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Supplemental Information

Insights on the Conformational Ensemble of Cyt C Reveal a Compact State during Peroxidase Activity

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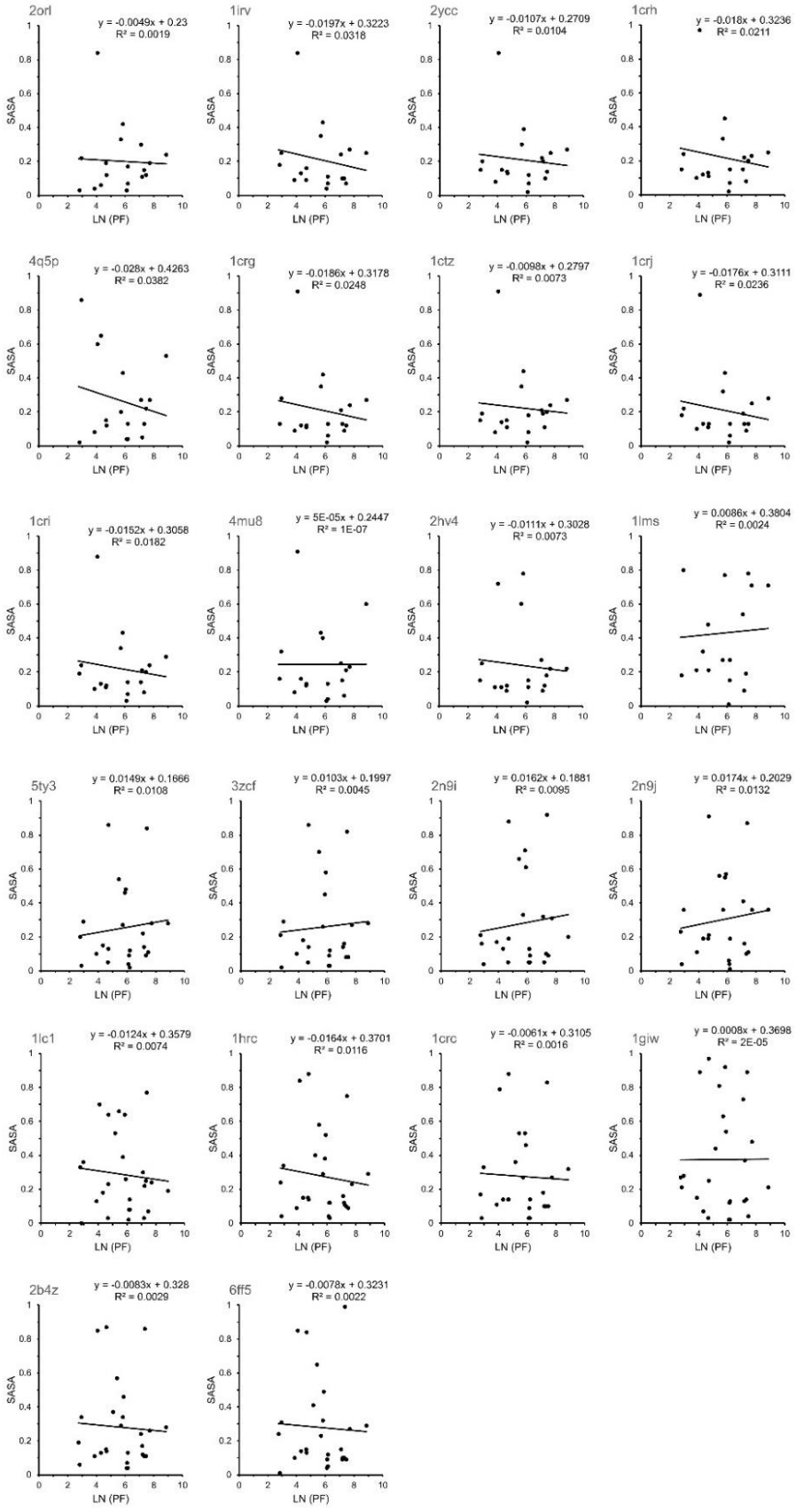

Figure S1. Several protection factor calculations show the SASA values from several cyt c structures do not correlate well with the FPOP modifications on cyt c suggesting there are multiple conformers in solution.

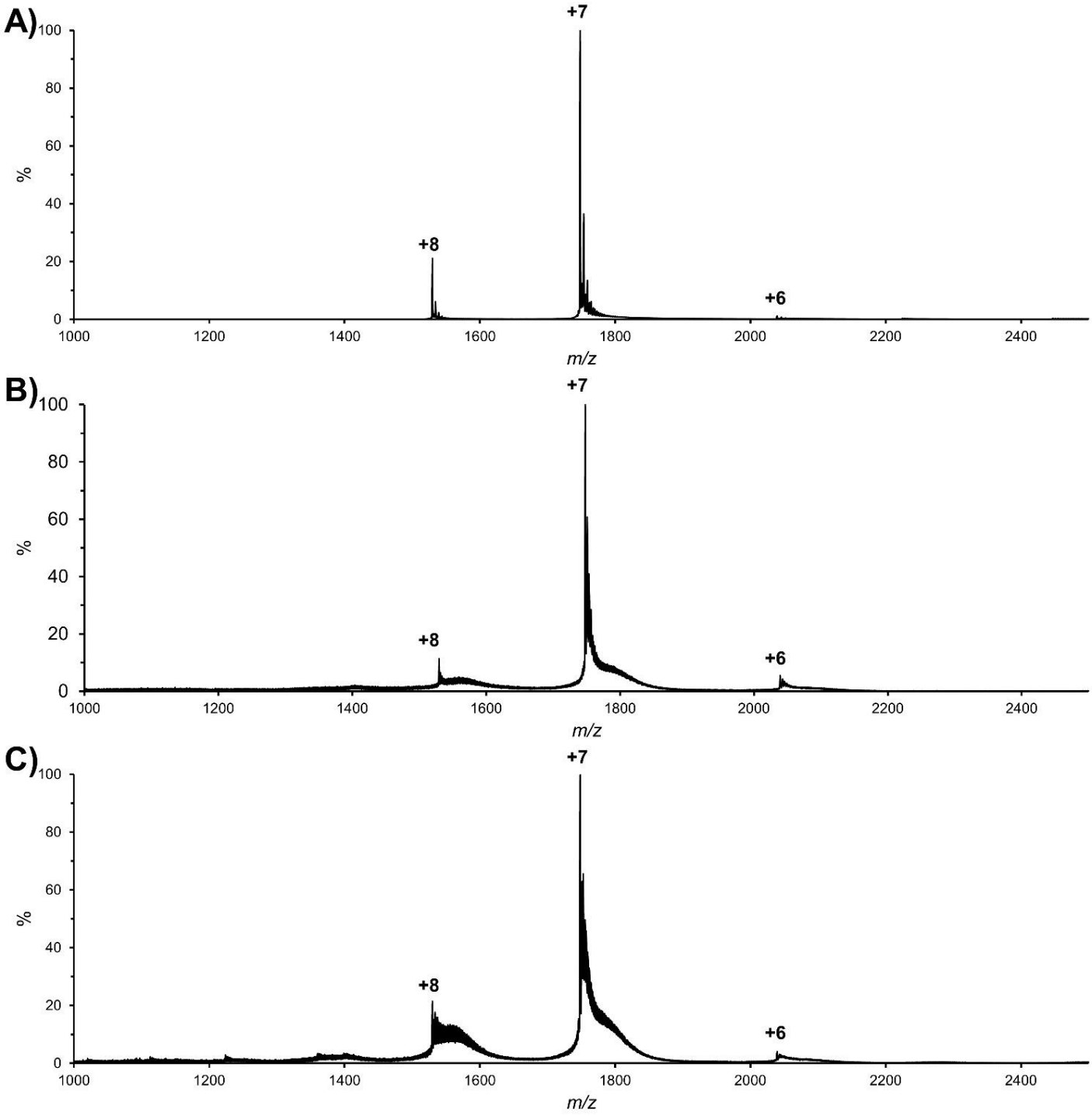

Figure S2. (A) Mass spectra of cyt c control in 100 mM ammonium acetate, (B) mass spectra with the addition of hydrogen peroxide, (C) and the mass spectra after laser irradiation.

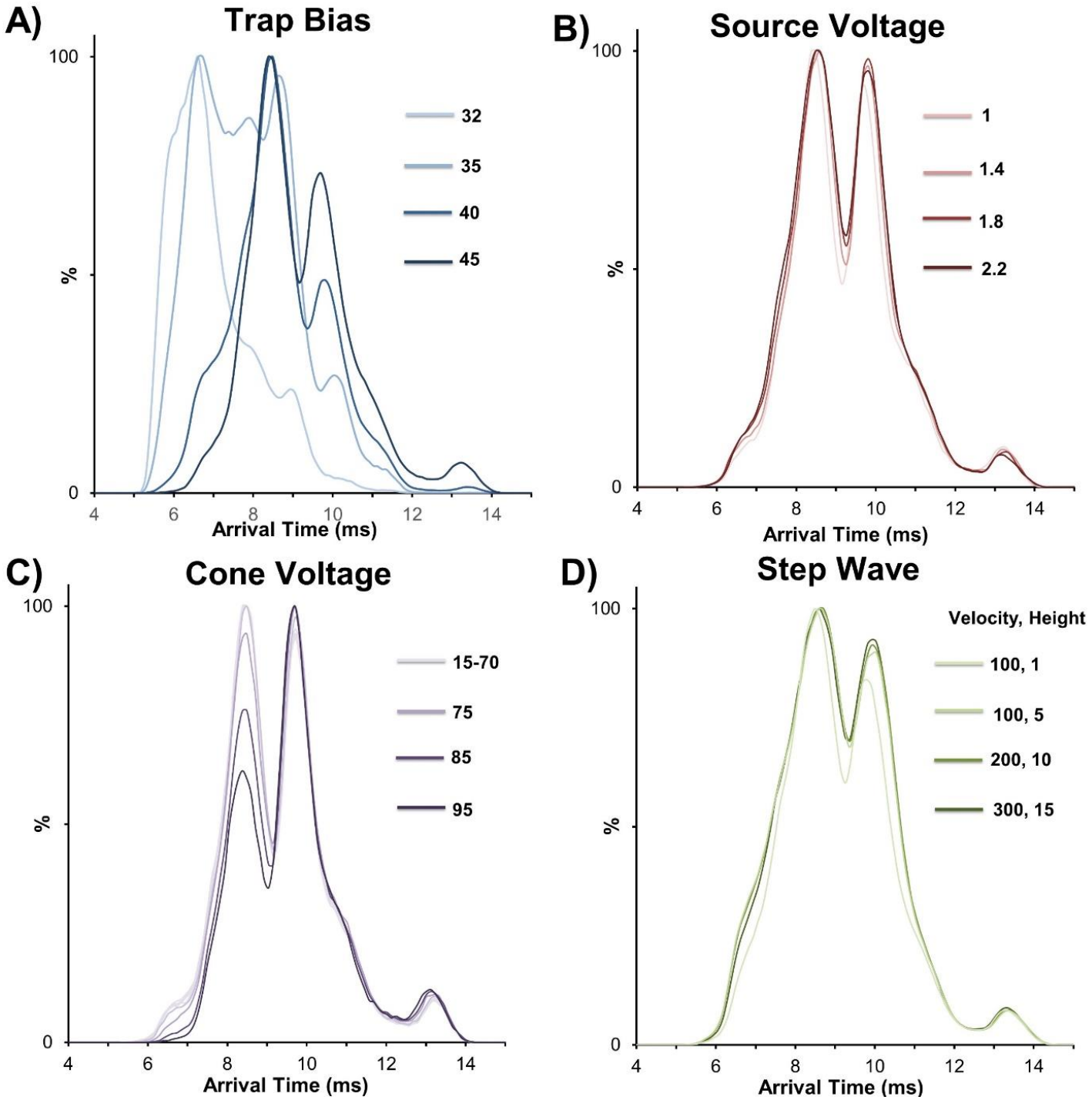

Figure S3: Changes in the trap bias (A), source voltage (B), cone voltage (C) , and step wave (D) all show the 7+ charge state of cyt c with multiple conformers. (A) The largest difference is observed with changes in the trap bias. Below a trap bias of 45 major signal reduction is observed. No signal remains with a trap bias below 32. Each trap bias setting tested all retained multiple conformers after IMS. For all other experiments, a trap bias of 45 is used. (B) The arrival time distributions show minimal differences using a source voltage between 1-2.2 kV. All other experiments use a source voltage of 1.8 kV. (C) The arrival time distribution of conformers remain constant with the cone voltage set between 15-70 V followed by a steady decrease in the compact conformer with further increased cone voltage. All other experiments use a cone voltage of 40 V. (D) Changes in the step wave velocity and height show minimal effects on the arrival time distribution. All other experiments use a step wave velocity of 300 ms and height of 15 V.

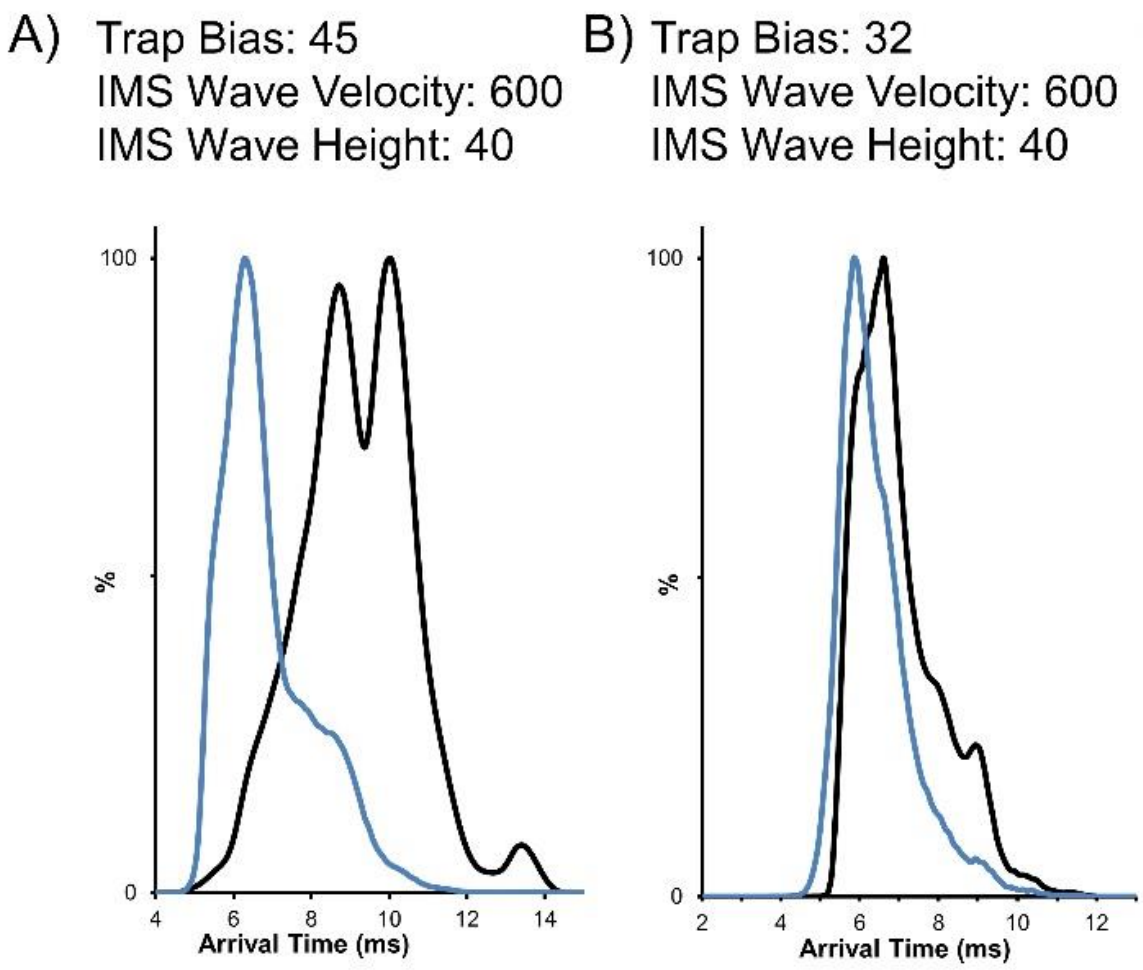

Figure S4: The arrival time distribution of cyt c's 7+ charge state showing a structural compaction remains even in the softest conditions. For each spectrum, the black trace is before exposure to H_2O_2 and the blue trace is 30 minutes after H_2O_2 exposure. (A) Has a trap bias of 45 with the IMS wave velocity of 600 m/s and IMS wave height of 40. Before H_2O_2 exposure three distinct conformers are observed. (B) Decreasing the trap bias to 32 and keeping the same IMS wave velocity and height two distinct conformers are observed.

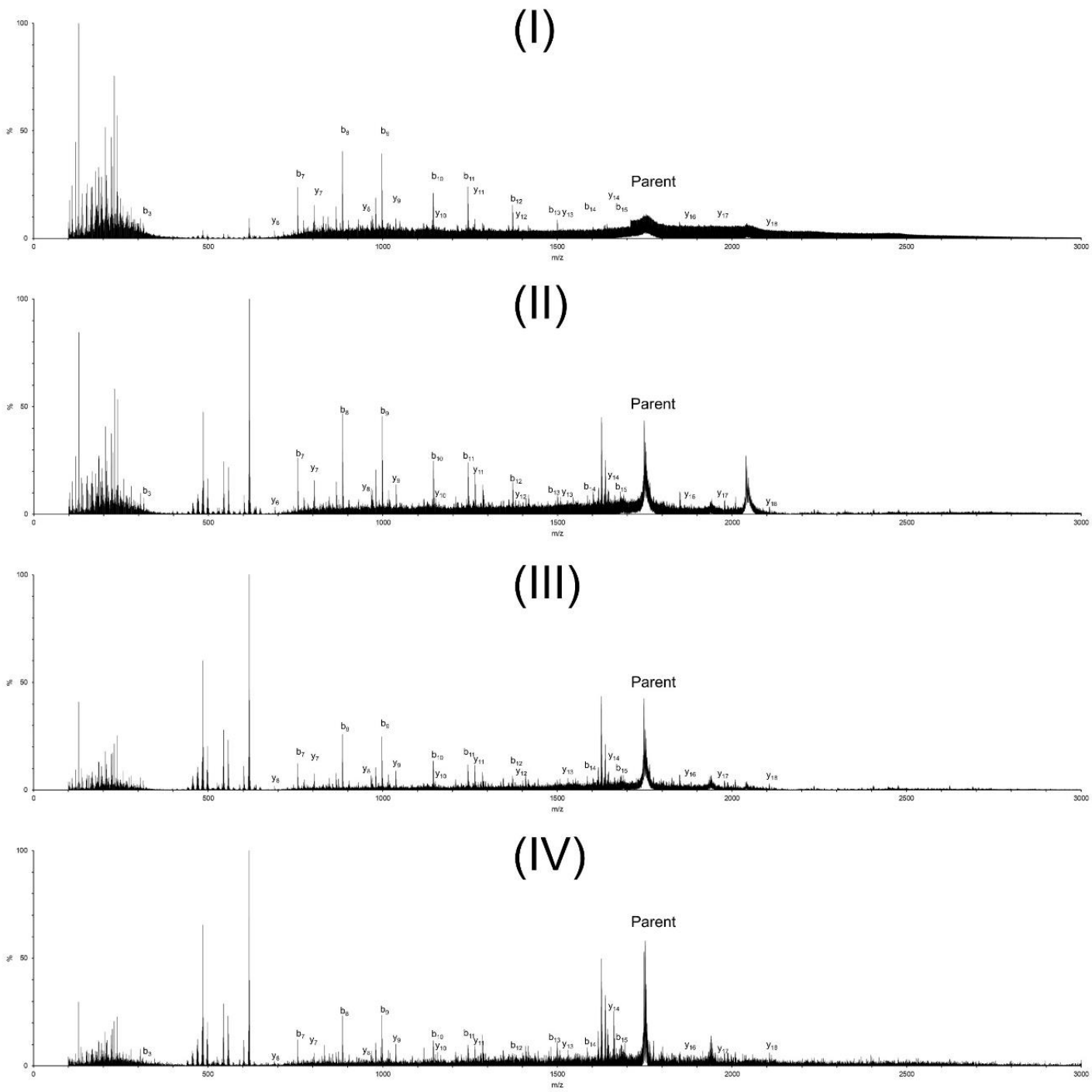

Figure S5: Ions produced using CID in transfer cell following IMS separation for each conformer. Most intense singly charged b and y ions are labeled. Multiply charged ions are not labeled.

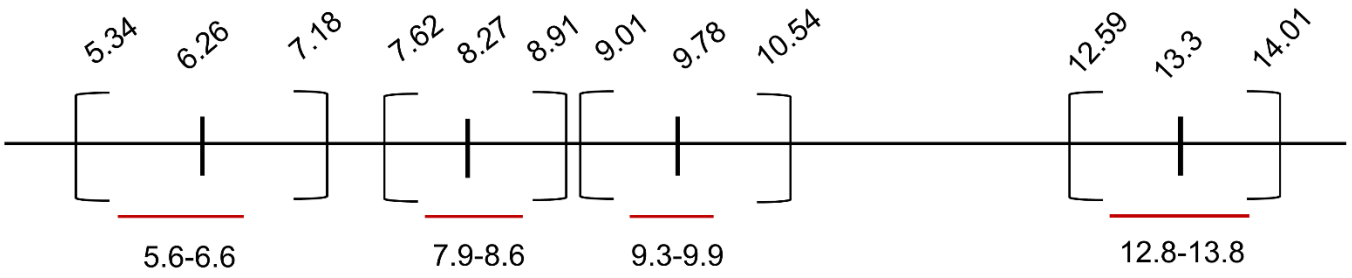

Figure S6: Gaussian distributions of the arrival time distribution (units labeled in ms) for each conformer of cyt c calculated in Origin. The gaussian center (vertical line) and width at half max (depicted in brackets) are labeled for each conformer. The area selected for mass analysis is labeled with the red line.

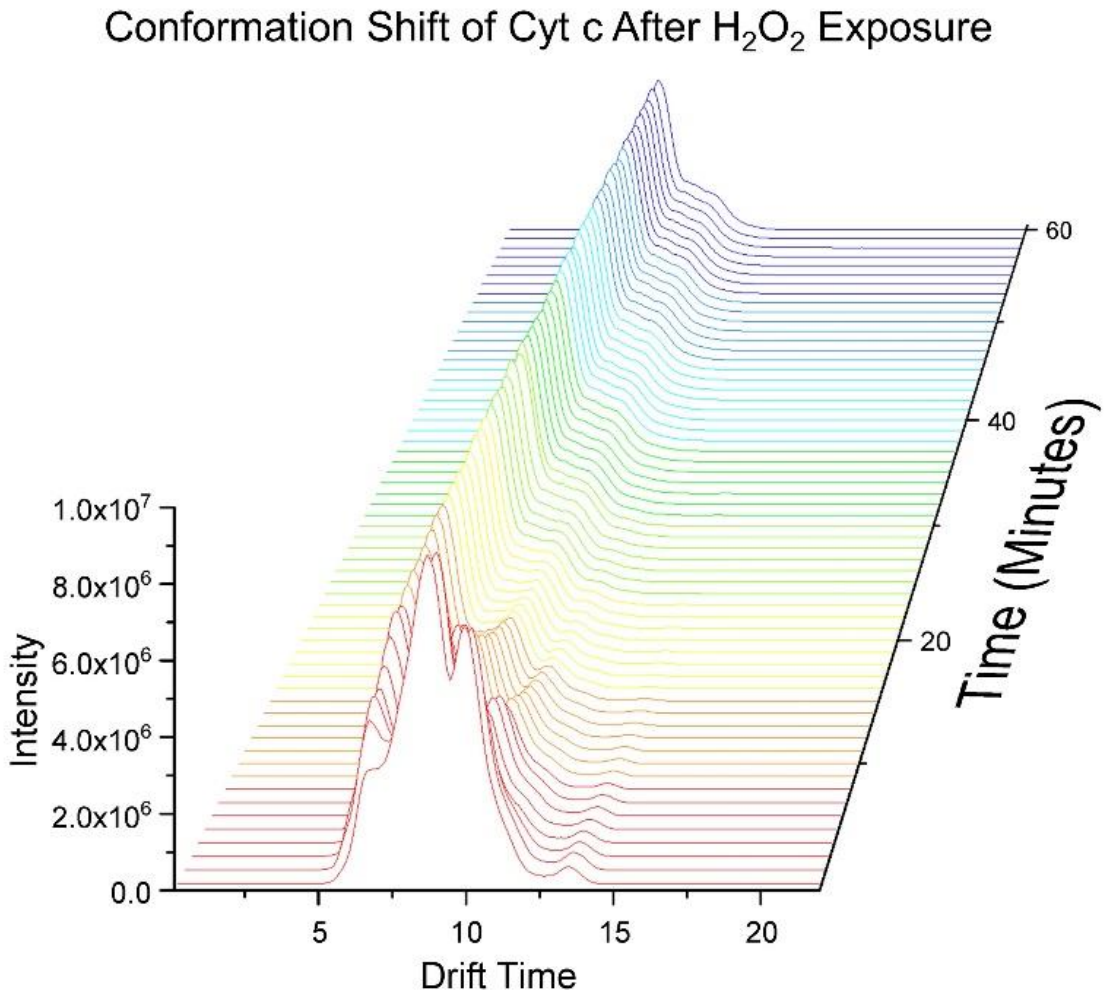

Figure S7: Arrival time distribution of 7+ charge state of cyt c after being exposed to 1 mM H_2O_2. First acquisition was collected 3 minutes after H_2O_2 exposure, and a new acquisition was collected for 30 seconds every minute for 60 minutes.

Table S1. Distance of the modified residues to the heme or Y67. The shortest distance of each modified residue to the Heme center or Y67 is found using pymol. The Extent of modification for the H_2O_2 control and after laser irradiation (Sample). By subtracting the control from sample the extent of FPOP modification is calculated. Modifications detected with bottom-up proteomics for each residue is listed.

Modified Residue	Distance to Heme (Å)	Distance to Y67 (Å)	Control GeoMean	Control GSD	Sample GeoMean	Sample GSD	FPOP GeoMean	%FPOP GSD	Modifications Detected
H26	12.8	14.9	0.3703	0.0661	2.1361	1.092	1.7659	1.094	+16, +5, -23, -22
K27	11	15.7	0.026	0.0129	0.2525	0.2026	0.2265	0.203	+14, +16
P30	7.5	9.4	0.0042	0.0017	0.017	0.0044	0.0128	0.0047	+14, +16
N31	10.8	13.8	0.0046	0.0022	0.0158	0.0039	0.0112	0.0045	+16
L32	8	10.6	0.0046	0.0025	0.0179	0.0056	0.0133	0.0061	+14, +16
H33	16.9	19	0.0299	0.0084	0.082	0.0384	0.0521	0.0393	+16, -23, -22, +5, -10
L35	10.5	11.2	0.0018	0.0003	0.0106	0.0027	0.0087	0.0027	+14, +16
F36	12.7	13.1	0.0786	0.0265	0.1328	0.0745	0.0542	0.079	+48, +32, +16
R38	14	13.7	0.0054	0.0013	0.0124	0.0024	0.007	0.0027	+16, -43
K39	17.1	14.6	0.0022	0.0016	0.0439	0.0268	0.0417	0.0016	+16, -1
Q42	14.9	13.3	0.0069	0.0012	0	0			+16
A43	13.3	13.9	0.005	0.0018	0.0299	0.0302	0.0248	0.0303	+16, +14
P44	16.7	17.6	0.0046	0.0041	0.0175	0.016	0.0129	0.0166	+16, +14
F46	8.2	9.8	0.0192	0.0117	0.0674	0.0221	0.0483	0.025	+48, +32, +16
Y48	9.4	9.6	0.0497	0.0205	0.0868	0.0257	0.0371	0.0329	+48, +32, +16
D50	16.5	13.7	0.0104	0.0092	0.0956	0	0.0852	0.0092	+16
A51	12.8	9.1	0.0043	0.0012	0.0738	0	0.0695	0.0012	+16
N52	8.1	6.7	0.0048	0.0012	0.3354	0	0.3306	0.0012	+16
K53	14.8	13	0.0378	0	0.3737	0.0309	0.3359	0	+16, -1
W59	9.8	7.5	0.5839	0.2685	0.1807	0.0322			+48, +16
E61	16.9	16.1	0.3251	0.2199	0.1397	0.0344			+16
E62	18.8	16.4	0.3251	0.2199	0.1089	0.0242			+16
L64	10.8	9.5	0.6397	0.0113	0.1038	0.0406			+16
M65	15.2	14	1.1067	0.1961	0.2904	0.7323			+16
Y67	4.8	NA	0.7467	0.4338	0.1568	0.479			+48, +16
K72	10.9	8	8.0236	0	6.9208	0			-1
K73	16.5	12.7	8.0236	0	6.9208	0			-1
M80	2.3	3.1	12.6563	3.6598	18.0497	20.1586	5.3935	4.2136	+32, +16
I81	9.1	9.8	0.6068	0.4636	7.0608	9.4496	6.4541	4.4195	+16, +14
E92	17.8	17.6	0	0	0.0096	0	0.0096	0	-28
D93	16.8	18.1	0	0	0.0096	0	0.0096	0	-28
I95	12.1	11.9	0	0	0.0115	0	0.0115	0	+14
A96	16.2	17.7	0	0	0.0133	0.0094	0.0133	0.0094	+16
Y97	14	17.5	0.0017	0	0.1754	0.027	0.1737	0.027	+48, +32, +16
L98	10.9	12.5	0	0	0.0056	0	0.0056	0	+16, +14

Table S2. Conditions for native MS analysis.

Capillary (kV)	1.8
Source Temperature (°C)	50
Sampling Cone	30
Trap Collision Energy	4
Transfer Collision Energy	6
IMS Wave Velocity (m/s)	600
IMS Wave Height (V)	40