UV-POSIT: Web-based Tools for Rapid and Facile Structural Interpretation of Ultraviolet Photodissociation (UVPD) Mass Spectra

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Supplemental Information

a)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
a.a.	Position	All ions	N-terminal	C-terminal	a	a+	b	c	x	x+	y	y-	y--	z
R	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Q	2	429	219	210	0	219	0	0	0	0	0	87	0	123
I	3	749	595	154	200	321	74	0	0	0	0	56	0	98
R	4	594	457	137	0	375	82	0	22	0	0	38	0	77
I	5	1344	1122	222	614	508	0	0	81	0	0	93	0	48
A	6	944	568	376	0	504	21	43	50	0	0	191	0	135
F	7	1041	765	276	0	765	0	0	201	0	0	75	0	0
Q	8	633	357	276	0	326	0	31	76	0	0	200	0	0
N	9	741	344	397	0	249	48	47	84	0	0	169	0	144
R	10	624	422	202	10	369	8	35	36	0	0	0	0	166
R	11	508	213	295	0	213	0	0	0	0	0	295	0	0
M	12	247	109	138	0	109	0	0	36	0	0	102	0	0
R	13	425	250	175	96	51	28	75	66	0	0	41	0	68
W	14	266	266	0	266	0	0	0	0	0	0	0	0	0
R	15	403	106	297	100	0	6	0	0	0	0	129	0	168
R	16	0	0	0	0	0	0	0	0	0	0	0	0	0

b)

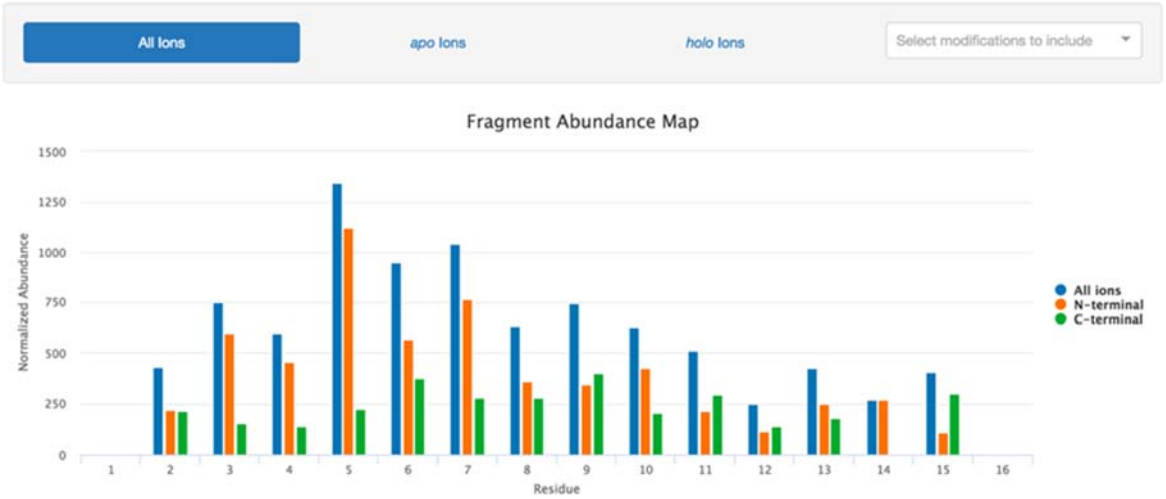

Figure S1: Representative data visualization interface for the fragment abundance utility for the 5+ charge state of peptide RQIRIAFQNRRMRWRR activated by a single 1.5 mJ laser pulse (via 193 nm UVPD).

a) Data tabulation output showing abundances of fragment ions associated with each residue position, grouped by ion type and by C-terminal (x,y,z)/N-terminal (a,b,c) origin.

b) Data visualization output showing abundances for all identified fragments, (blue bars) as well as fragment ions containing the N-terminus (a,b,c) and fragment ion containing the C-terminus (x,y,z) associated with each residue position.

a)

A	B	C	D	E	F	G	H
a.a.	position	1+	2+	3+	4+	5+	6+
R	1	89.38868	0.0	0.0	0.0	0.0	0.0
Q	2	75.8833	0.0	0.0	0.0	0.0	0.0
I	3	0.0	0.0	0.0	0.0	0.0	0.0
R	4	0.0	276.5045	0.0	0.0	0.0	0.0
I	5	0.0	596.5724	0.0	0.0	0.0	0.0
A	6	0.0	272.2974	0.0	0.0	0.0	0.0
F	7	0.0	394.15546	0.0	0.0	0.0	0.0
Q	8	0.0	81.48063	0.0	0.0	0.0	0.0
N	9	0.0	0.0	0.0	0.0	0.0	0.0
R	10	0.0	0.0	389.09594	0.0	0.0	0.0
R	11	0.0	0.0	200.89713	0.0	0.0	0.0
M	12	0.0	0.0	0.0	0.0	0.0	0.0
R	13	0.0	0.0	0.0	184.759	0.0	0.0
W	14	0.0	0.0	0.0	247.654	0.0	0.0
R	15	0.0	0.0	0.0	0.0	47.81111	0.0
R	16	0.0	0.0	0.0	0.0	0.0	0.0

b)

Figure S2: Representative data visualization interface for the charge state analysis utility. Data is visualized here for the 5+ charge state of peptide RQIRIAFQNRRMRWRR activated by a single 1.5 mJ laser pulse (via 193 nm UVPD) with the software set to search for a-type and x-type ions. a) Data tabulation output for N-terminal a-type ions. The results include the identity of the amino acid and residue position (with the N-terminal residue given the number 1) for each a-type ion, as well as the abundance of the ion for each charge state (a value of 0 means that the ion was not found at the given charge state). The abundance values contain excessive significant digits, but they are extracted directly from ProSight output without truncation or rounding.
b) Visualization output with bars to represent the observed charge states of the fragment ions.

a)

Position	Charge	Residue	HEM value	HB value	Non-HB value	Score	Click to Visualize
2	1	Q	0.25	0.25	0.57	4.69E-04	Click to Visualize
3	1	I	0.46	0.25	0.47	2.57E-04	Click to Visualize
4	1	R	0.29	0.37	0.76	1.27E-03	Click to Visualize
5	1	I	0.88	0.25	0.47	9.43E-06	Click to Visualize
7	1	F	0.48	0.33	0.63	4.81E-04	Click to Visualize
8	1	Q	0.53	0.25	0.57	8.16E-04	Click to Visualize
9	1	N	0.71	0.33	0.6	2.08E-04	Click to Visualize
9	2	N	1	0.33	0.6	7.62E-03	Click to Visualize
10	2	R	0.58	0.37	0.76	3.86E-04	Click to Visualize
11	2	R	0.6	0.37	0.76	2.78E-04	Click to Visualize
12	2	M	0.63	0.39	0.79	1.33E-03	Click to Visualize
13	2	R	0.7	0.37	0.76	1.05E-03	Click to Visualize
14	2	A	0.66	0.3	0.63	1.30E-03	Click to Visualize
15	2	R	0.88	0.37	0.76	1.45E-03	Click to Visualize
15	3	R	0.79	0.37	0.76	8.37E-03	Click to Visualize

b)

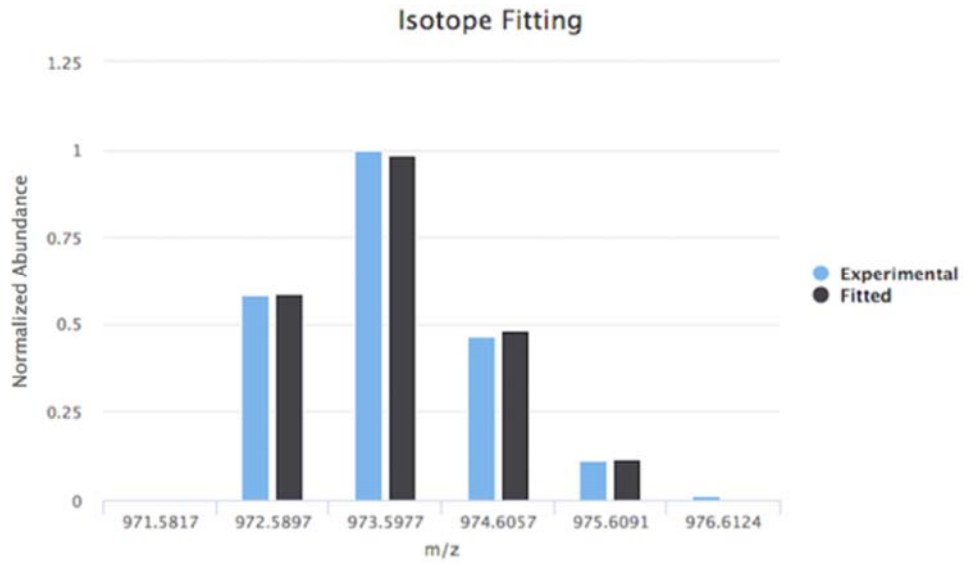

Figure S3: Representative data visualization interface for the hydrogen elimination monitoring (HEM) utility. Data is displayed here for the 3+ charge state of peptide RQIRIAFQNRRMRWRR activated by a single 1.5 mJ laser pulse (via 193 nm UVPD).
a) Data tabulation output for all computed HEM values. The data table gives the experimentally-measured HEM values (abundance of a-type ion as a fraction of total a and a+1 ions for each amino acid located at the C-terminus of an a-type ion) and standard HEM reference values for hydrogen bonded (HB) and non-hydrogen bonded (NHB) conditions (from ref. 8). For example, the seventh residue of the peptide is F, so the HEM value is given for the a_7 ion which terminates in F. Each fitting is assigned a score (listed in the column entitled Score) based on the peak-fit function [17]. Truncation of some of the entries to single significant digit (i.e. 0.7 instead of 0.70) occurs owing to an Excel formatting default.
b) Visualization output for the a_7^+ ion, showing a comparison between the experimental isotope distribution and the fitted distribution computed by the software.

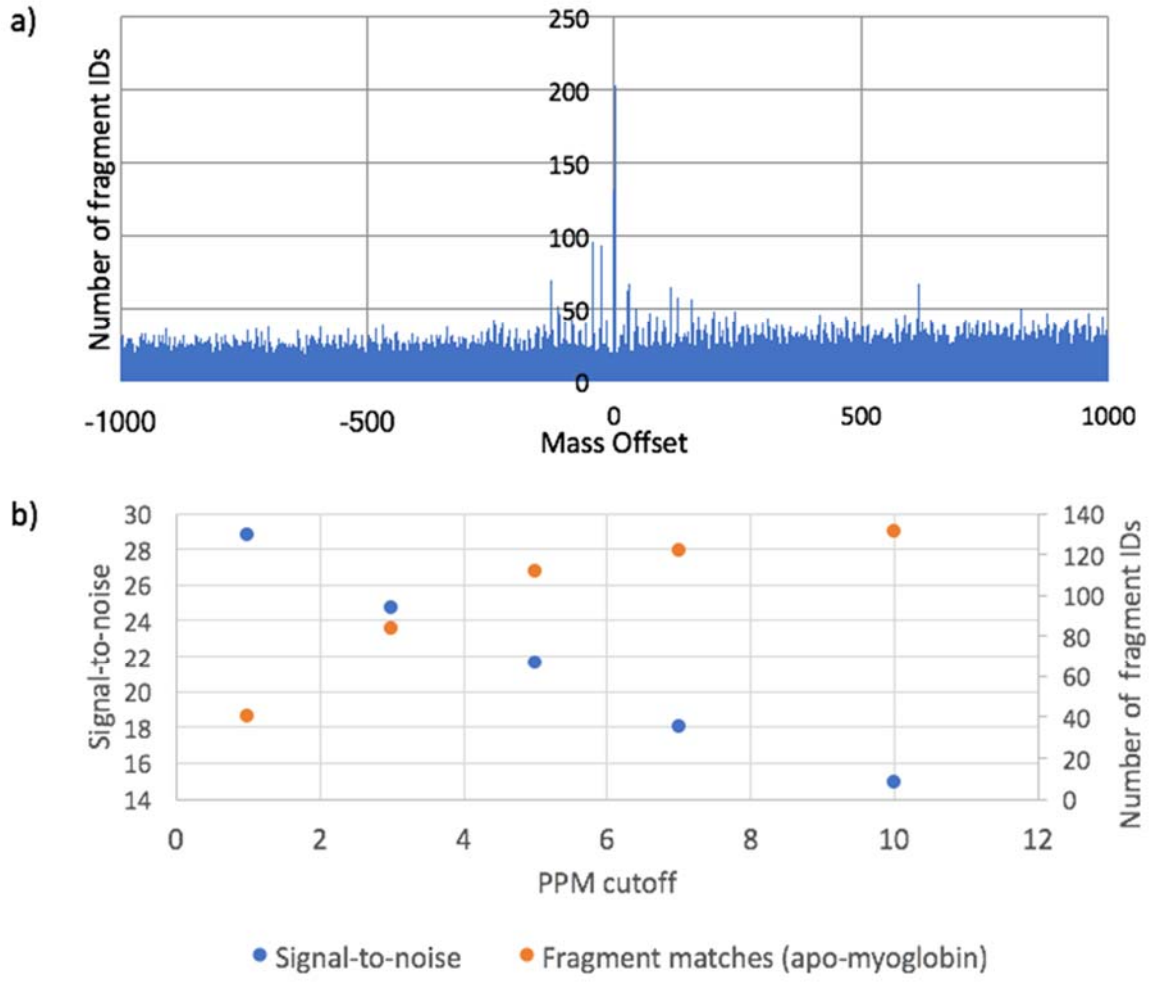

Figure S4:

a) Representative mass-offset search output for *holo*-myoglobin with the error tolerance set to 10 ppm. High noise (suggesting false positive fragment identifications) is observed when arbitrary mass shifts (offsets) are appended to each fragment. For example, 40 peaks are identified as fragments when a mass shift of +285.2 Da is applied, which does not correspond to any expected modification. 63 peaks are identified as fragments when a mass shift of +616.17 is applied, confirming this mass offset which corresponds to attachment of the heme ligand. Positive and negative mass shifts are referenced relative to the protein with no mass shift (0 offset).

b) Graph showing signal-to-noise from the iterative search plot (left axis, blue points) and the number of fragments assigned to *apo*-myoglobin (right axis, orange points) as the ppm error tolerance is narrowed.