

## Supporting Information For:

# A Universal Score for Deconvolution of Intact Protein and Native Electrospray Mass Spectra

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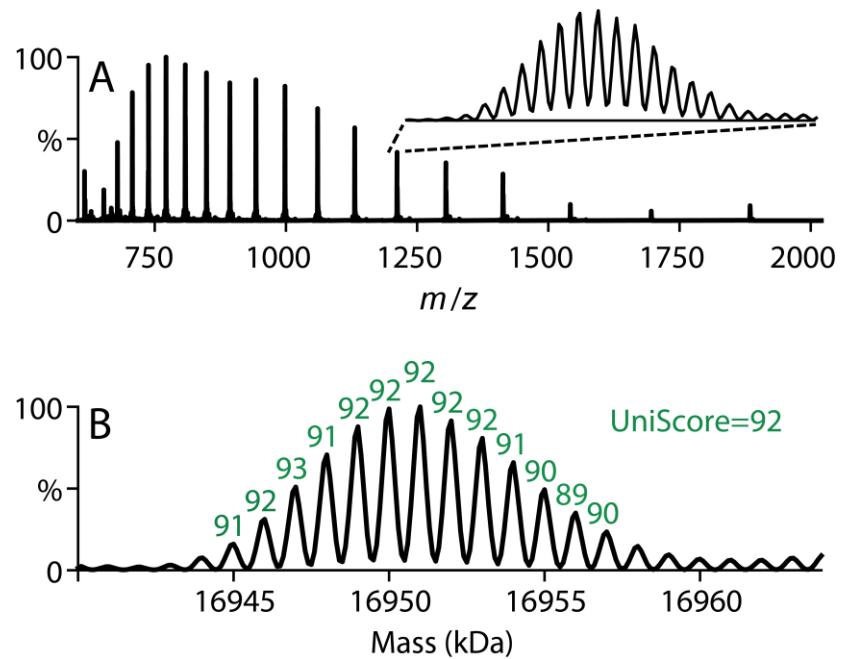
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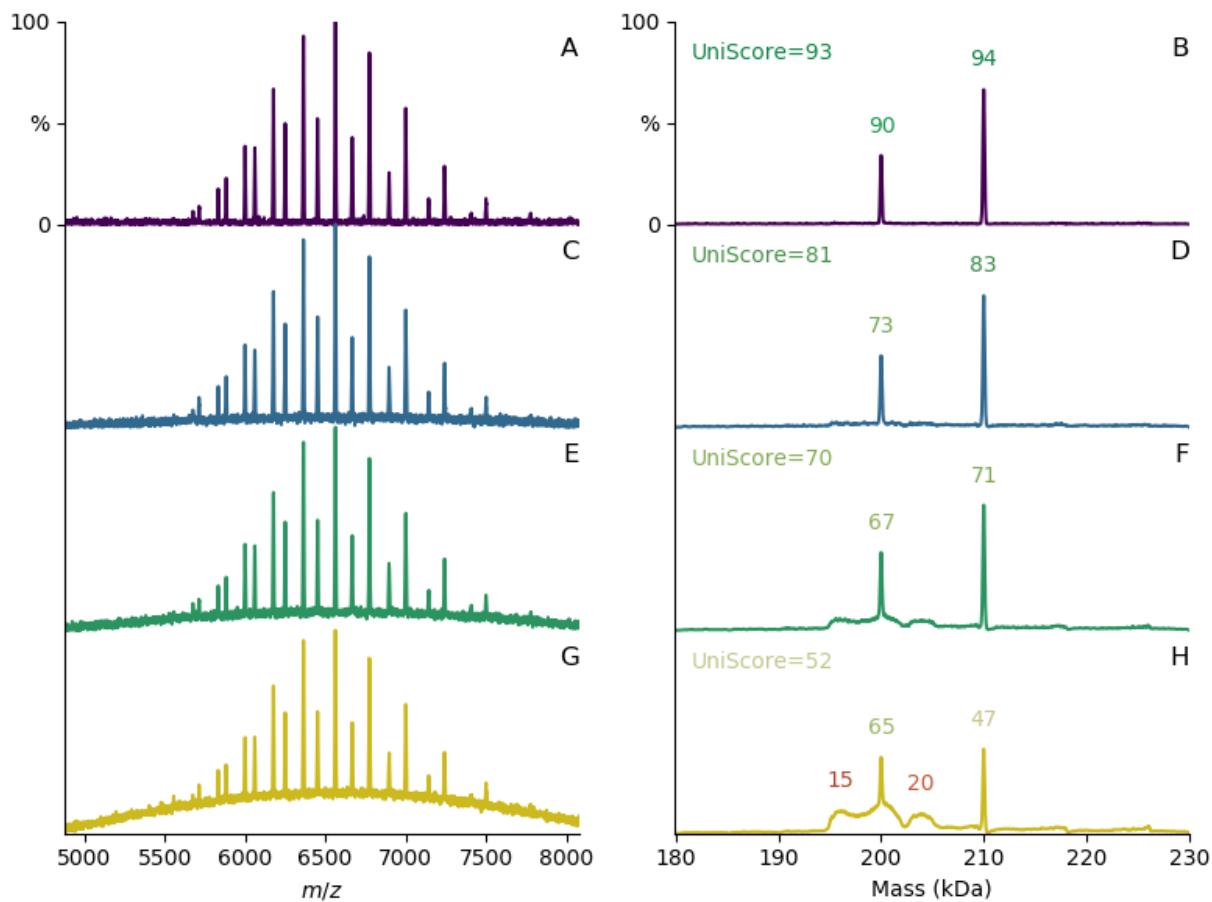
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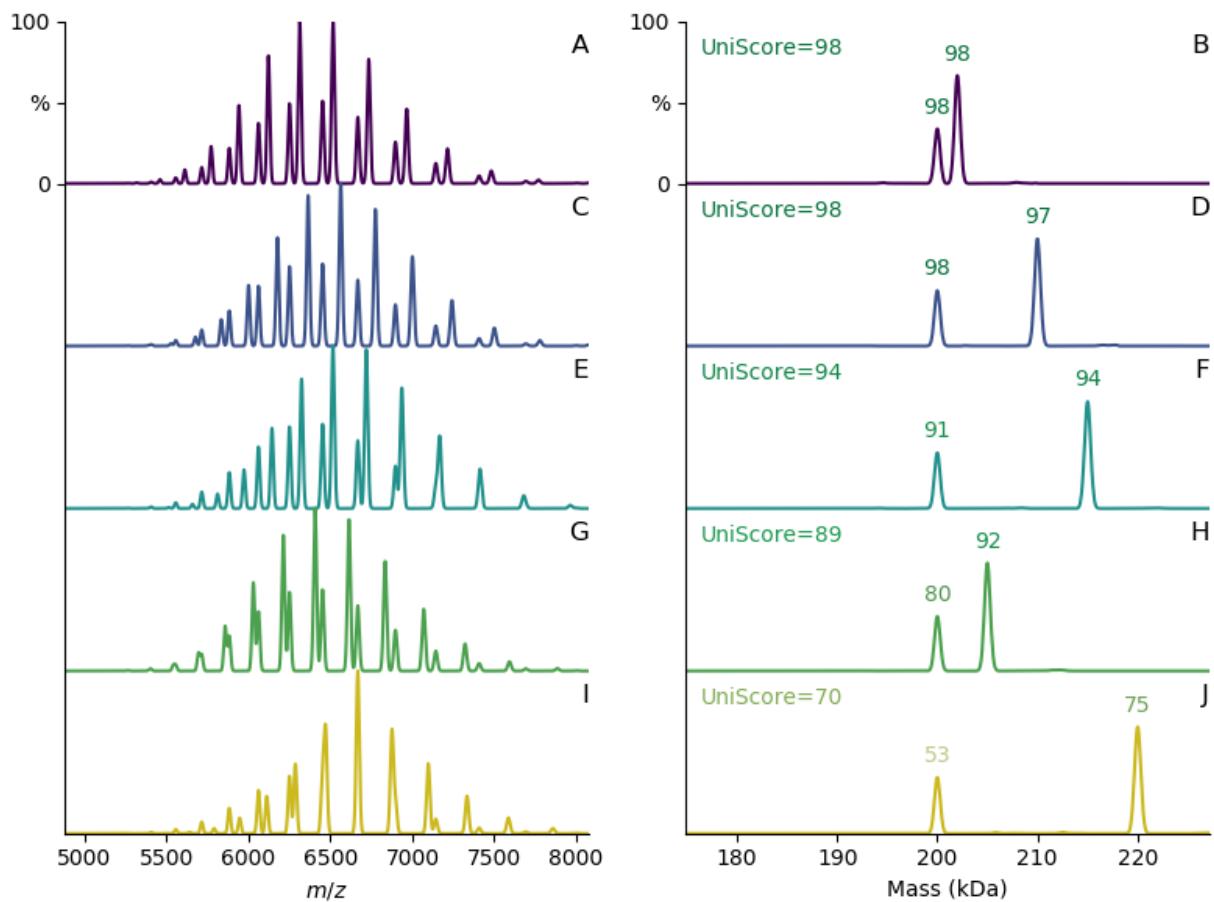
## SUPPLEMENTAL FIGURES



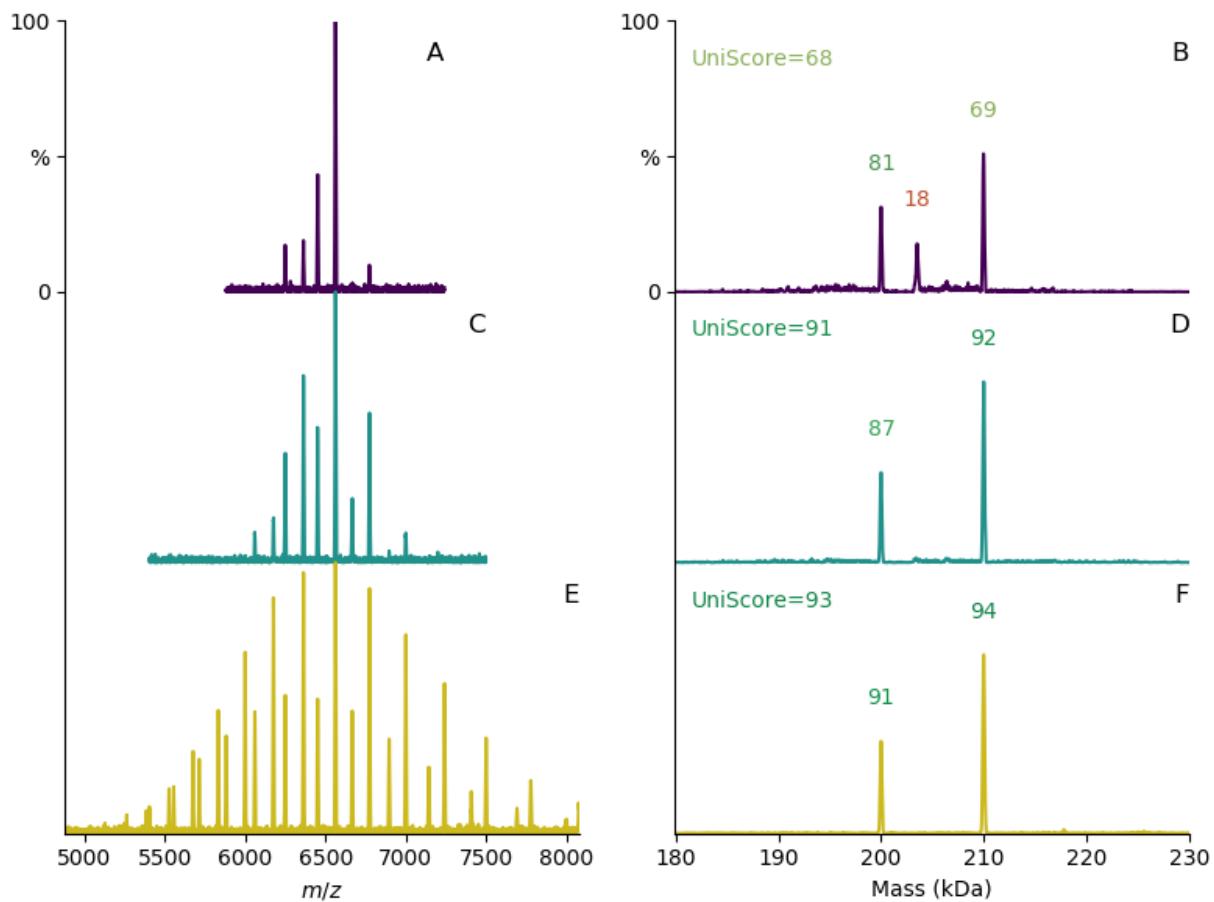
**Figure S1.** The denatured mass spectrum of myoglobin (A) and its deconvolved mass distribution (B) with annotated DScore and UniScore values. The inset shows the isotope distribution for the +14 charge state around 1212  $m/z$ . Each isotope peak is scored independently.



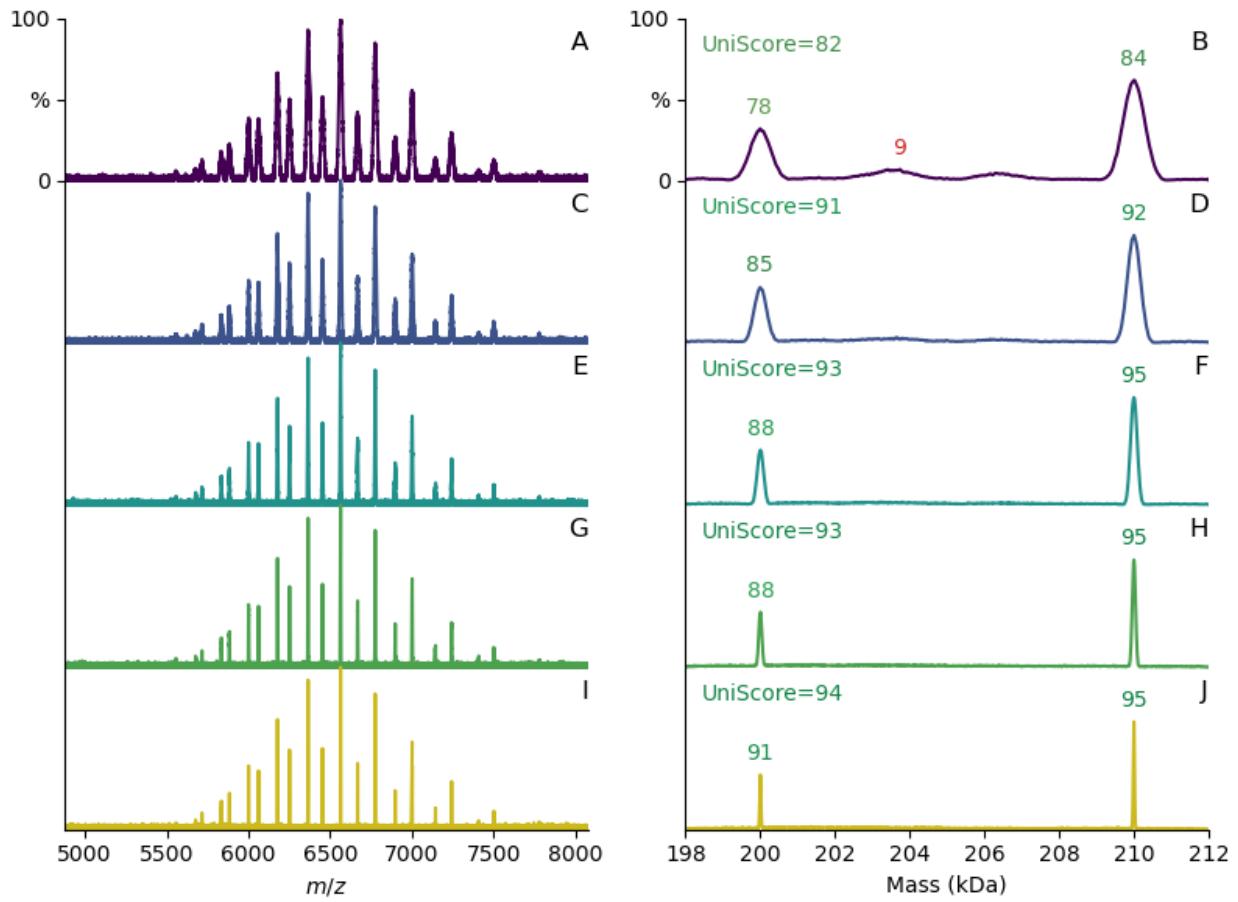
**Figure S2.** The mass spectra (A, C, E, G) and deconvolved mass distributions (B, D, F, H) for simulated spectra with baselines at 0, 5, 10, and 25% (from *top* to *bottom*) of the maximum intensity.



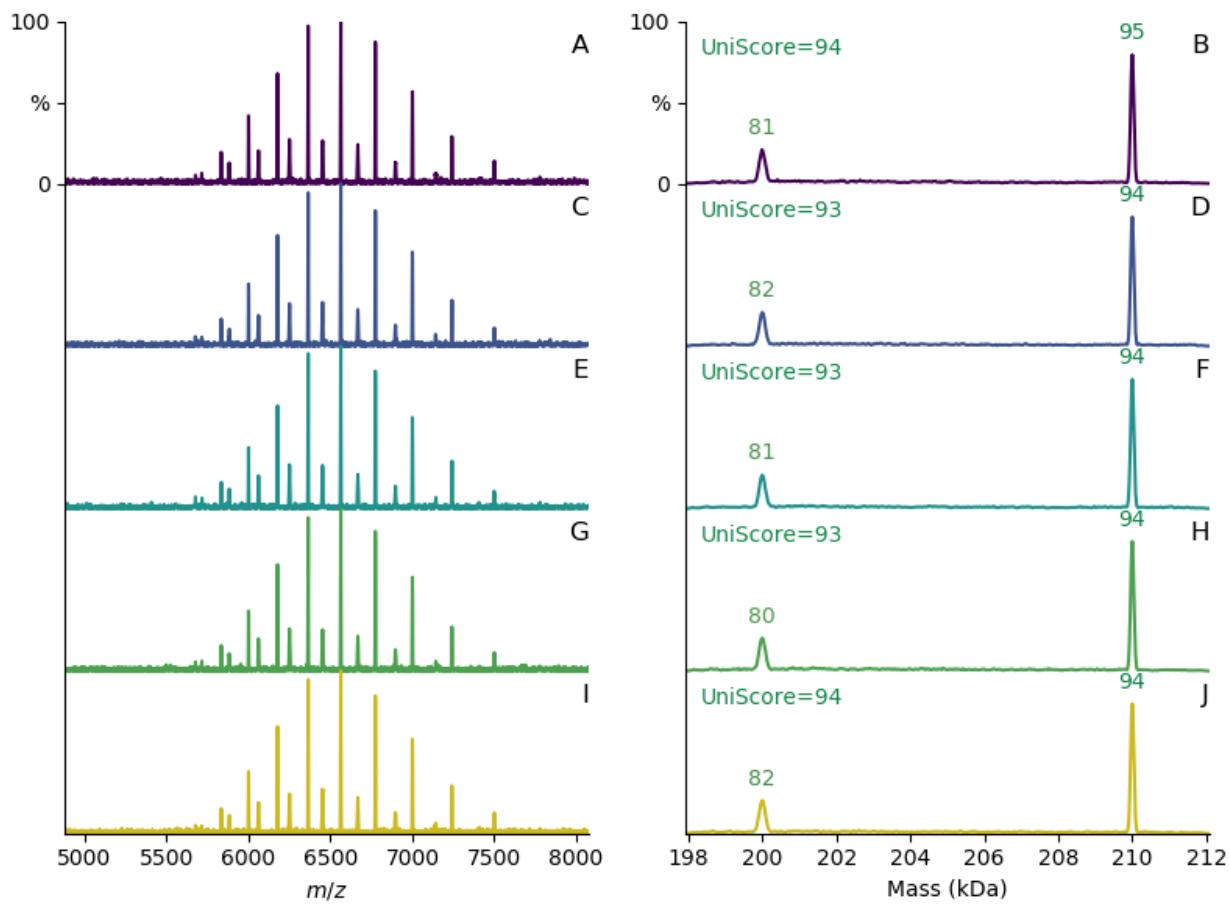
**Figure S3.** The mass spectra (A, C, E, G, I) and deconvolved mass distributions (B, D, F, H, J) for simulated spectra with masses chosen to give increasing degrees of peak overlap between charge states of different species from *top* to *bottom*.



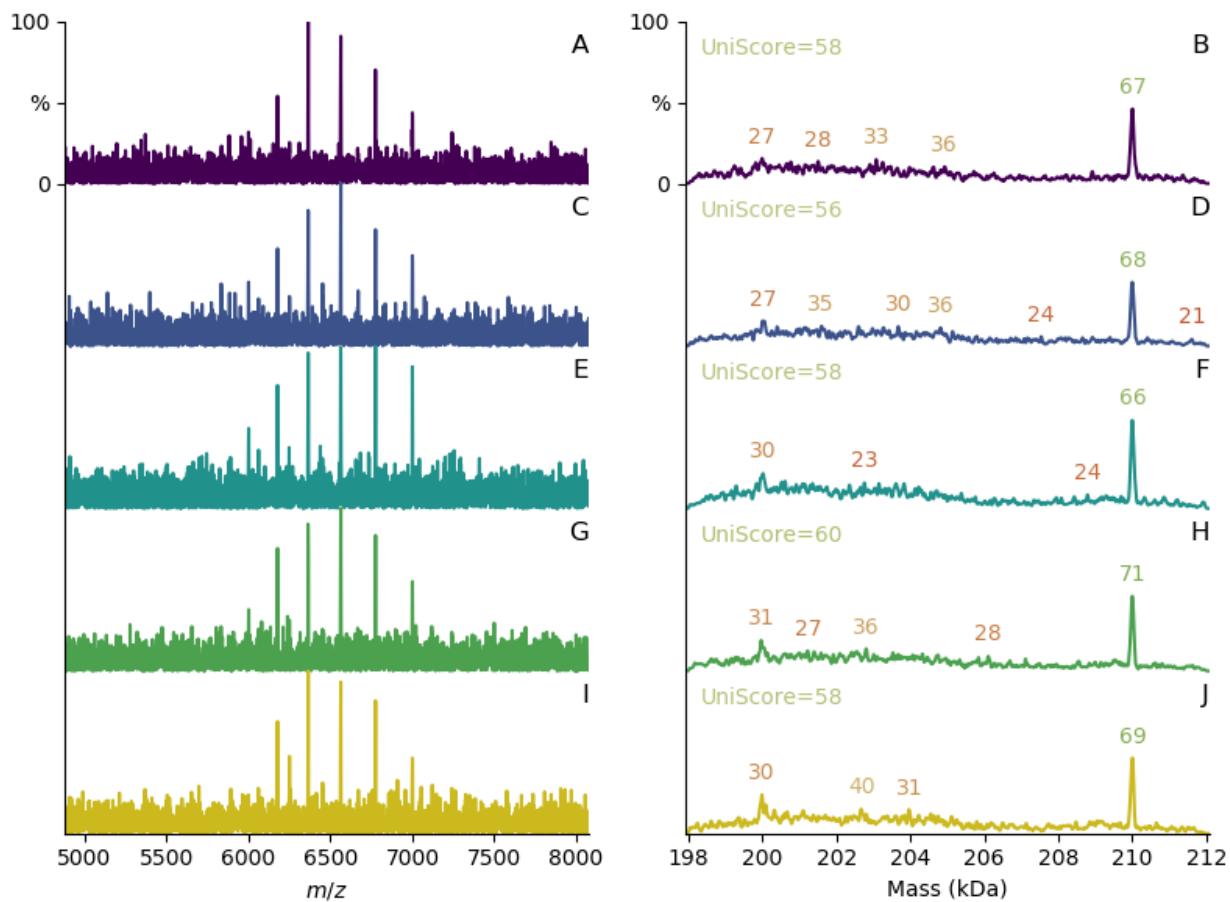
**Figure S4.** The mass spectra (A, C, E) and deconvolved mass distributions (B, D, F) for simulated spectra with charge state distributions having standard deviations of 0.5, 1, and 3 charges (from *top* to *bottom*).



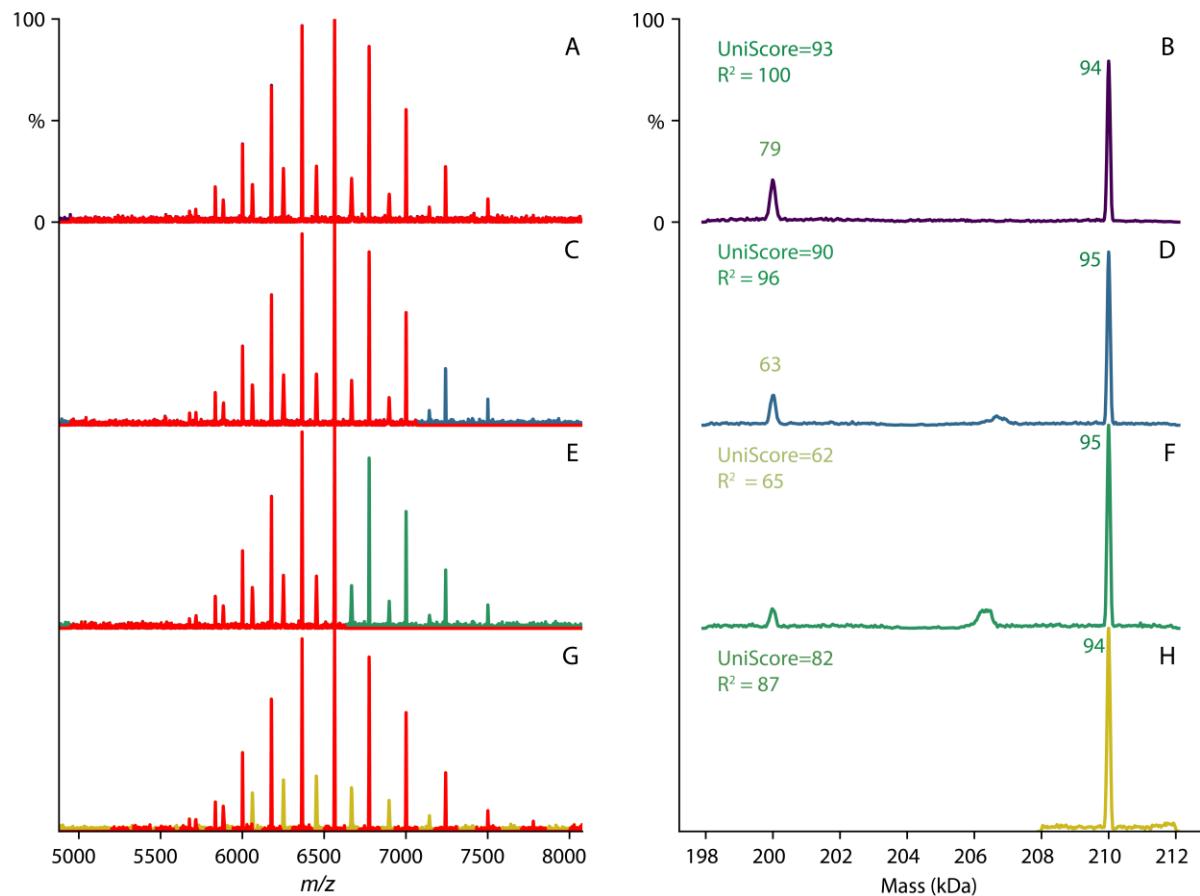
**Figure S5.** The mass spectra (A, C, E, G, I) and deconvolved mass distributions (B, D, F, H, J) for simulated spectra with resolutions ( $m/\Delta m_{FWHM}$ ) of 300, 500, 1000, 2000, and 5000 (from top to bottom). Simulated spectra were generated at 0.1  $m/z$  sampling to ensure the high-resolution data were sampled adequately.



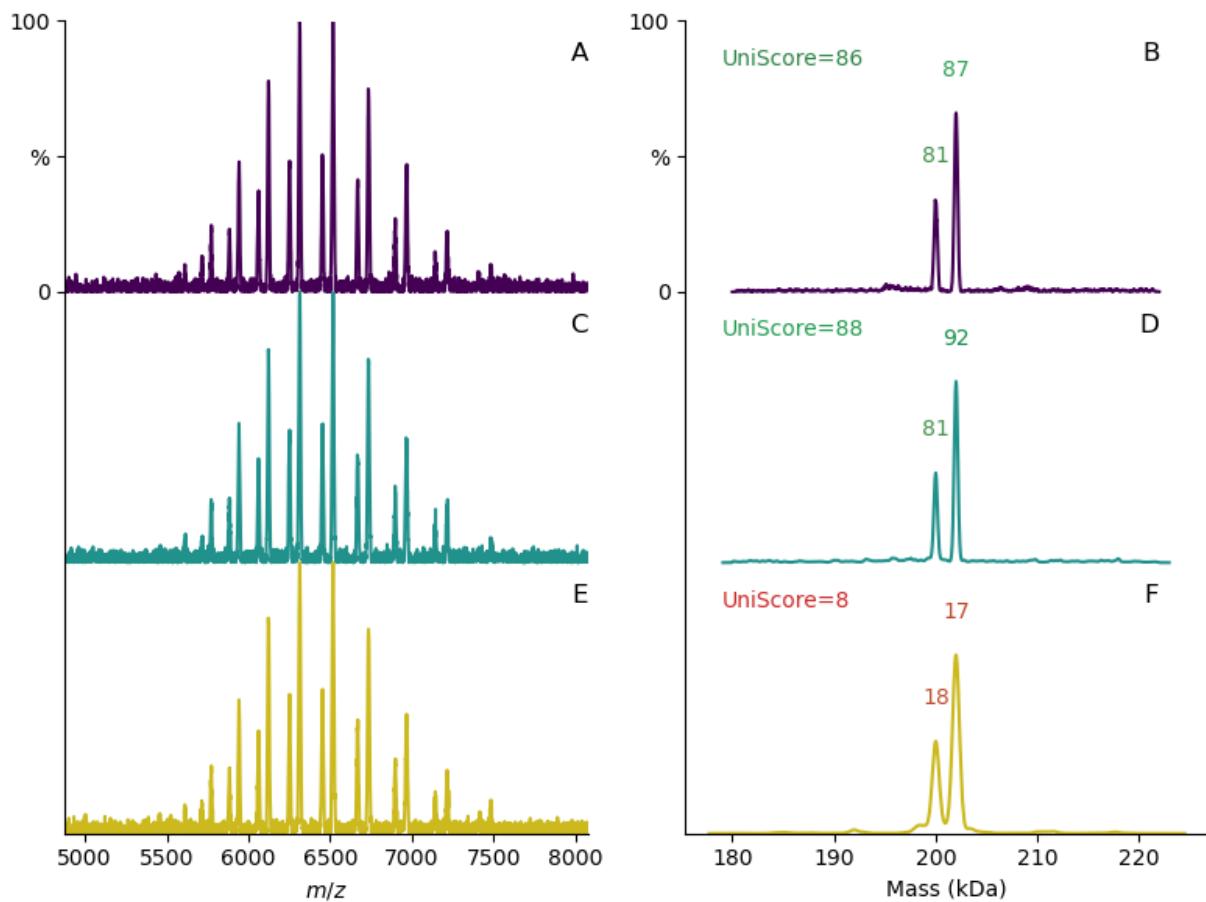
**Figure S6.** Five repeated simulations with different sampling of 1% noise. Mass spectra are shown in (A, C, E, G, I), and deconvolved mass distributions are in (B, D, F, H, J).



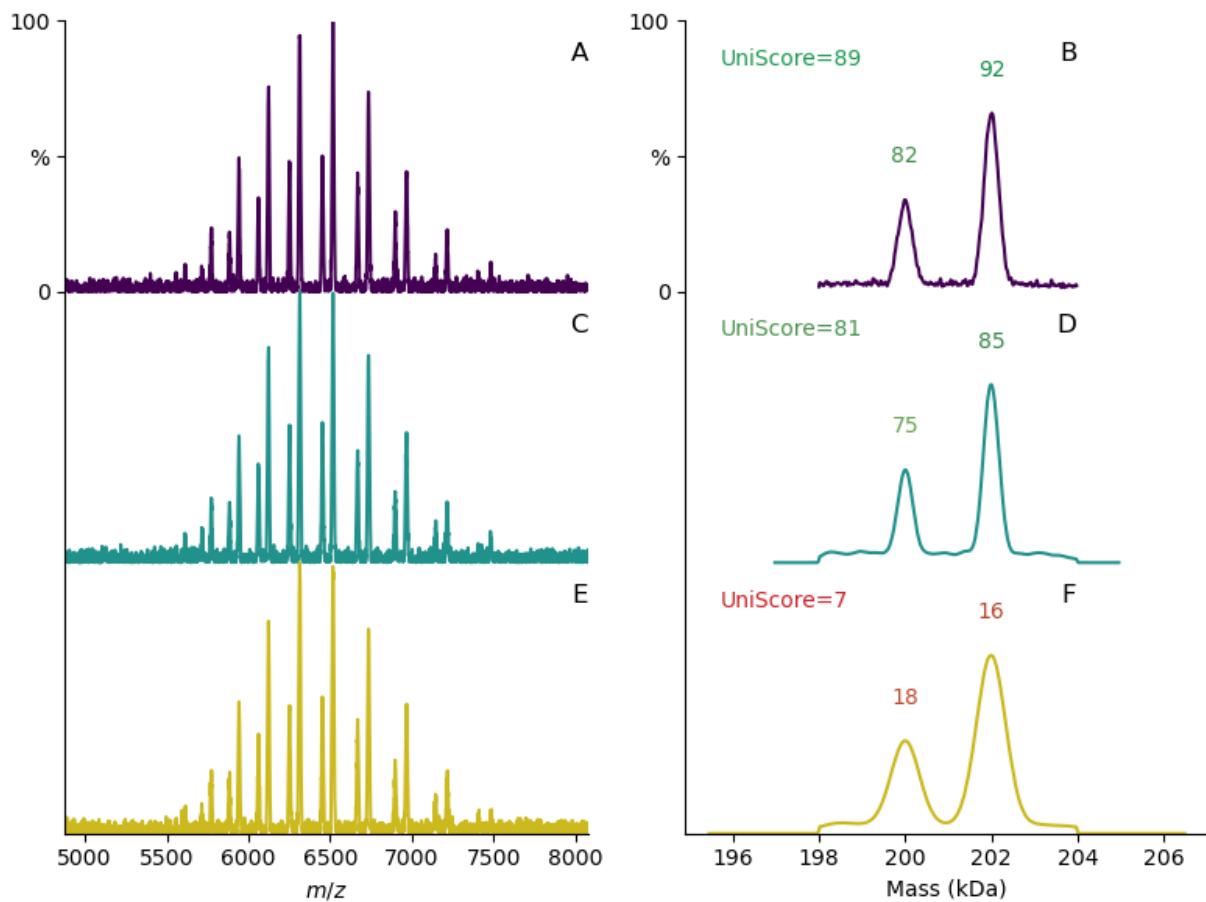
**Figure S7.** Five repeated simulations with different sampling of 10% noise. Mass spectra are shown in (A, C, E, G, I), and deconvolved mass distributions are in (B, D, F, H, I).



**Figure S8.** UniDec deconvolution (B, D, F, H) of the same simulated mass spectrum (A, C, E, G) shown in Figure S6 with the deconvolution limited in either charge range (D: 30–40; F: 32–40) or mass range (H: 208–212 kDa) to deliberately force the deconvolution to miss peaks. The  $R^2$  values are reported underneath the UniScore values, and the red lines over the mass spectra show the fit of the deconvolution to the data.



**Figure S9.** UniDec deconvolution (B, D, F) of the same simulated mass spectrum (A, C, E) with peak width settings of 0, 10, or 25  $m/z$  (from *top* to *bottom*) with a wider allowed mass range. An  $R^2$  of 0.45 caused the UniScore of F to be significantly below the average of the DScore values.



**Figure S10.** UniDec deconvolution (B, D, F) of the same simulated mass spectrum (A, C, E) from Figure S9 with peak width settings of 0, 10, or 25  $m/z$  (from *top* to *bottom*) with a narrower allowed mass range. An  $R^2$  of 0.45 caused the UniScore of F to be significantly below the average of the DScore values.

## SUPPLEMENTAL TABLES

**Table S1.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the BSA spectrum in Figure 2C.

Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
66426	100	97	100	98	100	100
132847	2.361097	90	93	96	100	100

**Table S2.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the AqpZ with bound POPC spectrum in Figure 2D.

Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
96339	1.711884	23	45	76	95	70
96727	1.419222	9	32	76	95	41
97036	1.037469	13	55	78	94	31
97645	3.024481	36	52	75	90	100
98849	100	87	92	95	100	100
99614	94.87508	84	91	92	100	100
100380	54.31229	81	92	87	100	100
101137	29.32114	78	91	86	100	100
101884	16.70846	72	85	85	100	100
102628	8.338605	66	87	82	100	93
103530	4.598929	59	79	85	100	88
104165	2.699479	38	71	73	99	74
104982	2.376222	8	49	94	83	22

**Table S3.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the HSP17.7 spectrum in Figure 2G.

Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
211840	100	89	92	97	100	100
213340	39.59328	89	93	96	100	100
214860	20.68344	76	80	95	100	100
216380	9.795226	80	86	94	100	100
217860	1.832505	30	37	84	99	100
219410	2.096386	6	7	81	99	100
220940	22.40217	71	78	91	100	100

**Table S4.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the POPC nanodisc spectrum in Figure 2H. Note: some peaks were not annotated in Figure 2H for readability but have been listed here.

Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
122600	11.5631	60	64	93	100	100
123370	16.25166	58	70	83	100	100
124120	10.70696	71	77	92	100	100
124890	20.48647	58	66	87	100	100
125650	30.12919	57	62	92	100	100
126410	22.16721	48	54	89	100	100
127170	32.07785	54	57	94	100	100
127930	51.9229	63	65	97	100	100
128690	54.79695	70	80	88	100	100
129450	67.06272	92	94	98	100	100
130200	78.69789	93	95	98	100	100
130960	87.84571	93	94	98	100	100
131720	96.82061	93	94	99	100	100
132490	96.68838	94	95	99	100	100
133250	100	93	95	98	100	100
134010	99.32728	92	94	98	100	100
134770	94.52711	92	95	97	100	100
135530	81.72271	94	95	99	100	100
136290	70.42657	88	89	98	100	100
137050	61.14621	78	80	98	100	100
137810	39.33954	53	59	89	100	100
138570	45.27899	51	54	94	100	100
139350	20.34439	51	73	71	100	100
140090	22.53033	71	82	86	100	100
140850	20.95547	78	82	95	100	100
141610	18.41286	73	75	97	100	100

**Table S5.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the myoglobin spectrum in Figure S1.

Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
16945	15.8418	91	97	95	98	100
16946	31.02489	92	98	94	99	100
16947	50.86786	93	99	94	99	100
16948	70.59354	91	99	93	99	100
16949	87.88331	92	99	93	100	100
16950	98.81132	92	100	93	99	100
16951	100	92	99	93	99	100
16952	91.43779	92	99	93	100	100
16953	80.72845	92	99	94	99	100
16954	65.79498	91	99	92	99	100
16955	49.17658	90	99	92	99	100
16956	34.84201	89	98	92	98	100
16957	23.34704	90	98	93	99	100

**Table S6.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the simulated spectra in Figure S9.

<b>Peak Width 0</b>						
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
199960	33.94664	81	88	93	100	100
202010	66.05336	87	91	96	100	100
<b>Peak Width 10</b>						
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
200000	33.10359	81	87	93	100	100
202000	66.89641	92	94	98	100	100
<b>Peak Width 25</b>						
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore
200000	34.07368	18	21	88	100	100
202000	65.92632	17	19	94	100	100

**Table S7.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the simulated spectra in Figure S10.

<b>Peak Width 0</b>							
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore	
199990	33.99058	82	100	82	100	100	
202010	66.00942	92	100	92	100	100	
<b>Peak Width 10</b>							
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore	
200000	34.2925	75	88	86	99	100	
202000	65.7075	85	93	92	99	100	
<b>Peak Width 25</b>							
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore	
200000	34.22122	18	21	84	100	100	
202000	65.77878	16	17	92	100	100	

**Table S8.** Mass, intensity, DScore, UScore, MScore, CSScore, and FScore values for deconvolution of the ADH spectrum with standard parameters (Figure 4B) and autotune (Figure 4C).

<b>Standard Deconvolution</b>							
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore	
142600	7.568445	8	8	93	100	100	
147900	100	73	74	98	100	100	
154500	5.685061	6	6	93	97	100	
163600	5.1549	7	9	84	98	100	
169900	5.506216	10	11	89	97	100	
<b>Autotuned</b>							
Mass	Intensity	DScore	UScore	MScore	CSScore	FScore	
147900	100	93	100	93	100	100	