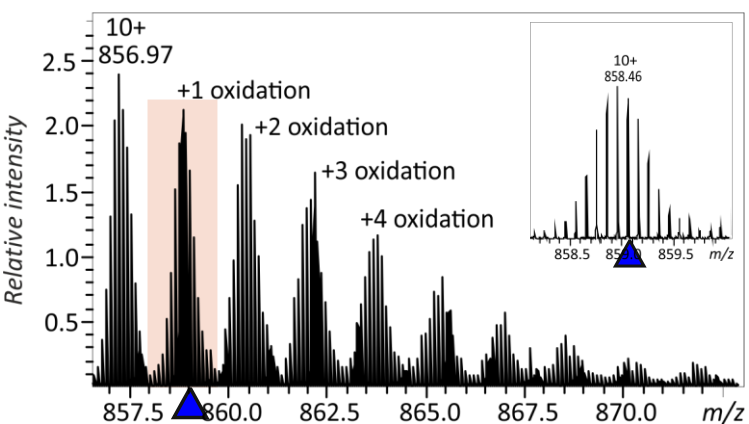
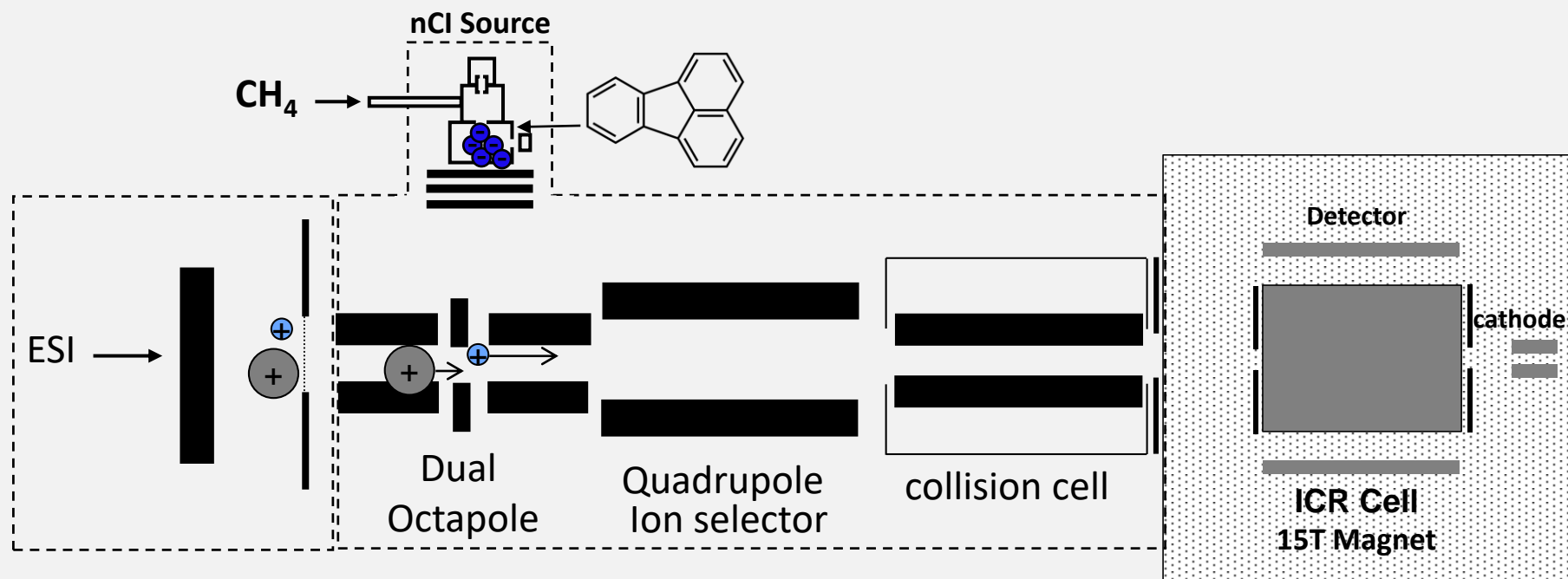
Collision-induced dissociation (CID)



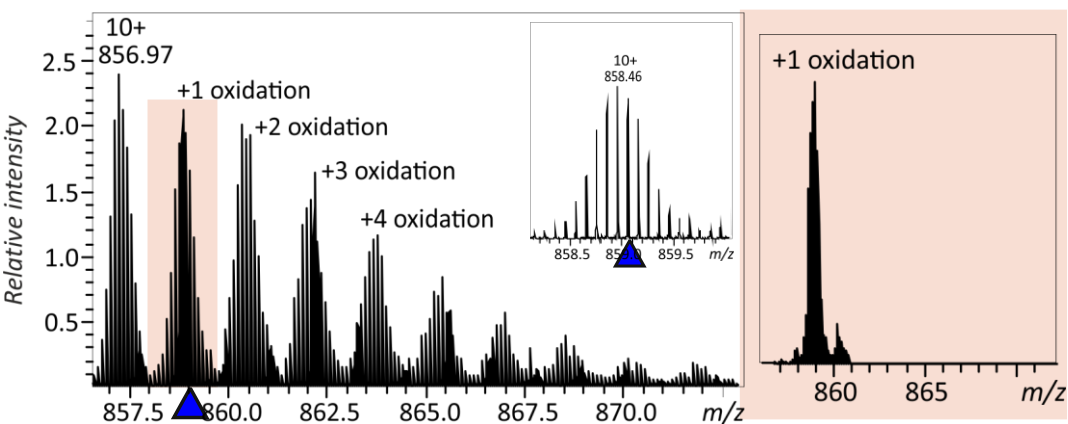
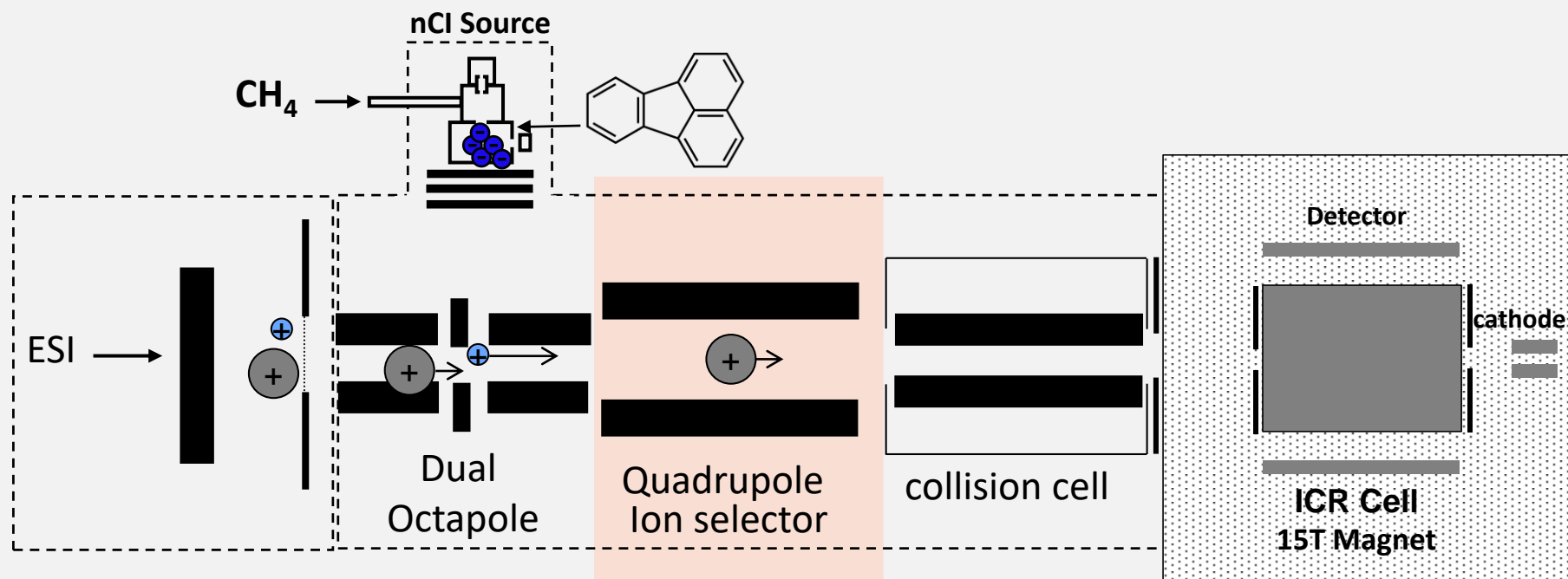
Collision-induced dissociation (CID)



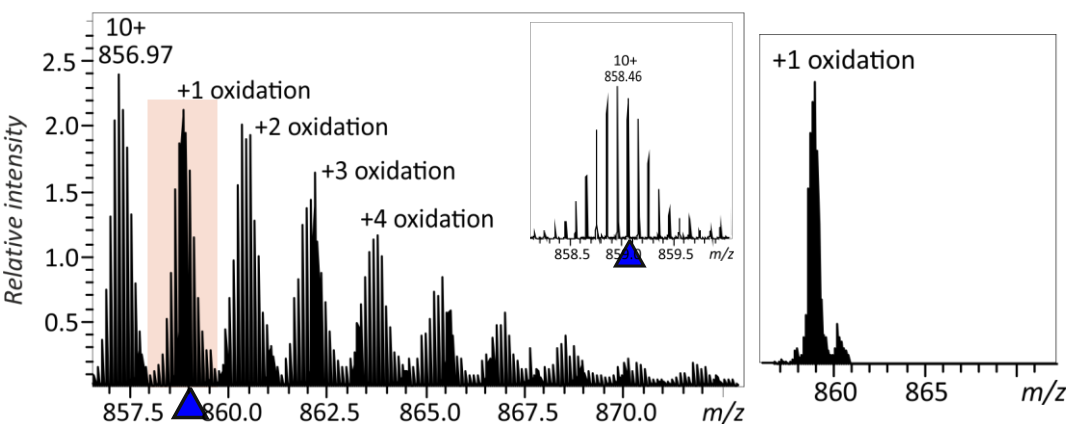
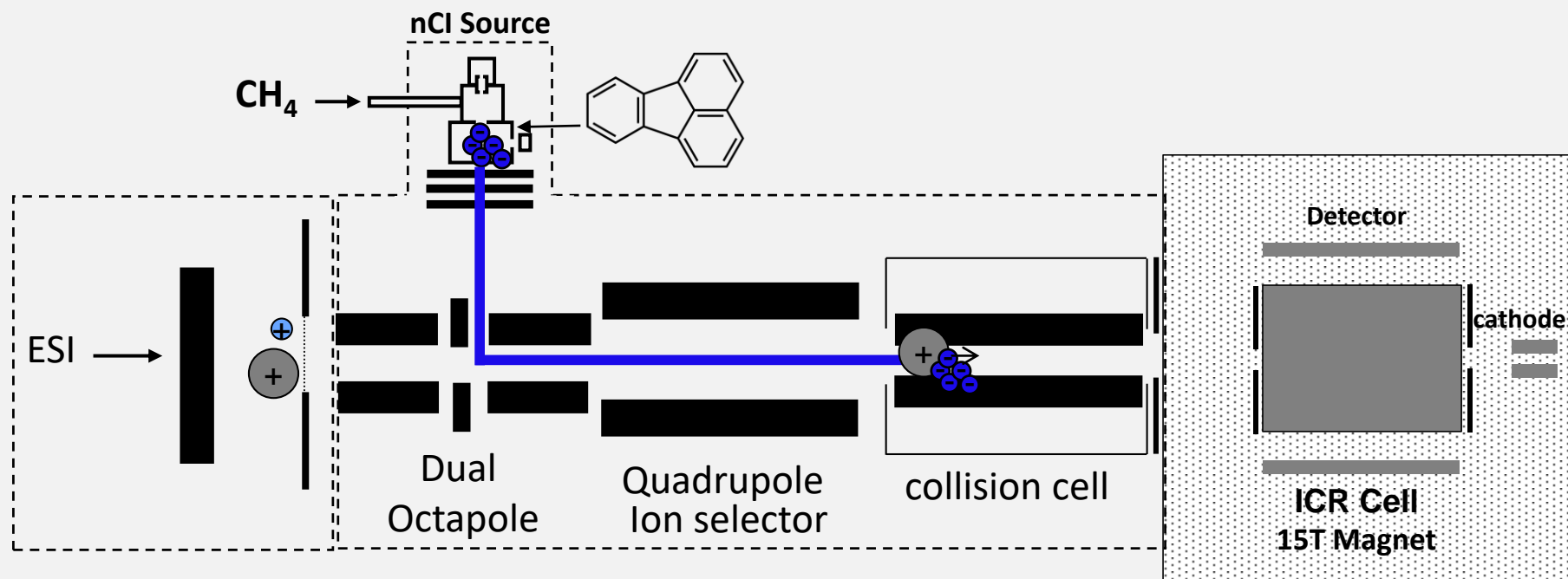
Electron-transfer dissociation (ETD)



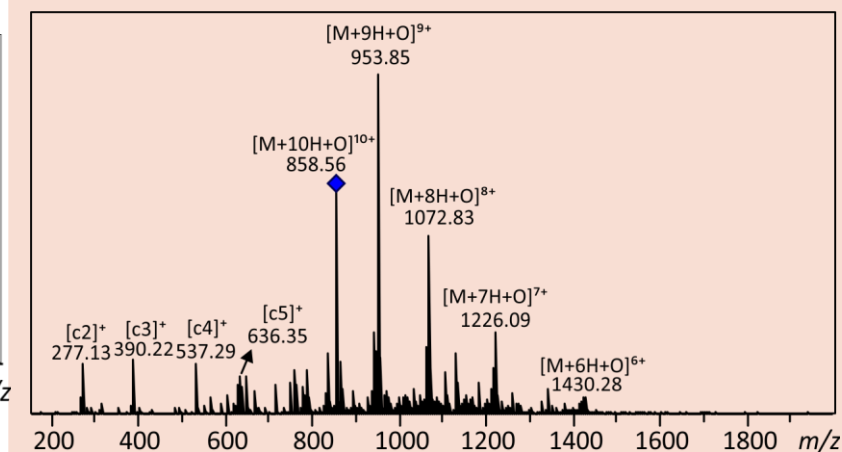
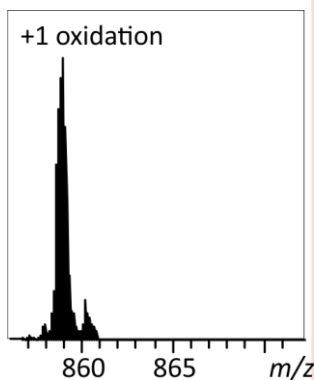
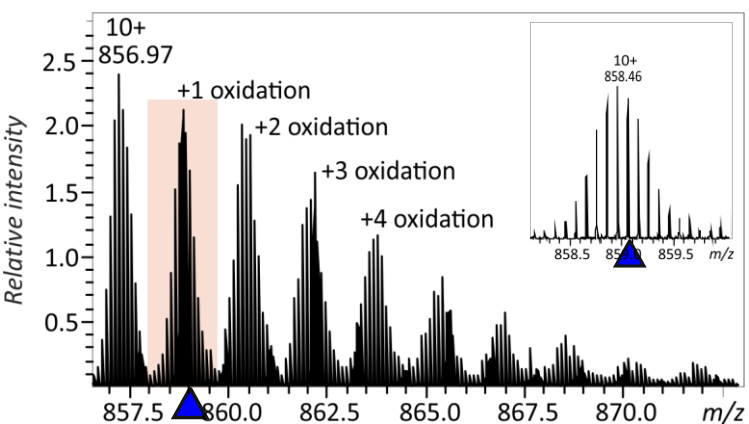
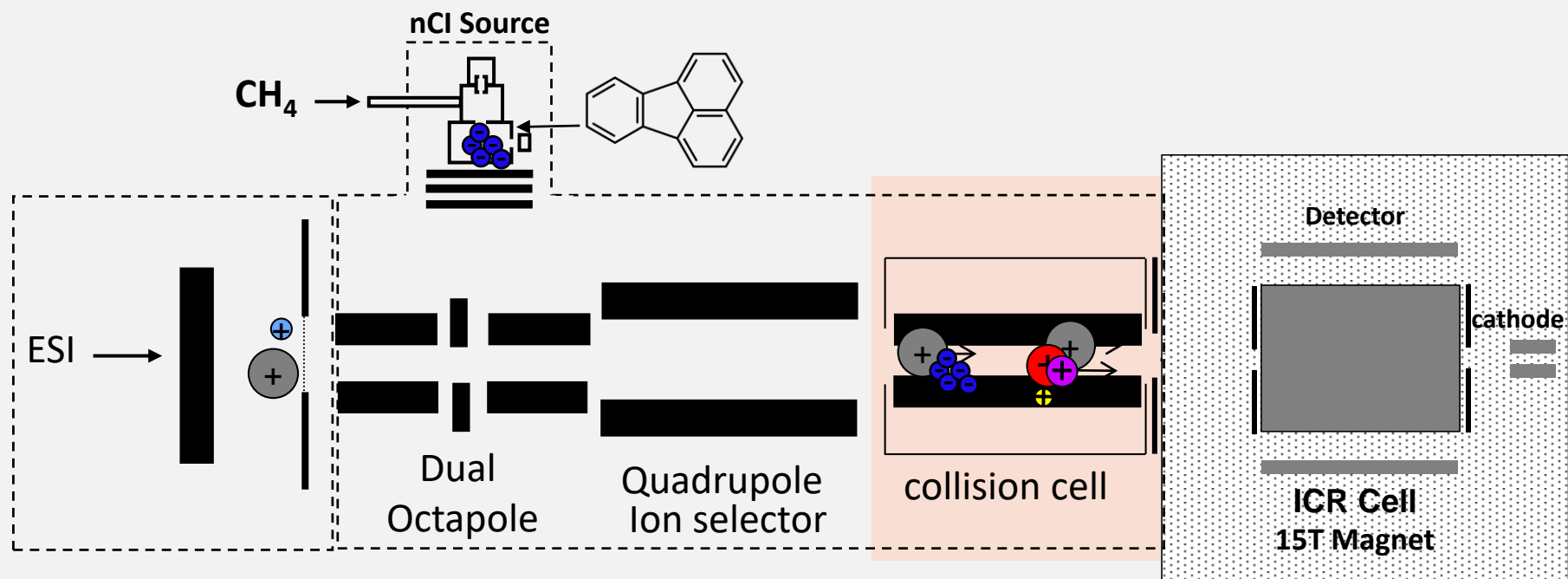
Electron-transfer dissociation (ETD)



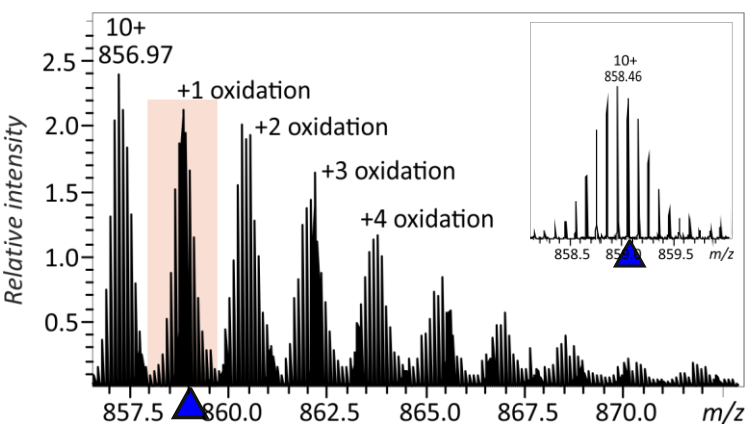
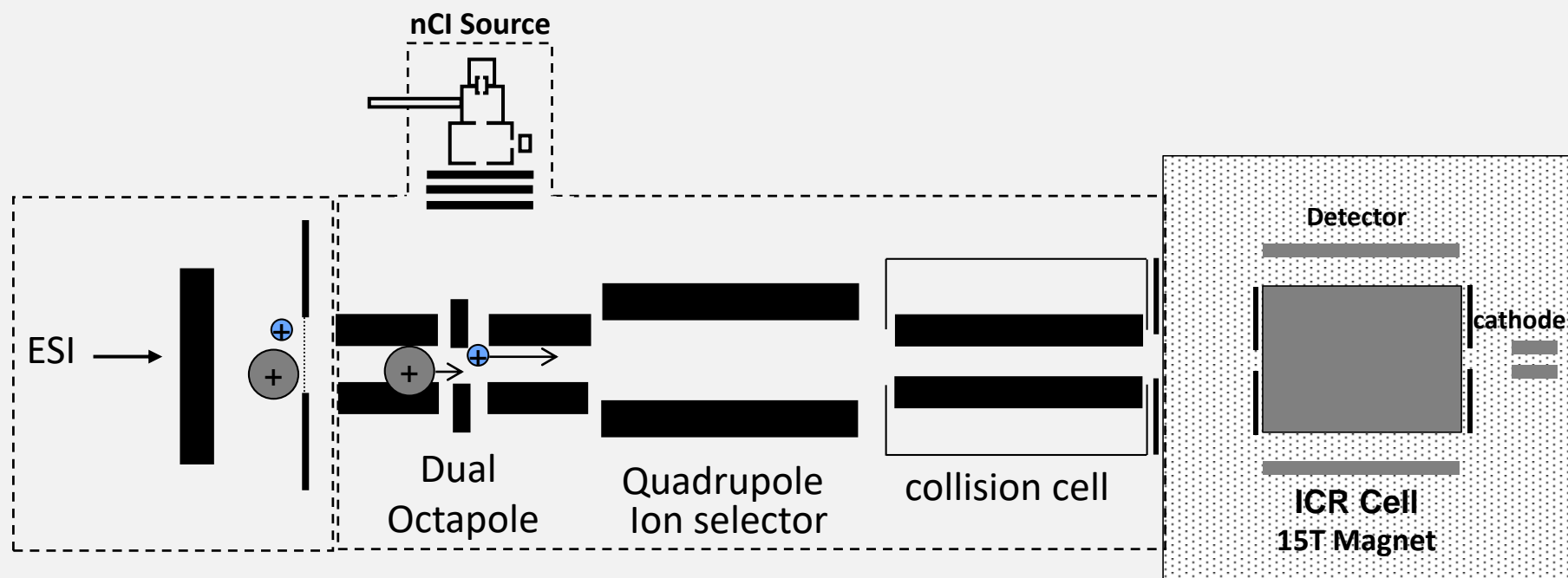
Electron-transfer dissociation (ETD)



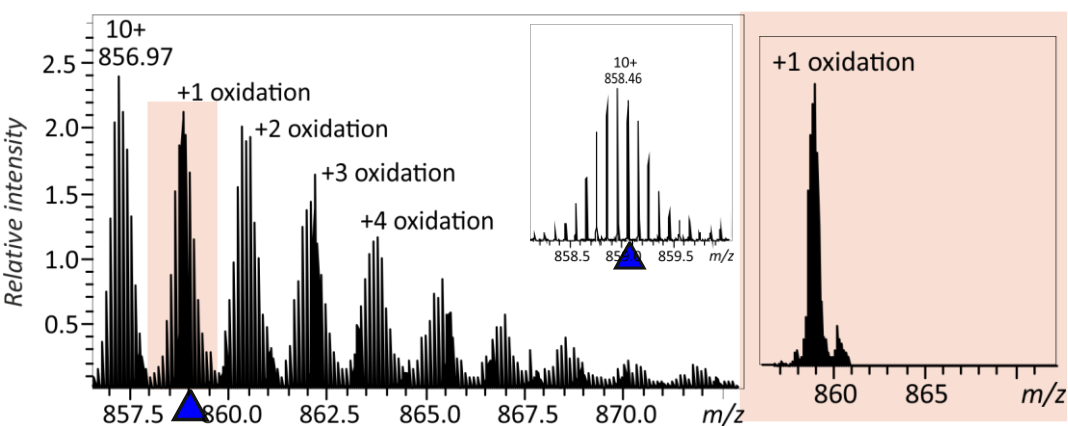
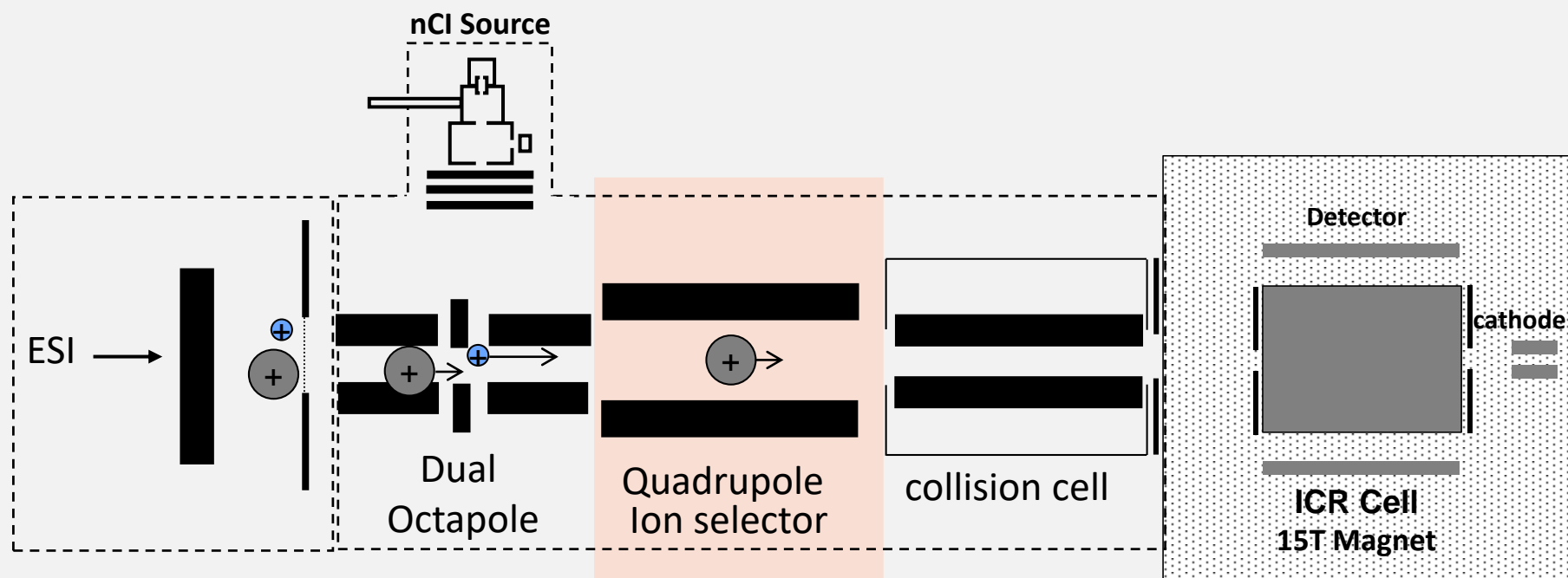
Electron-transfer dissociation (ETD)



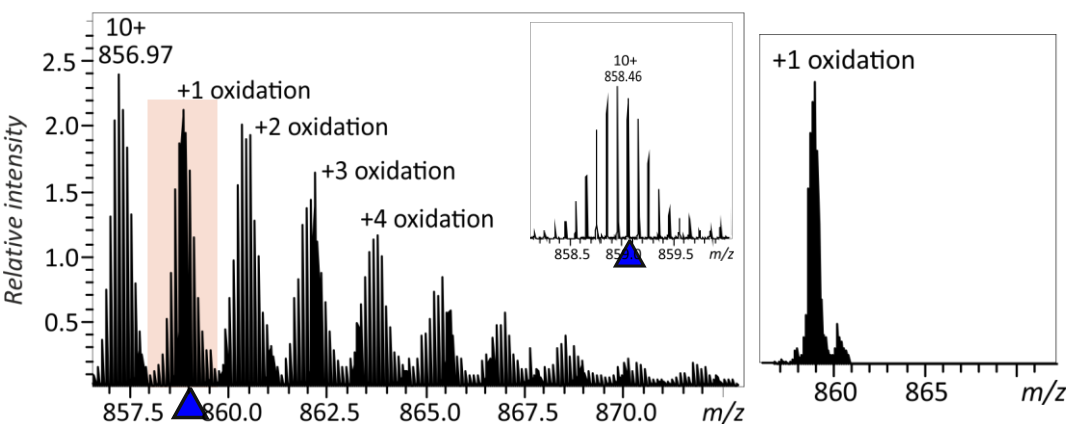
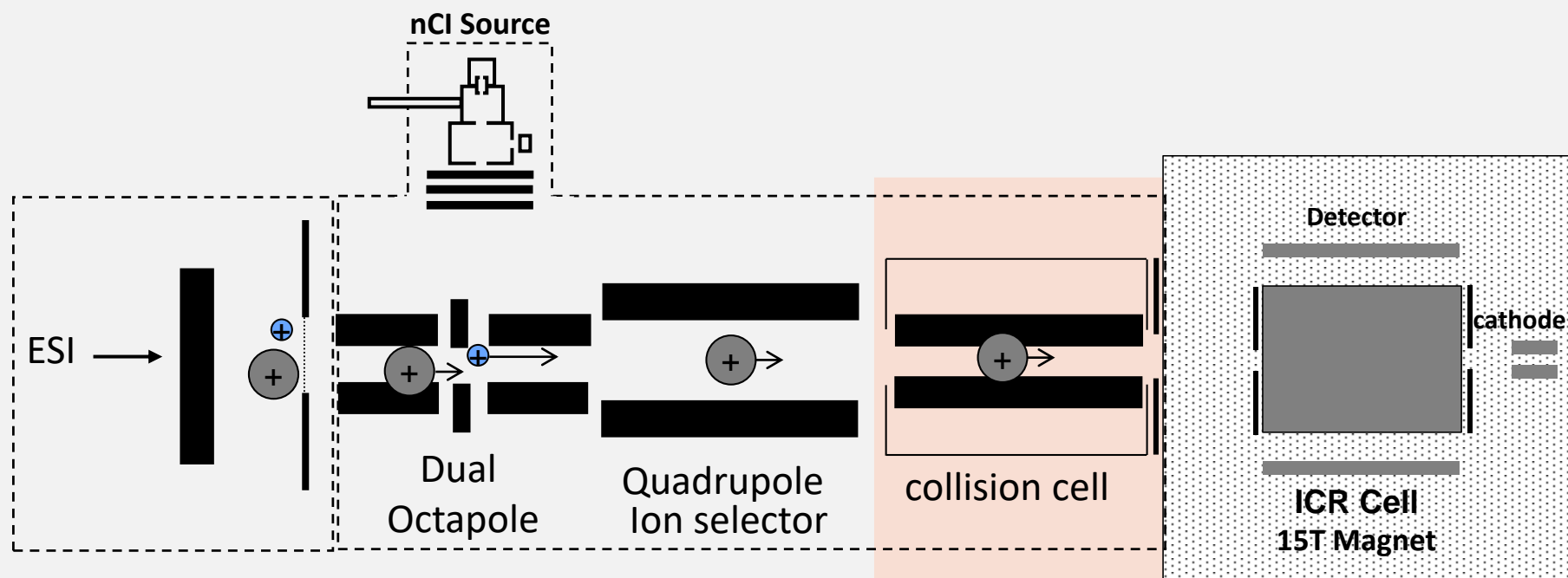
Electron-capture dissociation (ECD)



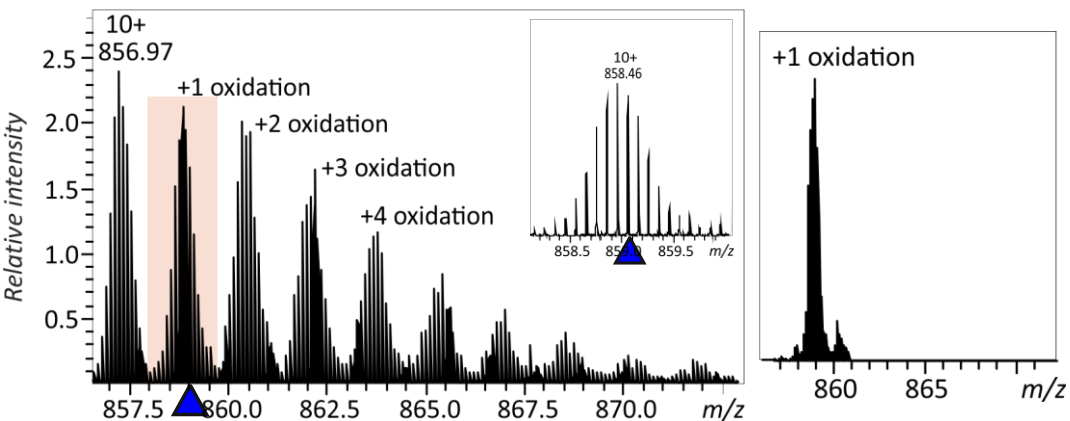
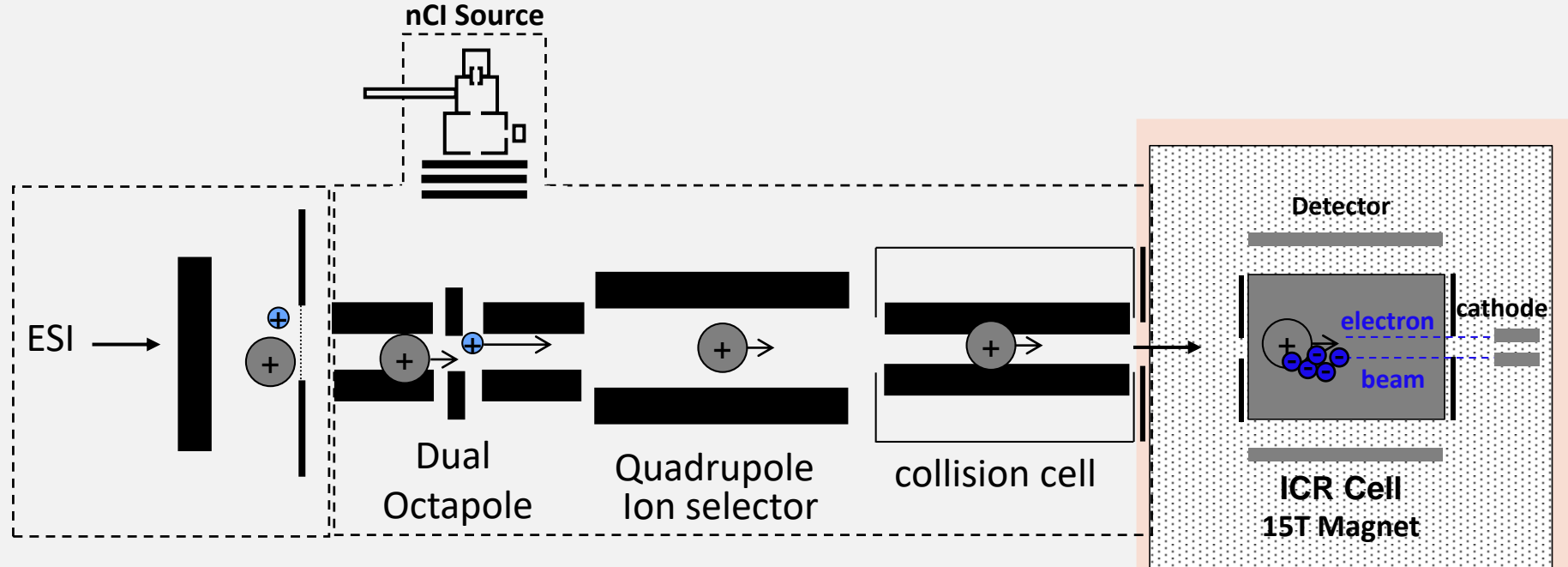
Electron-capture dissociation (ECD)



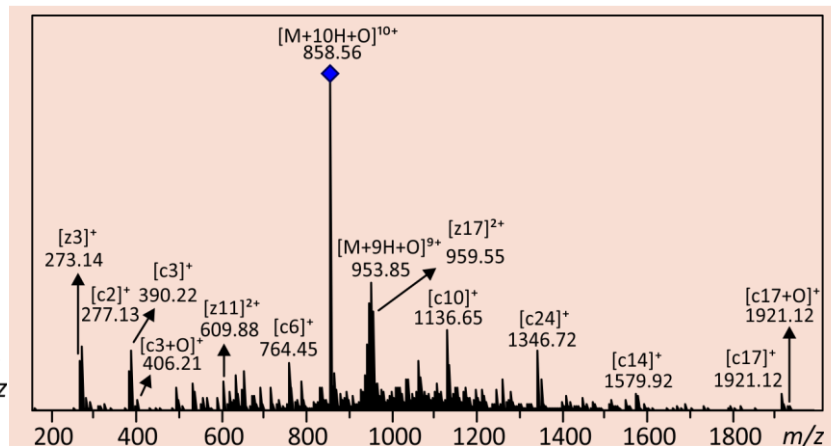
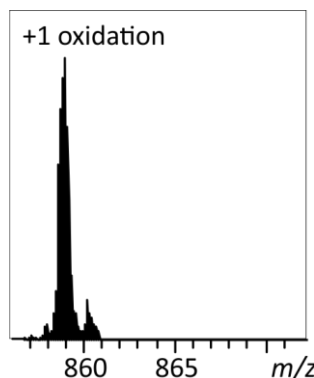
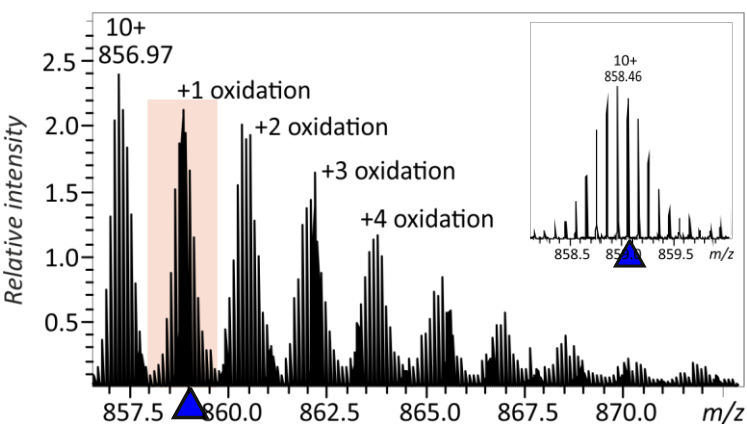
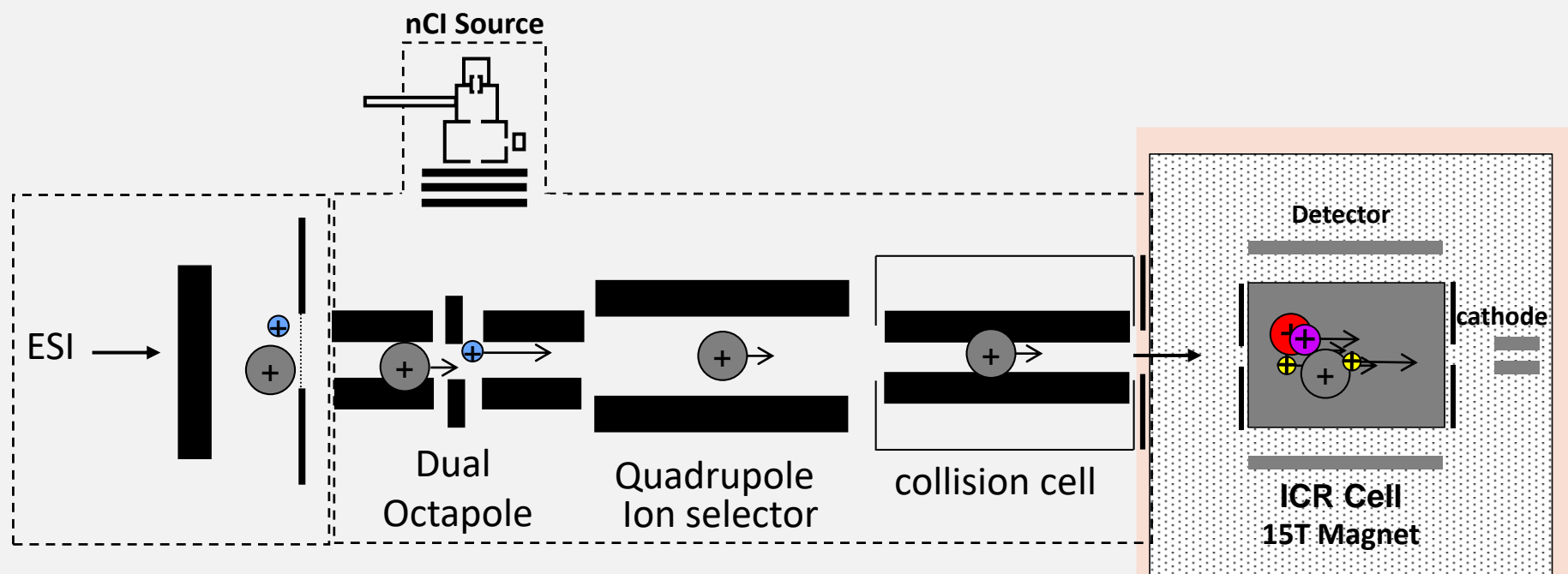
Electron-capture dissociation (ECD)



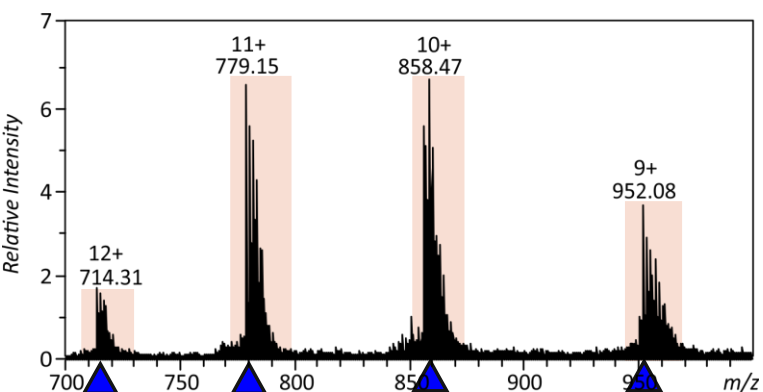
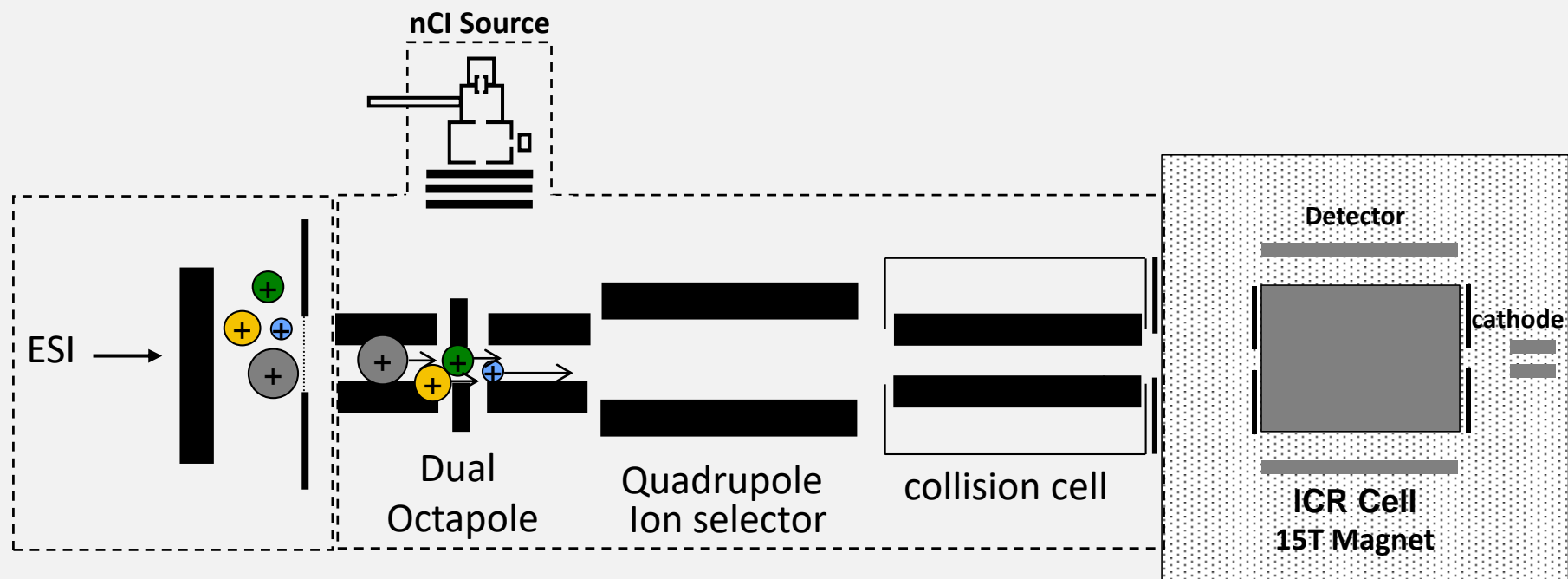
Electron-capture dissociation (ECD)



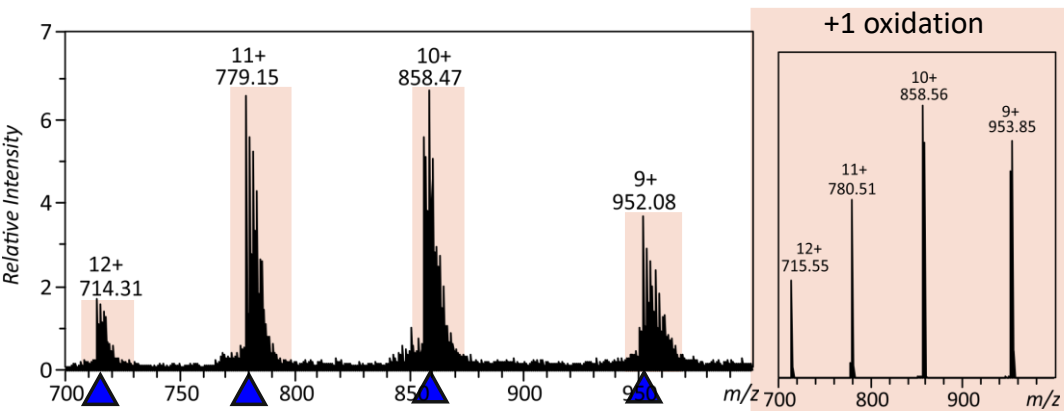
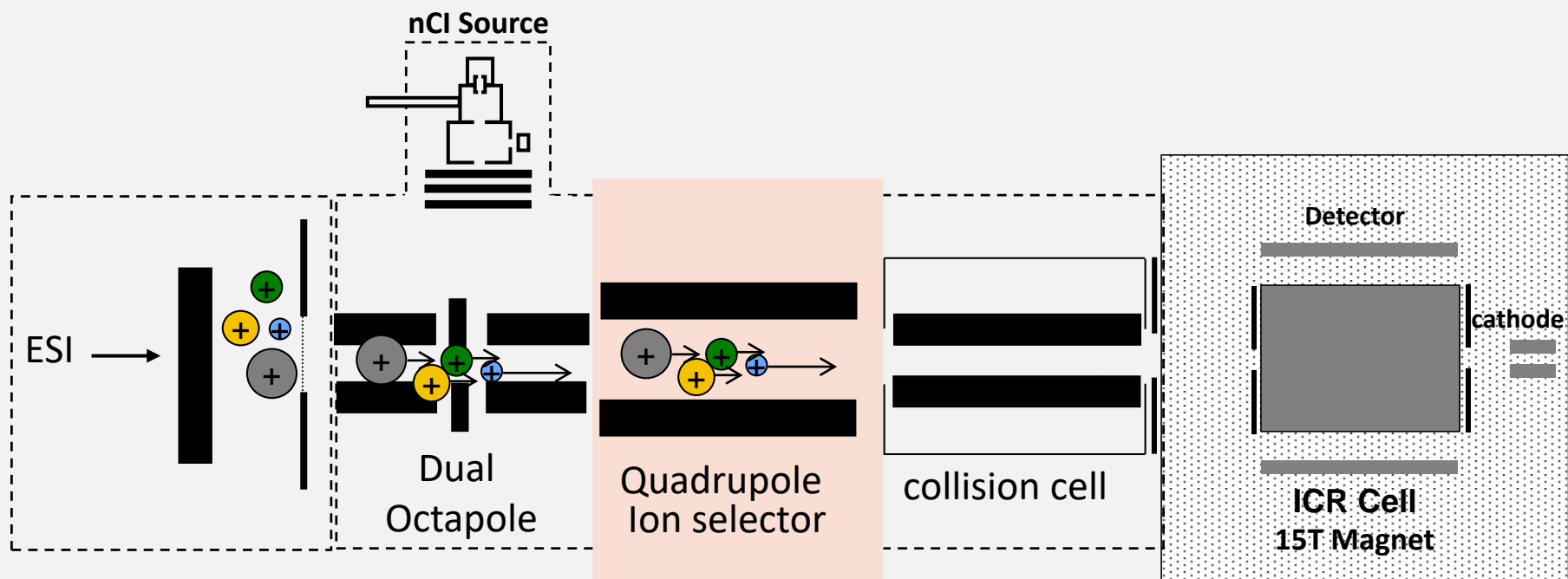
Electron-capture dissociation (ECD)



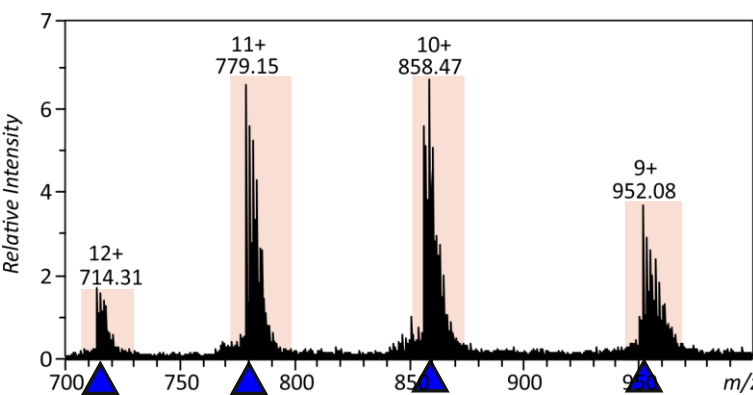
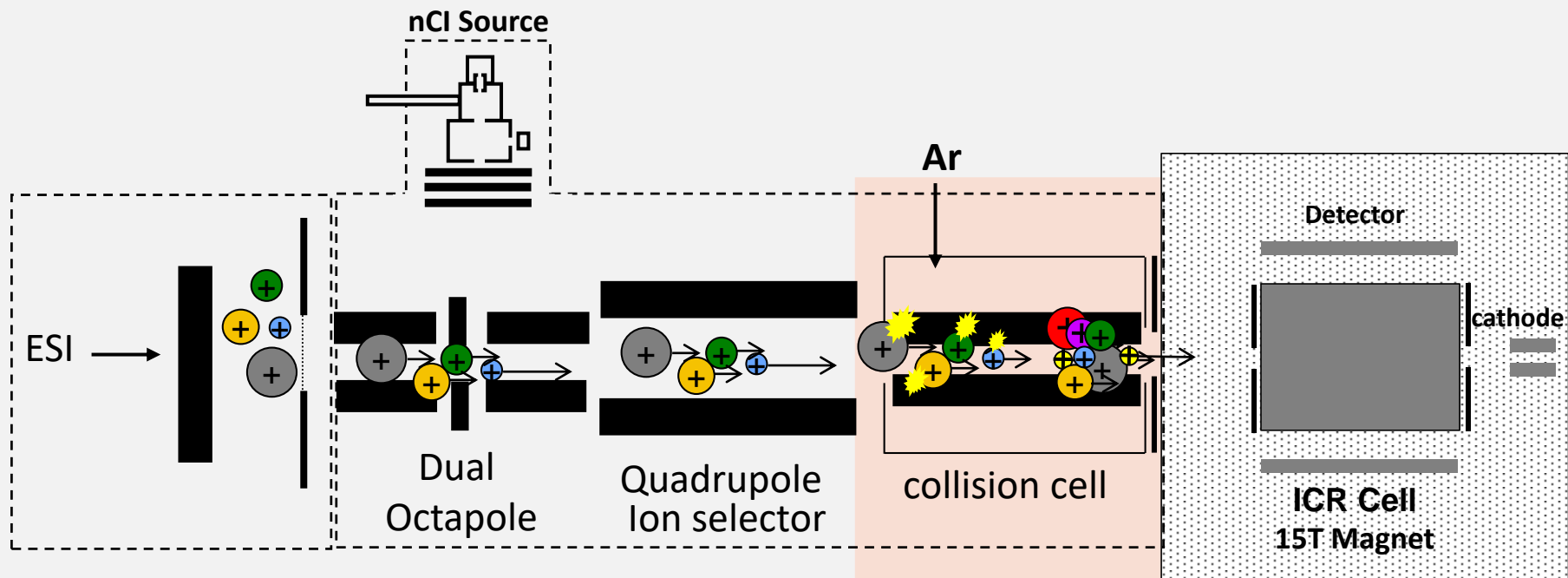
MultiCASI



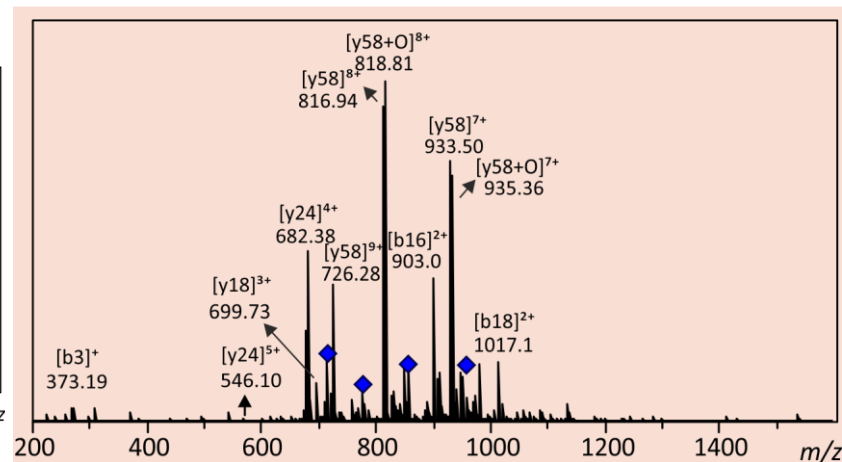
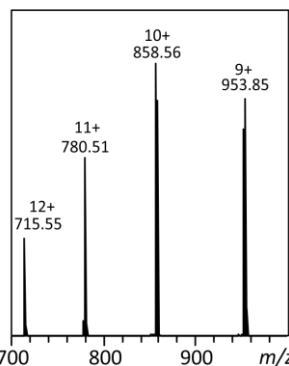
MultiCASI



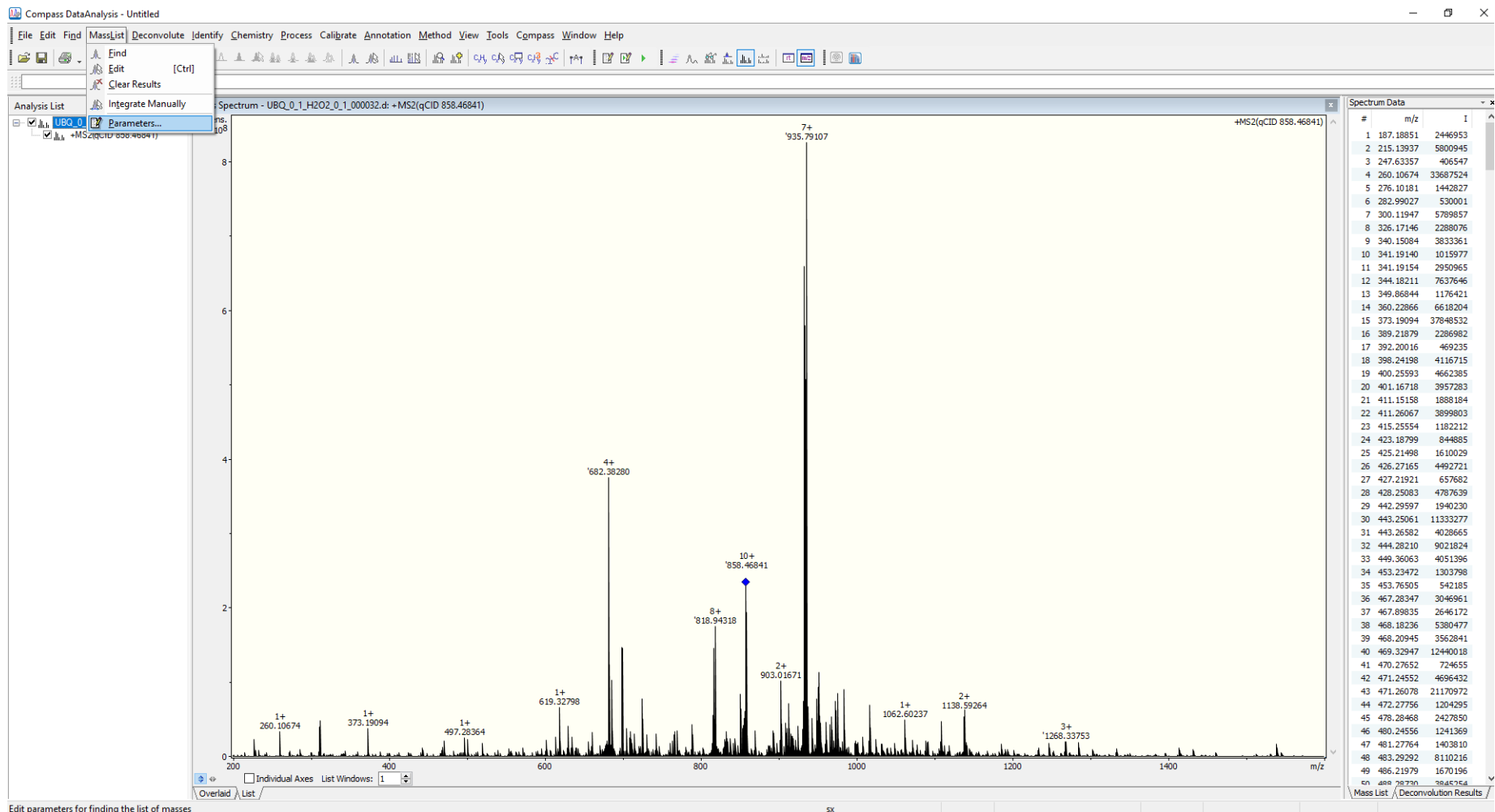
MultiCASI



+1 oxidation

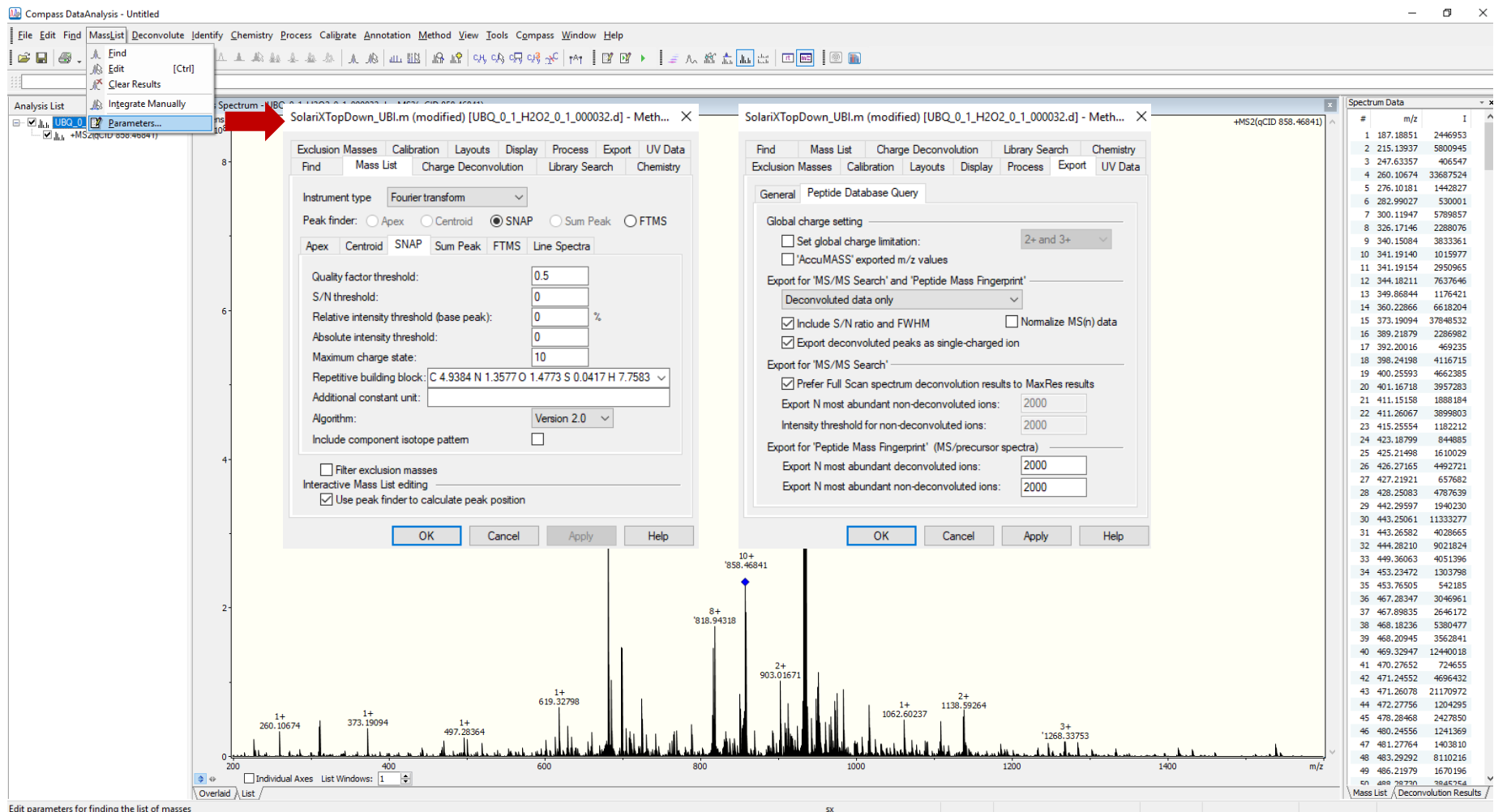


Data Analysis: ms2links Evaluation



Young MM Proc Natl Acad Sci U S A. 2000; 97:5802-6.
Schilling B J Am Soc Mass Spectrom. 2003;14:834-50.

Data Analysis: ms2links Evaluation



Data Analysis: ms2links Evaluation

Compass DataAnalysis - Untitled

File Edit Find Mass List Deconvolute Identify Chemistry Process Calibrate Annotation Method View Tools Cgpass Window Help

Analysis List: UBQ_0, Parameters...

Spectrum: SolarixTopDown_UBI.m (modified) [UBQ_0_1_H2O2_0_1_000032.d] - Meth...

Exclusion Masses Calibration Layouts Display Process Export UV Data

Find Mass List Charge Deconvolution Library Search Chemistry

Instrument type: Fourier transform

Peak finder: ☐ Apex ☐ Centroid ☒ SNAP ☐ Sum Peak ☐ FTMS

Apex Centroid SNAP Sum Peak FTMS Line Spectra

Quality factor threshold: 0.5

S/N threshold: 0

Relative intensity threshold (base peak): 0

Absolute intensity threshold: 0

Maximum charge state: 10

Repetitive building block: C 4.9384 N 1.3577 O 1.4773 S 0.0417 H 7.7589

Additional constant unit:

Algorithm: Version 2.0

Include component isotope pattern ☐

☐ Filter exclusion masses

Interactive Mass List editing ☒ Use peak finder to calculate peak position

OK Cancel Apply Help

Select Quality Factor threshold

Select max charge state

General Peptide Database Query

Find Mass List Charge Deconvolution Library Search Chemistry

Exclusion Masses Calibration Layouts Display Process Export UV Data

Global charge setting

☐ Set global charge limitation: 2+ and 3+

☐ 'AccuMASS' exported m/z values

Export for 'MS/MS Search' and 'Peptide Mass Fingerprint'

Deconvoluted data only

☒ Include S/N ratio and FWHM ☐ Normalize MS(n) data

☒ Export deconvoluted peaks as single-charged ion

Export for 'MS/MS Search'

☒ Prefer Full Scan spectrum deconvolution results to MaxRes results

Export N most abundant non-deconvoluted ions: 2000

Intensity threshold for non-deconvoluted ions: 2000

Export for 'Peptide Mass Fingerprint' (MS/precursor spectra)

Export N most abundant deconvoluted ions: 2000

Export N most abundant non-deconvoluted ions: 2000

OK Cancel Apply Help

Spectrum Data

#	m/z	I
1	187.18851	2446953
2	215.13937	5800945
3	247.63357	406547
4	260.10674	33687524
5	276.10181	1442827
6	282.99027	530001
7	300.11947	5789857
8	326.17146	2288076
9	340.15084	3833361
10	341.19140	1015977
11	341.19154	2950965
12	344.18211	7637646
13	348.86844	1176421
14	360.22866	6618204
15	373.19094	37848532
16	389.21879	2286982
17	392.20016	469235
18	398.24198	4118715
19	400.25593	4662385
20	401.16718	3957283
21	411.15158	1888184
22	411.26067	3899803
23	415.25554	1182212
24	423.18799	844885
25	425.21498	1610029
26	426.27165	4492721
27	427.21921	657682
28	428.25083	4787639
29	442.29597	1940230
30	443.25061	11333277
31	443.26582	4028665
32	444.28210	9021824
33	449.36063	4051396
34	453.23472	1303798
35	453.76505	542185
36	467.28347	3046961
37	467.89835	2646172
38	468.18236	5380477
39	468.20945	3562841
40	469.32947	12440018
41	470.27652	724655
42	471.24552	4696432
43	471.26078	21170972
44	472.27756	1204295
45	478.28468	2427850
46	480.24556	1241369
47	481.27764	1403810
48	483.29292	8110216
49	486.21979	1670196
50	488.28730	3845254

Mass List / Deconvolution Results

Edit parameters for finding the list of masses

Overlay List

Individual Axes List Windows: 1

OK Cancel Apply Help

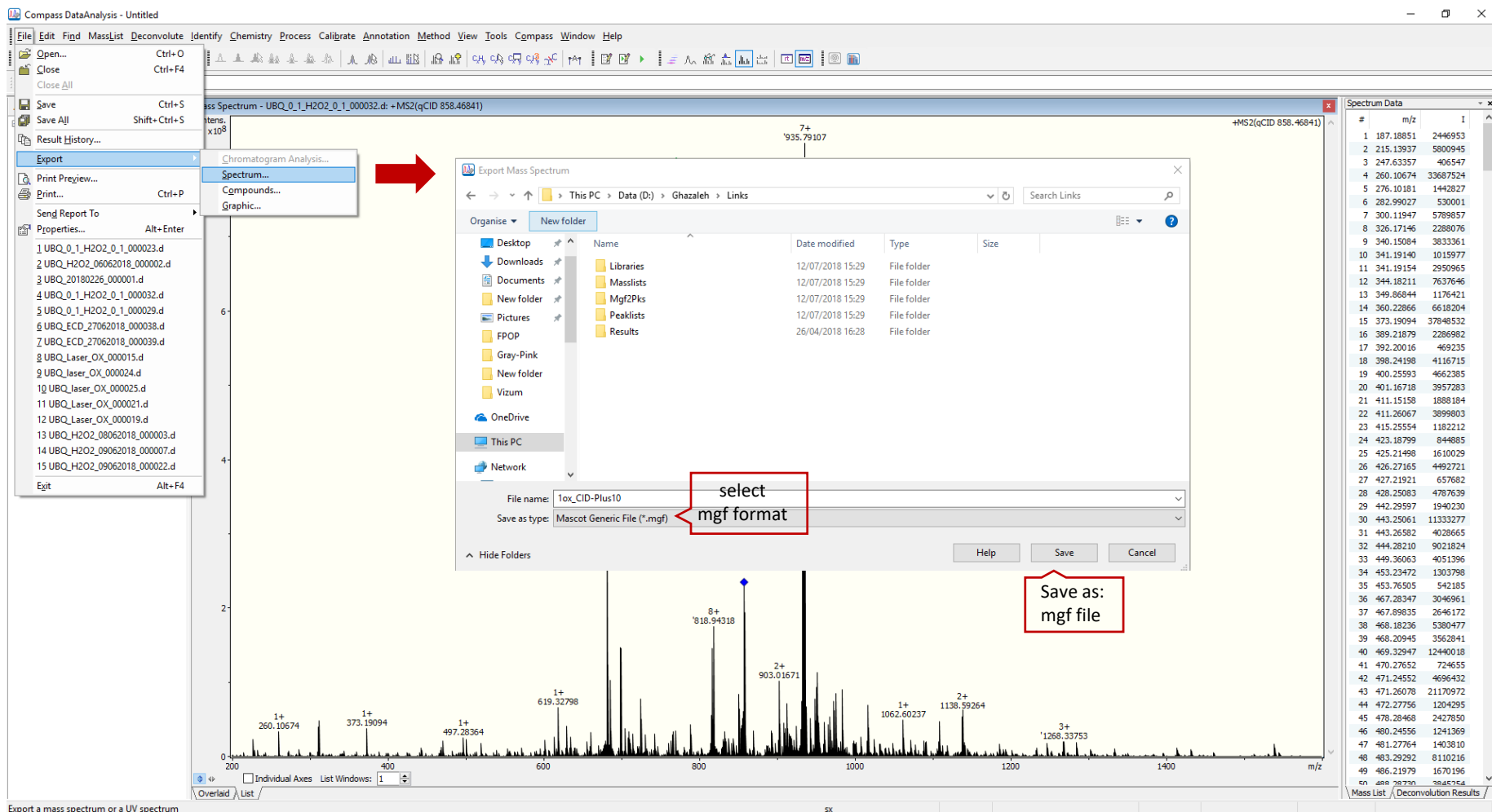
Mass List / Deconvolution Results

Data Analysis: ms2links Evaluation

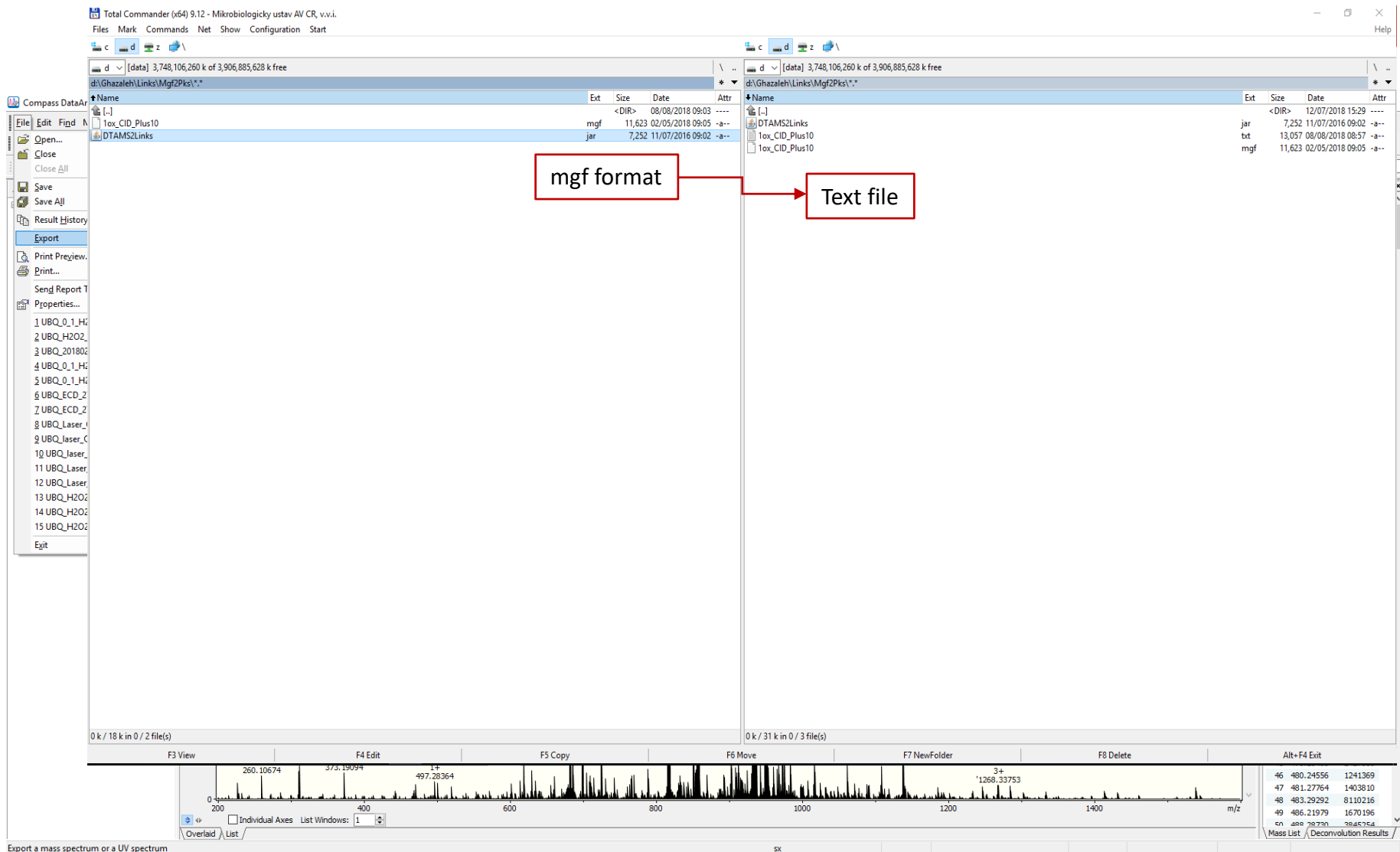
Data file format:

For an MS/MS Ions Search, the data file contain one or more MS/MS peak lists.

In the Mascot generic format, (MGF), each MS/MS dataset → pairs of mass and intensity values



Data Analysis: ms2links Evaluation



Data Analysis: ms2links Evaluation

Total Commander (x64) 9.12 - Mikrobiologický ústav AV ČR, v.v.i.

Files Mark Commands Net Show Configuration Start

Compass Data Analysis

File Edit Find

Open... Close Close All Save Save All Result History Export Print Preview Print... Send Report T Properties...

1 UBQ_0_1_H2 2 UBQ_H2O2 3 UBQ_201802 4 UBQ_0_1_H2 5 UBQ_0_1_H2 6 UBQ_ECD_2 7 UBQ_ECD_2 8 UBQ_Laser_1 9 UBQ_Laser_C 10 UBQ_Laser 11 UBQ_Laser 12 UBQ_Laser 13 UBQ_H2O2 14 UBQ_H2O2 15 UBQ_H2O2

Exit

0 k / 18 k in 0 / 2 file(s)

F3 View

Export a mass spectrum or a UV spectrum

Ext Size Date Attr

<DIR> 08/08/2018 09:03 ---- 1ox_CID_Plus10 mgf 11,623 02/05/2018 09:05 -a-- DTAMS2Links jar 7,252 11/07/2016 09:02 -a--

mgf format

Text file

Mass of ions

Intensity of ions

File	Edit	Format	View	Help
1	229.118636	Y	7197043	###
1	243.08015	Y	4209797	###
1	260.106752	Y	28636670	###
1	326.171449	Y	1495552	###
1	342.202823	Y	2652776	###
1	344.182097	Y	4888717	###
1	360.228653	Y	4267283	###
1	373.19089	Y	27950146	###
1	389.218812	Y	2019773	###
1	400.255875	Y	3776125	###
1	401.167184	Y	3786421	###
1	411.151471	Y	1508507	###
1	428.25088	Y	3324779	###

F8 Delete

Alt+F4 Exit

46 480.24556 1241369 47 481.27764 1403810 48 483.29292 8110216 49 486.21979 1670196 50 488.28730 3846754

Mass List Deconvolution Results

Data Analysis: ms2links Evaluation

Total Commander (x64) 9.12 - Mikrobiologicky ustav AV CR, v.v.i.

Files Mark Commands Net Show Configuration Start

c d z \

d [data] 3,748,364,632 k of 3,906,885,628 k free

d:\Ghazaleh\Links\.*

Name	Ext	Size	Date	Attr
[.]				
[Libraries]				
[Masslists]				
[Mgf2Pks]				
[Peaklists]				
[Results]				
ADSL				
ang_I				
ang_II				
apro_EIA_PE_112103				
apro_vp_112103				
aprotinin				
asap.new				
avgDists				
insulinbox				
io				
library				
links				
links				
Links_mod				
links_mod				
Links_mod_old				
melittinLib				
mod_table				
mod_table				
mod_table1				
mod_table2				
ms2links				
ms2links				
Ms2links				
ms2pro				
ms2proLC				
Myoglobin_horse				
peakRed				
peakRedQTOF				
prLogFile				
report				
rho_EMCS_CNBr				
Rho_ros_ctrl_011503_pk				
sortPeaks				
tmpPeaks				
ubiquitin				
wordpad				

ms2links.in - Notepad

File Edit Format View Help

```
fasta_file      ubiquitin.fasta
mod_table       mod_table
isotopic_masses yes
error_in_ppm    yes
error           3
write_library   no
internal_ions   yes
interfragment_xlink no
xlinks_only     no
include_rare_ions no
loss_of_H2O     no
loss_of_NH3     no
loss_of_CO      no
loss_of_C202    no
a_ions          no
b_ions          yes
c_ions          no
d_ions          no
w_ions          no
x_ions          no
y_ions          yes
z_ions          no
#seq_type       protein
```

0 k / 6,978 k in 0 / 37 file(s), 0 / 5 dir(s)

d:\Ghazaleh\Links\Mgf2Pks> fflu

F3 View F4 Edit F5 Copy F6 Mx

Data Analysis: ms2links Evaluation

Total Commander (x64) 9.12 - Mikrobiologicky ustav AV CR, v.v.i.

Files Mark Commands Net Show Configuration Start

c d z \

d [data] 3,748,364,632 k of 3,906,885,628 k free

d:\Ghazaleh\Links\.*

Name	Ext	Size	Date	Attr
[.]				
[Libraries]				
[Masslists]				
[Mgf2Pks]				
[Peaklists]				
[Results]				
ADSL				
ang_I				
ang_II				
apro_EIA_PE_112103				
apro_vp_112103				
aprotinin				
asap.new				
avgDists				
insulinbox				
io				
library				
links				
links				
Links_mod				
links_mod				
Links_mod_old				
melittinLib				
mod_table				
mod_table				
mod_table1				
mod_table2				
ms2links				
ms2links				
Ms2links				
ms2pro				
ms2proLC				
Myoglobin_horse				
peakRed				
peakRedQTOF				
prLogFile				
report				
rho_EMCS_CNBr				
Rho_ros_ctrl_011503_pk				
sortPeaks				
tmpPeaks				
ubiquitin				
wordpad				

ms2links.in - Notepad

File Edit Format View Help

```
fasta_file      ubiquitin.fasta
mod_table       mod_table
isotopic_masses yes
error_in_ppm    yes
error           3
write_library   no
internal_ions    yes
interfragment_xlink no
xlinks_only     no
include_rare_ions no
loss_of_H2O     no
loss_of_NH3     no
loss_of_CO      no
loss_of_C202    no
a_ions          no
b_ions          yes
c_ions          no
d_ions          no
w_ions          no
x_ions          no
y_ions          yes
z_ions          no
#seq_type       protein
```

Select internal ions for CID

Select b ions for CID

Select y ions for CID

0 k / 6,978 k in 0 / 37 file(s), 0 / 5 dir(s)

d:\Ghazaleh\Links\Mgf2Pks> ffuu

F3 View F4 Edit F5 Copy F6 Mx

Data Analysis: ms2links Evaluation

Total Commander (x64) 9.12 - Mikrobiologicky ustav AV CR, v.v.i.

Files Mark Commands Net Show Configuration Start

c d z \

d [data] 3,748,364,632 k of 3,906,885,628 k free

d:\Ghazaleh\Links*

Name Ext

[..]
[Libraries]
[Masslists]
[Mgf2Pks]
[Peaklists]
[Results]
ADSL
ang_I
ang_II
apro_EIA_PE_112103
apro_vp_112103
aprotinin
asap.new
avgDists
insulinbox
io
library
links
links
links_mod
links_mod
links_mod_old
melittinLib
mod_table
mod_table
mod_table1
mod_table2
ms2links
ms2links
ms2pro
ms2proLC
Myoglobin_horse
peakRed
peakRedQTOF
prLogFile
report
rho_EMCS_CNBr
Rho_ros_ctrl_011503_pk
sortPeaks
tmpPeaks
ubiquitin
wordpad

ms2links.in - Notepad

File Edit Format View Help

fasta_file ubiquitin.fasta
mod_table mod_table
isotopic_masses yes
error_in_ppm yes
error 3
write_library no
internal_ions yes
interfragment_xlink no
xlinks_only no
include_rare_ions no
loss_of_H2O no
loss_of_NH3 no
loss_of_CO no
loss_of_C202 no
a_ions no
b_ions yes
c_ions no
d_ions no
w_ions no
x_ions no
y_ions yes
z_ions no
#seq_type protein

Select internal ions for CID

Select b ions for CID

Select y ions for CID

ubiquitin.fasta - Notepad

File Edit Format View Help

>sp|P02248|UBIQ_HUMAN Ubiquitin
MQIFVKLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG

mod_table - Notepad

File Edit Format View Help

#MOD	1	A	73.0289	*	*	DEPC
##### MODIFICATIONS #####						
#MOD	1	M	15.994914	*	*	ox-M
#MOD	1	W	15.994914	*	*	ox-W
#MOD	1	LIRKMYFH	15.994914	*	*	1 OX
#MOD	1	C	105.05785	*	*	PE
#MOD	2	C	105.05785	*	*	PE
#MOD	1	C	238.22967	322	*	Palm
#MOD	1	C	238.22967	323	*	Palm
#MOD	2	C	238.22967	322	*	Palm
#MOD	2	C	238.22967	323	*	Palm
#MOD	1	N	1095.3968	2	*	Glyc
#MOD	1	N	1095.3968	15	*	Glyc
#MOD	2	N	1095.3968	2	*	Glyc
#MOD	2	N	1095.3968	15	*	Glyc

0 k / 6,978 k in 0 / 37 file(s), 0 / 5 dir(s)

d:\Ghazaleh\Links\Mgf2Pks> ffuu

F3 View F4 Edit F5 Copy F6 Mx

Data Analysis: ms2links Evaluation

Total Commander (x64) 9.12 - Mikrobiologicky ustav AV CR, v.v.i.

Files Mark Commands Net Show Configuration Start

c d z \

d [data] 3,748,364,632 k of 3,906,885,628 k free

d:\Ghazaleh\Links*

Name

ms2links.in - Notepad

File Edit Format View Help

fasta_file ubiquitin.fasta

mod_table mod_table

isotopic_masses yes

error_in_ppm yes

error 3

write_library no

internal_ions yes **Select internal ions for CID**

interfragment_xlink no

xlinks_only no

include_rare_ions no

loss_of_H2O no

loss_of_NH3 no

loss_of_CO no

loss_of_C2O2 no

a_ions no

b_ions yes **Select b ions for CID**

c_ions no

d_ions no

w_ions no

x_ions no

y_ions yes **Select y ions for CID**

z_ions no

#seq_type protein **# Inactivate for unmodified**

ubiquitin.fasta - Notepad

File Edit Format View Help

>sp|P02248|UBIQ_HUMAN Ubiquitin

MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG

mod_table - Notepad

File Edit Format View Help

#MOD	1	A	73.0289	*	*	DEPC
##### MODIFICATIONS #####						
#MOD	1	M	15.994914	*	*	ox-M
#MOD	1	W	15.994914	*	*	ox-W
#MOD	1	LIRKMYFH	15.994914	*	1	OX
#MOD	1	C	105.05785	*	*	PE
#MOD	2	C	105.05785	*	*	PE
#MOD	1	C	238.22967	322	*	Palm
MOD	1	LIRKMYFH	15.994914	*	1	OX
MOD	1	LIRKMYFH	15.994914	*	2	OX
#MOD	1	N	1095.3968	2	*	Glyc
#MOD	1	N	1095.3968	15	*	Gly
#MOD	2	N	1095.3968	2	*	Gly
#MOD	2	N	1095.3968	15	*	Gly

Choose number of modification

0 k / 6,978 k in 0 / 37 file(s), 0 / 5 dir(s)

d:\Ghazaleh\Links\Mgf2Pks> ffuu

F3 View F4 Edit F5 Copy F6 Mx

Data Analysis: Result ms2links Evaluation

```

10x_CID_Plus10_results.txt - Notepad
File Edit Format View Help

LIRKMYFH      15.995  0      1      OX

1 modifications read in.
MS2Link parameters are:

      Fasta file name = ubiquitin.fasta
      Allowed mass error (ppm) = 3.00
      Internal ions?   yes

Including b ions.
Including y ions.
Generating isotopic mass list...
>sp|P02248|UBIQ_HUMAN Ubiquitin
Length: 76
MQIFVKTLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRRLGG
Attempting to autodetect sequence type...
Input : protein sequence
Search results :

Expno    C13MHobs    MHcalc    Err    Ion    Seq Tag    Int
1        260.1068    260.1069    0.6    b2      MQ        28636670
1        326.1714    326.1716    0.5    b(17-19) VEP        1495552
1        342.2028    342.2029    0.3    b(15-17) LEV        2652776
1        344.1821    344.1822    0.2    b(14-16) TLE        4888717
1        344.1821    344.1822    0.2    b(22-24) TIE        4888717
1        360.2287    360.2287    0.2    b(3-5)   IFV        4267283
1        373.1909    373.1909    0.2    b3       MQI        27950146
1        520.2595    520.2594    0.1    b4       MQIF       13311483
1        528.2305    528.2306    0.1    b(17-21) VEPSD     917427
1        536.2543    536.2543    0.0    b4+10X   MQIF       4048708
1        614.3878    614.3878    0.0    b(8-13) LTGKTI    15184581
1        619.3279    619.3278    0.2    b5       MQIFV     41329364
1        635.3228    635.3228    0.1    b5+10X   MQIFV     11080986
    
```

Data Analysis: Result ms2links Evaluation

```
1ox_CID_Plus10_results.txt - Notepad
File Edit Format View Help

LIRKMYFH      15.995  0      1      0X

1 modifications read in.
MS2Link parameters are:

      Fasta file name = ubiquitin.fasta
      Allowed mass error (ppm) = 3.00
      Internal ions? yes

Including b ions.
Including y ions.
Generating isotopic mass list...
>sp|P02248|UBIQ_HUMAN Ubiquitin
Length: 76
MQIFVKTLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRLLRG
Attempting to autodetect sequence type...
Input : protein sequence
Search results :
```

Expno	C13MHobs	MHcalc	Err	Ion	Seq Tag	Int
1	260.1068	260.1069	0.6	b2	MQ	28636670
1	326.1714	326.1716	0.5	b(17-19)	VEP	1495552
1	342.2028	342.2029	0.3	b(15-17)	LEV	2652776
1	344.1821	344.1822	0.2	b(14-16)	TLE	4888717
1	344.1821	344.1822	0.2	b(22-24)	TIE	4888717
1	360.2287	360.2287	0.2	b(3-5)	IFV	4267283
1	373.1909	373.1909	0.2	b3	MQI	27950146
1	520.2595	520.2594	0.1	b4	MQIF	13311483
1	528.2305	528.2306	0.1	b(17-21)	VEPSD	917427
1	536.2543	536.2543	0.0	b4+10X	MQIF	4048708
1	614.3878	614.3878	0.0	b(8-13)	LTGKTI	15184581
1	619.3279	619.3278	0.2	b5	MQIFV	41329364
1	635.3228	635.3228	0.1	b5+10X	MQIFV	11080986

Data Analysis: Result ms2links Evaluation

1ox_CID_Plus10_results.txt - Notepad

File Edit Format View Help

LIRKMYFH 15.995 0 1 0X

1 modifications read in.
MS2Link parameters are:

Fasta file name = ubiquitin.fasta
Allowed mass error (ppm) = 3.00
Internal ions? yes

Including b ions.
Including y ions.
Generating isotopic mass list...
>sp|P02248|UBIQ_HUMAN Ubiquitin
Length: 76
MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRGG

Attemptin...
Input : p
Search re

Experimental mass of ions
Theoretical mass of ions

Ions sequence
Intensity of ions

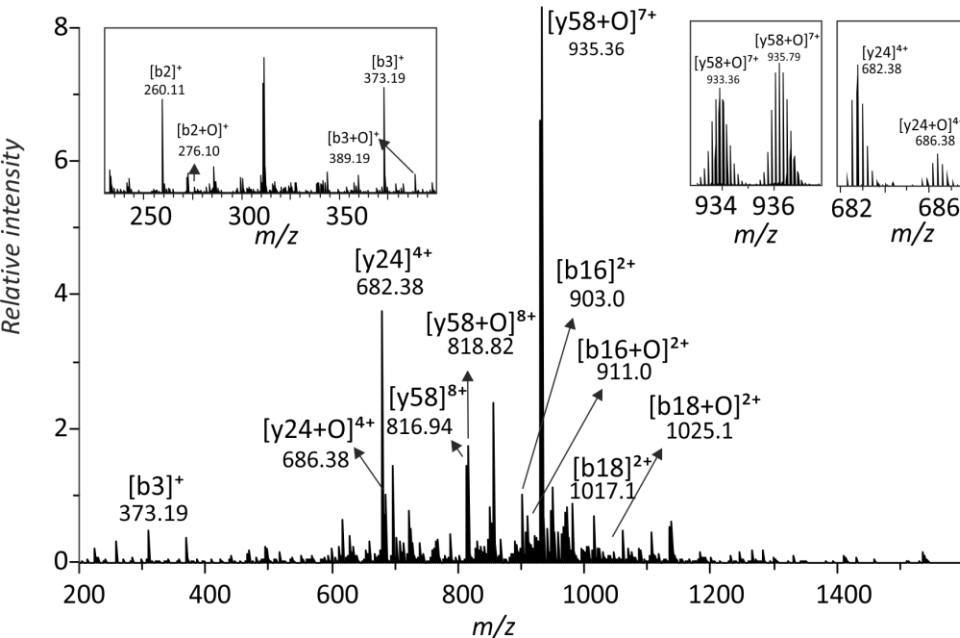
Expno	C13MHobs	MHcalc	Err	Ion	Seq Tag	Int
1	260.1068	260.1069	0.6	b2	MQ	28636670
1	326.1714	326.1716	0.5	b(17-19)	VEP	1495552
1	342.2028	342.2029	0.3	b(15-17)	LEV	2652776
1	344.1821	344.1822	0.2	b(14-16)	TLE	4888717
1	344.1821	344.1822	0.2	b(22-24)	TIE	4888717
1	360.2287	360.2287	0.2	b(3-5)	IFV	4267283
1	373.1909	373.1909	0.2	b3	MQI	27950146
1	520.2595	520.2594	0.1	b4	MQIF	13311483
1	528.2305	528.2306	0.1	b(17-21)	VEPSD	917427
1	536.2543	536.2543	0.1	b4+10X	MQIF	4048708
1	614.3878	614.3878	0.1	b(8-13)	LTGKTI	15184581
1	619.3279	619.3279	0.1	b5	MQIFV	41329364
1	635.3228	635.3228	0.1	b5+10X	MQIFV	11080986

Detected of unmodified and modified ions

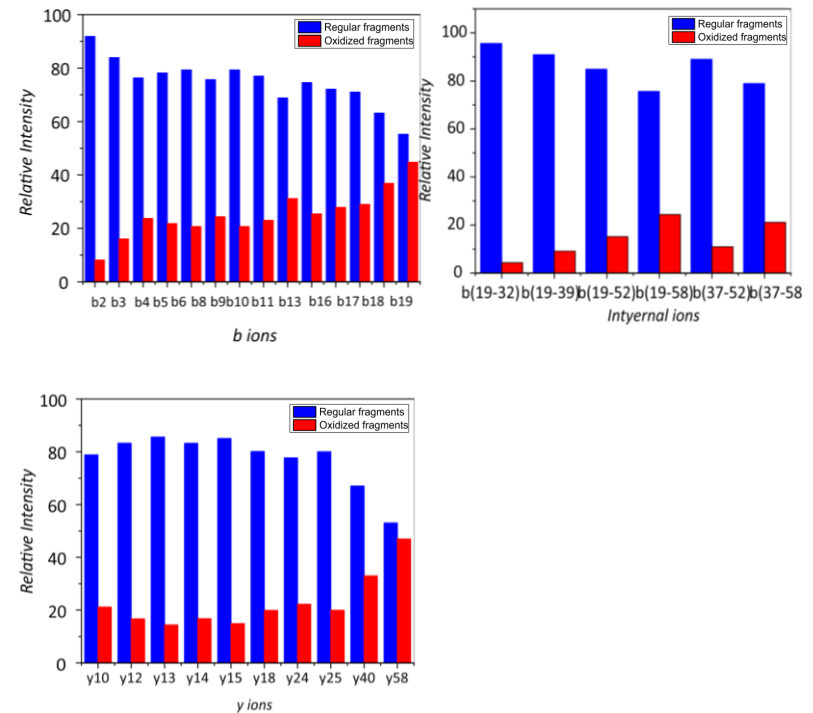
Read the intensities of signals for the modified (I_{ox}) and the unmodified species (I) for each.

Extent of Modification

- CID of single oxidized ubiquitin



- Plot of the yield of oxidized residues in ubiquitin

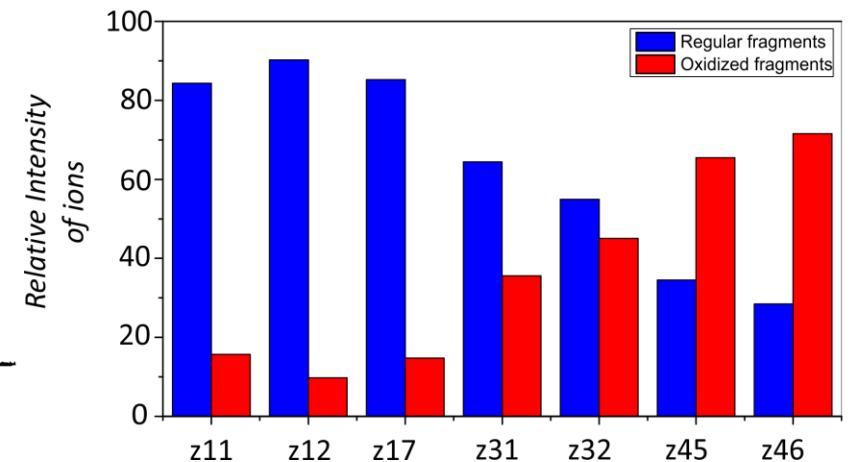
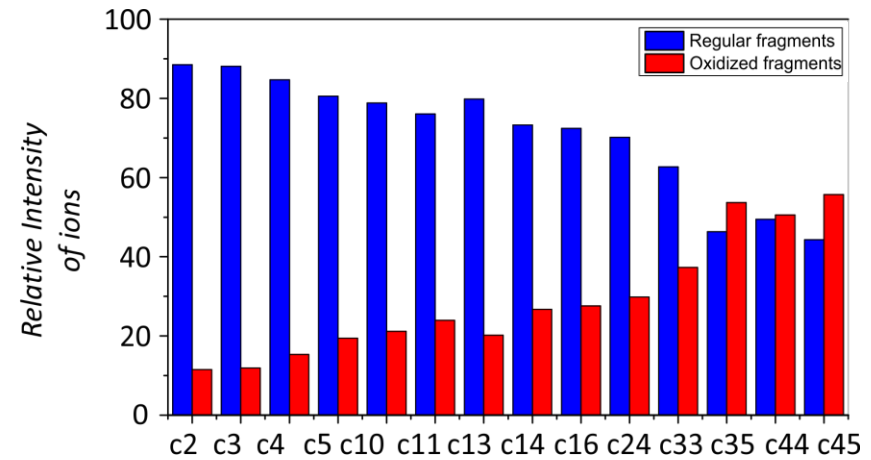
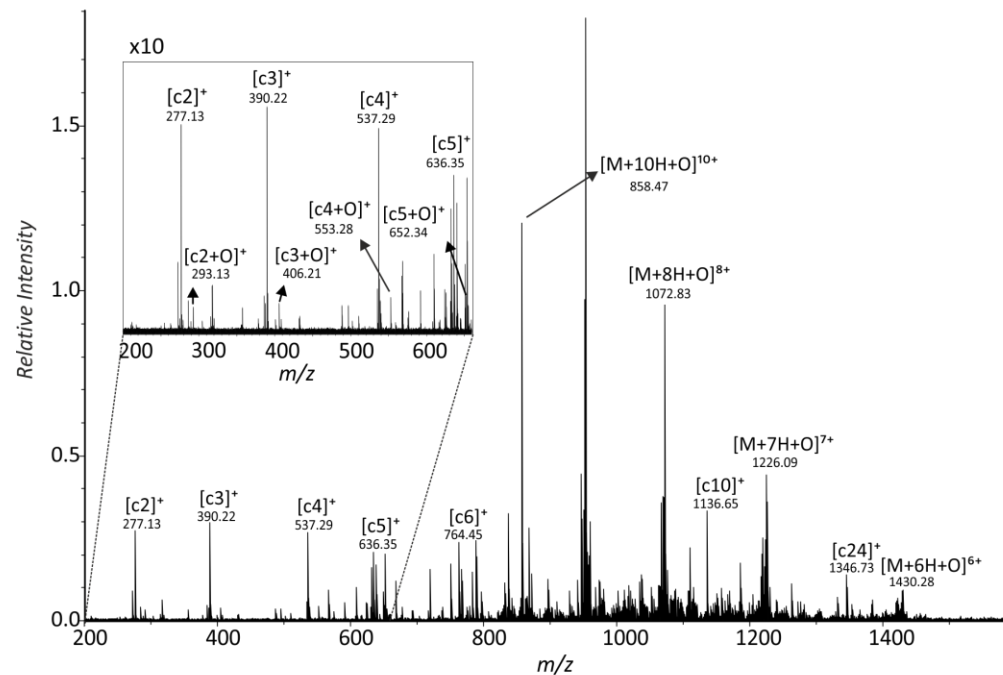
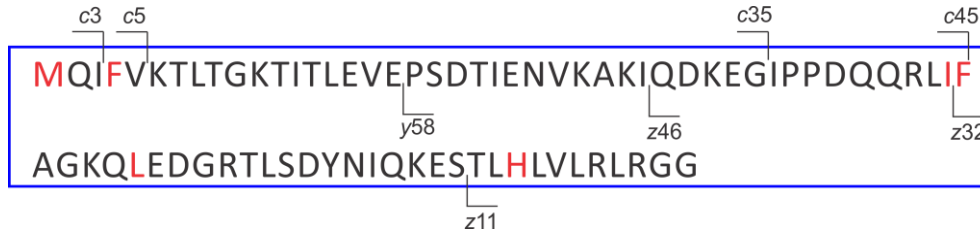


Extent of Modification

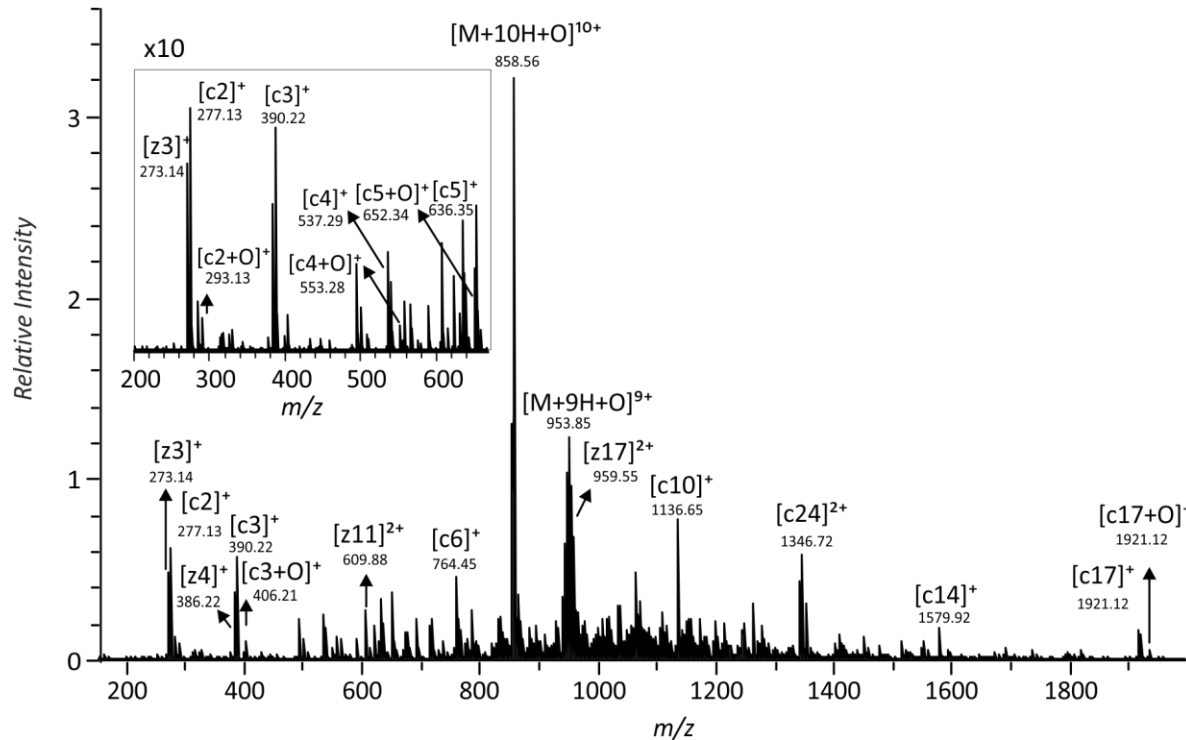
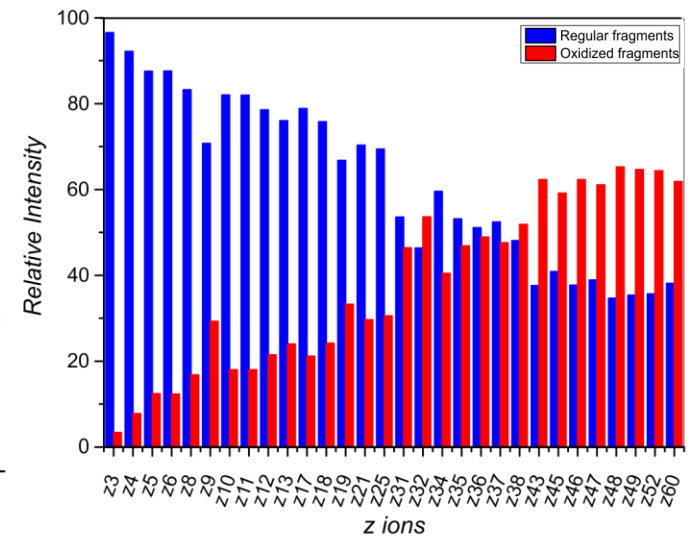
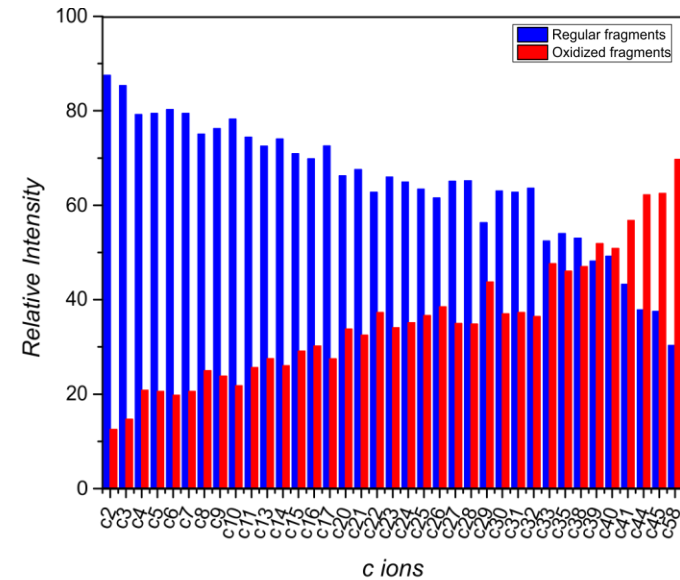
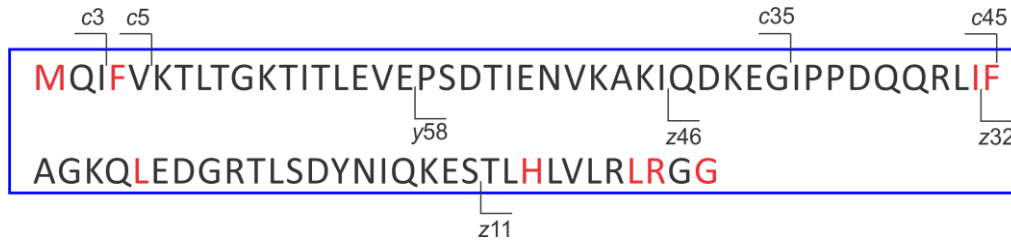
- Read the intensities of signals for the modified (I_{ox}) and the unmodified species (I) for each
- The extents of modification: were calculated by using the following equation:

$$\text{Extent of Modification} = \frac{\sum I_{ox}}{(\sum I_{ox} + I)}$$

ETD of single oxidized ubiquitin

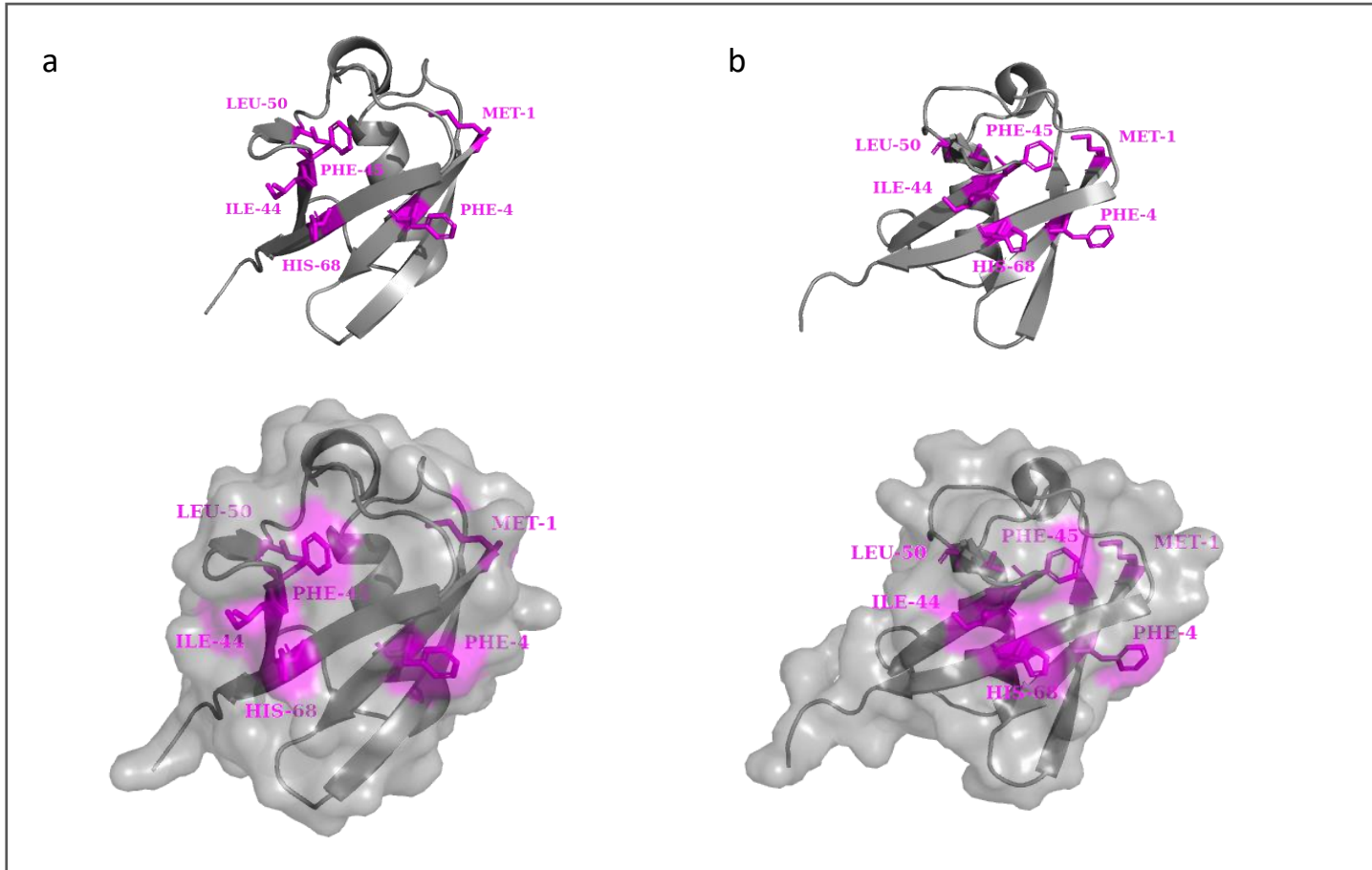


ECD of single oxidized ubiquitin



Crystal structure of oxidized ubiquitin_CID and ETD

¹MQI⁴FVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRL⁴⁴I⁴⁵F
AGKQ⁵⁰L⁵¹EDGRTLSDYNIQKESTL⁶⁸H⁶⁹LVLRLRGG

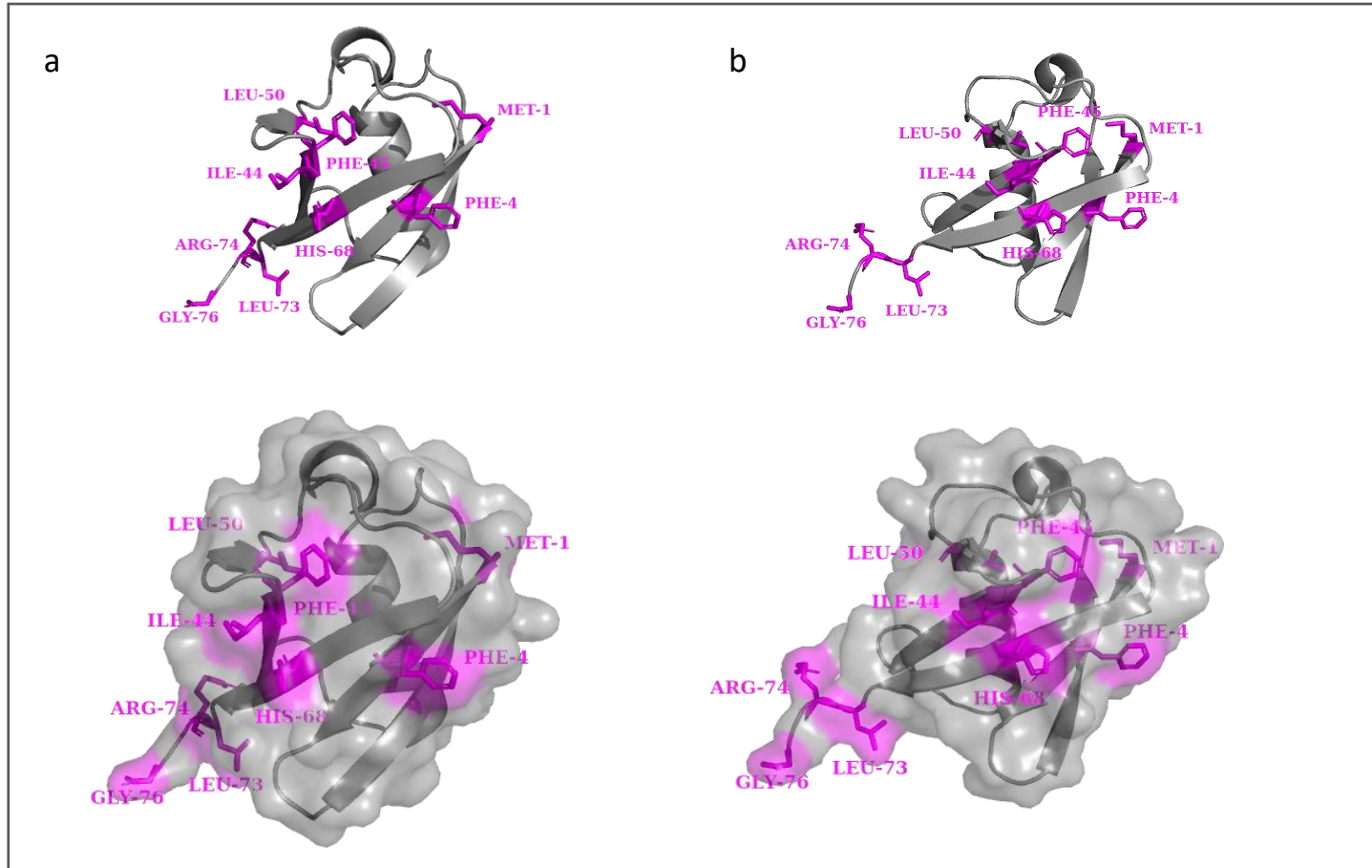


Colored residue → side-chain residues that are modified (violet)

Modified structure from ref. Kumar V. et al. J. Mol. Biol. 194, 531-544 (1987).

Crystal structure of oxidized ubiquitin_ECD

¹MQI⁴FVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRL⁴⁴|⁴⁵F
AGKQ⁵⁰LEDGRTLSDYNIQKESTL⁶⁸HLVLR⁷³L⁷⁴RG⁷⁶G



Colored residue → side-chain residues that are modified (violet)

Modified structure from ref. Kumar V. et al. J. Mol. Biol. 194, 531-544 (1987).

ACKNOWLEDGEMENT



EU FT-ICR MS

GHAZALEH YASSAGHI, LUKAS SLAVATA, ZDENEK KUKACKA, PETR POMPACH,
PETR MAN, DANIEL KAVAN, MICHAL ROSULEK, RUZENA LISKOVA



DANIELE FABRIS, WILL MCINTIRE, MIKE MILLER, MATEO SCALABRIN



GARY KRUPPA, JOE SCHOENIGER, MALIN YOUNG



***H2020 EUROPEAN NETWORK OF FOURIER-TRANSFORM ION-CYCLOTRON-
RESONANCE MASS SPECTROMETRY CENTERS - PROJECT AGREEMENT NO.731077***

CZECH SCIENCE FOUNDATION (GRANT NUMBERS 16-24309S)

***THE MINISTRY OF EDUCATION OF THE CZECH REPUBLIC (PROJECT LH15010; PROGRAMS "NPU II" -
LQ1604 AND LM2015043 CIISB FOR CMS BIOCEV - LTC17065) AND THE EUROPEAN REGIONAL
DEVELOPMENT FUNDS (BIOCEV - CZ.1.05/1.1.00/02.0109)***