Electronic Supplementary Material (ESI) for Analyst.

Supplementary Material

Desalting Protein Ions in Native Mass Spectrometry Using Supercharging Reagents

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Table S-1. Average charge and average number of sodium ions adducted to individual charge states calculated from mass spectra of 5 μM protein in 10 mM ammonium bicarbonate, 1 mM NaCl with no supercharging reagent, with 1.5% *m*-NBA, or with 2.5% sulfolane. Data for the most abundant charge state is given in bold.

Protein	Average Charge	4+	5+	6+	7+	8+	9+	10+	11+
Ubiquitin	5.33 ± 0.01	3.2 ± 0.2	**3.5 ± 0.1**	3.5 ± 0.1	2.0 ± 0.4	0.48 ± 0.02	0.12 ± 0.02	0.00 ± 0.00	
***m*-NBA**	7.1 ± 0.1	1.6 ± 0.4	0.9 ± 0.2	1.3 ± 0.3	**1.2 ± 0.2**	0.00 ± 0.00	0.00 ± 0.00		
sulfolane	6.3 ± 0.1	0.5 ± 0.1	0.53 ± 0.03	**0.44 ± 0.03**	0.13 ± 0.01				
Barstar	5.5 ± 0.1	2.8 ± 0.3	**3.5 ± 0.4**	4.7 ± 0.5					
***m*-NBA**	7.1 ± 0.1	1.1 ± 0.2	1.29 ± 0.04	1.3 ± 0.2	**1.4 ± 0.2**	1.1 ± 0.1	0.89 ± 0.03		
sulfolane	5.7 ± 0.1	0.9 ± 0.4	1.0 ± 0.3	**1.0 ± 0.4**	0.45 ± 0.01				
Cytochrome *c*	6.5 ± 0.1		2.4 ± 0.5	4.2 ± 0.1	**2.5 ± 0.2**	0.6 ± 0.1	0.99 ± 0.04	0.85 ± 0.03	0.82 ± 0.03
***m*-NBA**	9.42 ± 0.04		1.1 ± 0.2	0.78 ± 0.02	0.72 ± 0.01	1.03 ± 0.01	**0.93 ± 0.01**	0.17 ± 0.01	0.17 ± 0.01
sulfolane	8.6 ± 0.2			1.3 ± 0.2	0.87 ± 0.02	0.23 ± 0.01	**0.17 ± 0.01**		
Ribonuclease A	6.9 ± 0.1			3.7 ± 0.1	**4.5 ± 0.2**	2.9 ± 0.8			
***m*-NBA**	9.16 ± 0.03				0.4 ± 0.3	1.1 ± 0.1	**1.2 ± 0.2**	1.3 ± 0.5	0.3 ± 0.1
sulfolane	7.7 ± 0.1			0.9 ± 0.1	0.7 ± 0.1	**0.5 ± 0.1**	0.1 ± 0.1		
α-Lactalbumin	6.8 ± 0.1			3.1 ± 0.5	**3.3 ± 0.5**	2.3 ± 0.3	2.1 ± 0.2	1.0 ± 0.2	0.5 ± 0.2
***m*-NBA**	8.9 ± 0.1			1.1 ± 0.3	1.9 ± 0.5	1.9 ± 0.2	**1.7 ± 0.1**		
sulfolane	7.2 ± 0.2				**1.0 ± 0.3**	0.54 ± 0.03	0.00 ± 0.00		
Lysozyme	7.5 ± 0.1			2.9 ± 0.2	2.8 ± 0.3	**1.7 ± 0.1**	0.59 ± 0.03	0.35 ± 0.04	0.7 ± 0.1
***m*-NBA**	9.9 ± 0.1			0.6 ± 0.4	0.2 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	**0.60 ± 0.03**	0.23 ± 0.03
sulfolane	8.8 ± 0.3				0.7 ± 0.5	0.6 ± 0.3	**0.4 ± 0.1**	0.2 ± 0.2	
holo-Myoglobin	8.2 ± 0.1				3.5 ± 0.6	**3.9 ± 0.4**	4.9 ± 0.5		
***m*-NBA**	11.2 ± 0.2					0.50 ± 0.03	1.3 ± 0.1	1.2 ± 0.1	**0.79 ± 0.02**
sulfolane	9.8 ± 0.4				1.1 ± 0.5	1.1 ± 0.2	0.8 ± 0.2	**0.3 ± 0.1**	0.00 ± 0.00
apo-Myoglobin	--	--	--	--	**--**	--	--	--	--
***m*-NBA**	12.4 ± 0.6					0.9 ± 0.2	1.1 ± 0.1	0.76 ± 0.04	**0.71 ± 0.04**
sulfolane	9.2 ± 0.3				0.9 ± 0.3	0.67 ± 0.03	**0.28 ± 0.01**	0.00 ± 0.00	0.00 ± 0.00
B-lactalbumin	8.13 ± 0.04				3.6 ± 0.2	**4.0 ± 0.2**	4.4 ± 0.4	3.7 ± 0.5	2.4 ± 0.2
***m*-NBA**	10.41 ± 0.03				2.3 ± 0.3	2.4 ± 0.3	3.30 ± 0.02	**2.88 ± 0.03**	0.00 ± 0.00
sulfolane	8.5 ± 0.2						**1.9 ± 0.2**	0.7 ± 0.1	
Carbonic Anhydrase	9.8 ± 0.1					3.7 ± 0.7	4.0 ± 0.6	**4.5 ± 0.6**	4.8 ± 0.8
***m*-NBA**	13.5 ± 0.3								2.1 ± 0.3
sulfolane	11.7 ± 0.1						1.1 ± 0.2	1.4 ± 0.2	1.0 ± 0.1

Protein	12+	13+	14+	15+	16+	17+	18+	19+	20+
Ubiquitin									
***m*-NBA**									
sulfolane									
Barstar									
***m*-NBA**									
sulfolane									
Cytochrome *c*	0.59 ± 0.04	0.30 ± 0.02	0.35 ± 0.03						
***m*-NBA**	0.00 ± 0.00								
sulfolane									
Ribonuclease A									
***m*-NBA**	0.00 ± 0.00								
sulfolane									
α-Lactalbumin	0.36 ± 0.04								
***m*-NBA**									
sulfolane									
Lysozyme	0.4 ± 0.2								
***m*-NBA**									
sulfolane									
holo-Myoglobin									
***m*-NBA**	0.76 ± 0.05	0.44 ± 0.03	0.6 ± 0.4	0.6 ± 0.2	1.0 ± 0.1	0.2 ± 0.3	0.6 ± 0.3		
sulfolane	0.00 ± 0.00	0.00 ± 0.00							
apo-Myoglobin	--	--	--	--	--	**--**	--	--	--
***m*-NBA**	0.76 ± 0.02	0.9 ± 0.1	0.88 ± 0.04	0.92 ± 0.04	0.76 ± 0.02	**0.87 ± 0.02**	0.79 ± 0.04	0.36 ± 0.03	0.00 ± 0.00
sulfolane	0.00 ± 0.00								
B-lactalbumin	2.0 ± 0.2	1.9 ± 0.1							
***m*-NBA**									
sulfolane									
Carbonic Anhydrase	6.6 ± 2.7								
***m*-NBA**	2.9 ± 0.5	**3.0 ± 0.5**	2.3 ± 0.1	1.42 ± 0.04	1.3 ± 0.1	0.82 ± 0.03	0.8 ± 0.4	1.0 ± 0.4	0.9 ± 0.6
sulfolane	**0.7 ± 0.2**	0.29 ± 0.01	0.00 ± 0.00	0.00 ± 0.00					

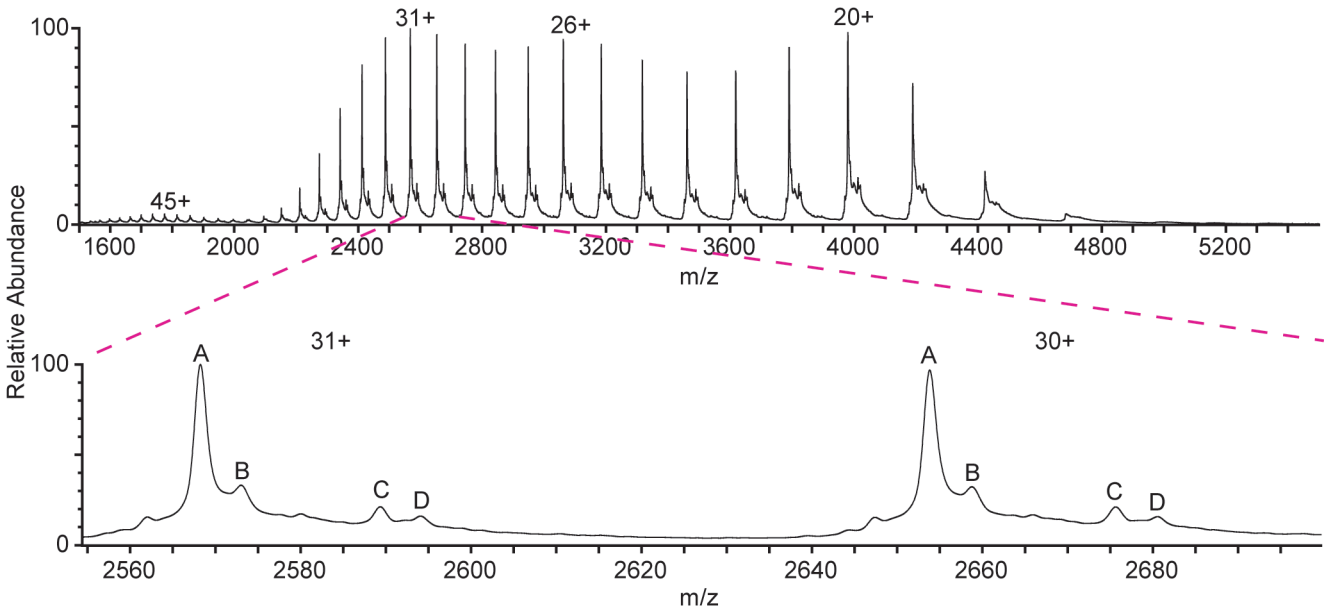

Figure S-1. nESI mass spectrum of 10 µM human holo-transferrin in 60:40 methanol: water with 2% acetic acid. Four glycoforms labeled A-D are present.

Table S-2. Experimental masses calculated from the spectrum in Figure S-1.

Experimental Apo-Transferrin Mass	Calculated Apo-Transferrin Mass	Glycosylation
79,584 ± 1	79,584 Da	2 Diantennary
79,731 ± 2	79,730 Da	+Fuc
80,240 ± 1	80,240 Da	1 Di, 1 Triantennary
80,386 ± 2	80,386 Da	+ Fuc

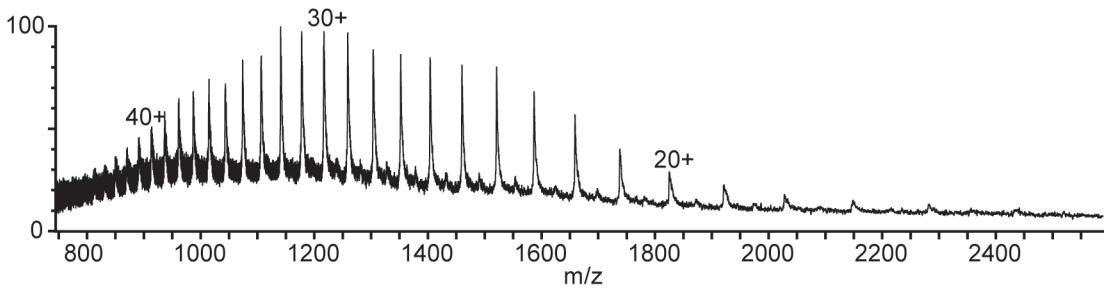

Figure S-2. nESI mass spectrum of 10 µM rabbit LDH in 60:40 methanol: water with 2% acetic acid.

Effects of supercharging reagents on S/N and LOD. Signal-to-noise ratio (S/N) and limit of detection (LOD) measurements for the most abundant protein ion for barstar, ubiquitin, α-lactalbumin, and ribonuclease A are given in Table S-3 and Figure S-3. The baseline amplitude was calculated by averaging signal intensity over a width of a *m*/*z* range of 0.5 in between ions contributing to chemical noise directly next to the most abundant protein ion in a spectrum, and the root mean square (RMS) electronic noise was calculated after baseline subtraction. The S/N was calculated as the ion signal intensity divided by the total noise (including chemical noise) over a *m*/*z* range of 20 directly next to the most abundant protein ion. A linear fit to the S/N data was used to determine the LOD (where S/N = 3) for each protein. Measurements for each protein were made using the same nanoelectrospray capillary to eliminate tip-to-tip variability, and the capillary was washed in between each sample with methanol and then water to prevent cross contamination between solutions.

For barstar, ubiquitin, and α-lactalbumin, the S/N of the most abundant ion formed with 1.5% *m*-NBA and 2.5% sulfolane is greater than without the supercharging reagents, and for barstar and ubiquitin in particular, the S/N with m-NBA is dramatically higher than without supercharging reagent and than with sulfolane (Table S-3 and Figure S-3). This result is likely due to the high chemical noise from sulfolane-salt clusters observed in mass spectra obtained from solutions with sulfolane (Table S-3). Supercharging reagents can also decrease the LOD for these protein ions formed from solutions containing sodium. The LOD for barstar from solutions containing sodium is a factor of 10 lower with *m*-NBA, the LOD for ubiquitin is halved with either *m*-NBA or sulfolane, and the LOD for α-lactalbumin is halved with sulfolane (Table S-3). However, there is little to no improvement in S/N or LOD with supercharging reagents for other proteins, such as ribonuclease A (Table S-3 and Figure S-3). The S/N of the most abundant ribonuclease A ion decreases with supercharging reagent, and the LOD for this protein more than doubles with sulfolane. Thus, even though significant desalting occurs for all four of these proteins with *m*-NBA and especially with sulfolane, the effect of these reagents on protein ion S/N and LOD depends on the protein.

Table S-3. Chemical noise data for 5 µM protein nanoelectrosprayed from 10 mM ammonium bicarbonate, 1 mM NaCl with no supercharging reagent, with 1.5% *m*-NBA, or with 2.5% sulfolane. "N/A" indicates that there is no chemical noise intensity next to the most abundant ion.

	Protein Ion Abundance	RMS Noise	Chemical Noise	Baseline	S/N	LOD (µM)
Barstar	1210	36	N/A	82	32	0.12
+*m*-NBA	1750	8	N/A	14	210	0.02
+sulfolane	2329	8	87	52	65	0.22
Ubiquitin	11882	36	N/A	57	326	0.49
+*m*-NBA	5242	4	N/A	14	1436	0.24
+sulfolane	6676	5	86	49	586	0.25
a-Lactalbumin	824	31	N/A	47	25	0.80
+*m*-NBA	172	3	N/A	5	31	0.50
+sulfolane	450	3	23	13	35	0.36
Ribonuclease A	1282	6	64	42	103	0.33
+*m*-NBA	1151	12	N/A	18	94	0.32
+sulfolane	899	5	35	20	60	0.82

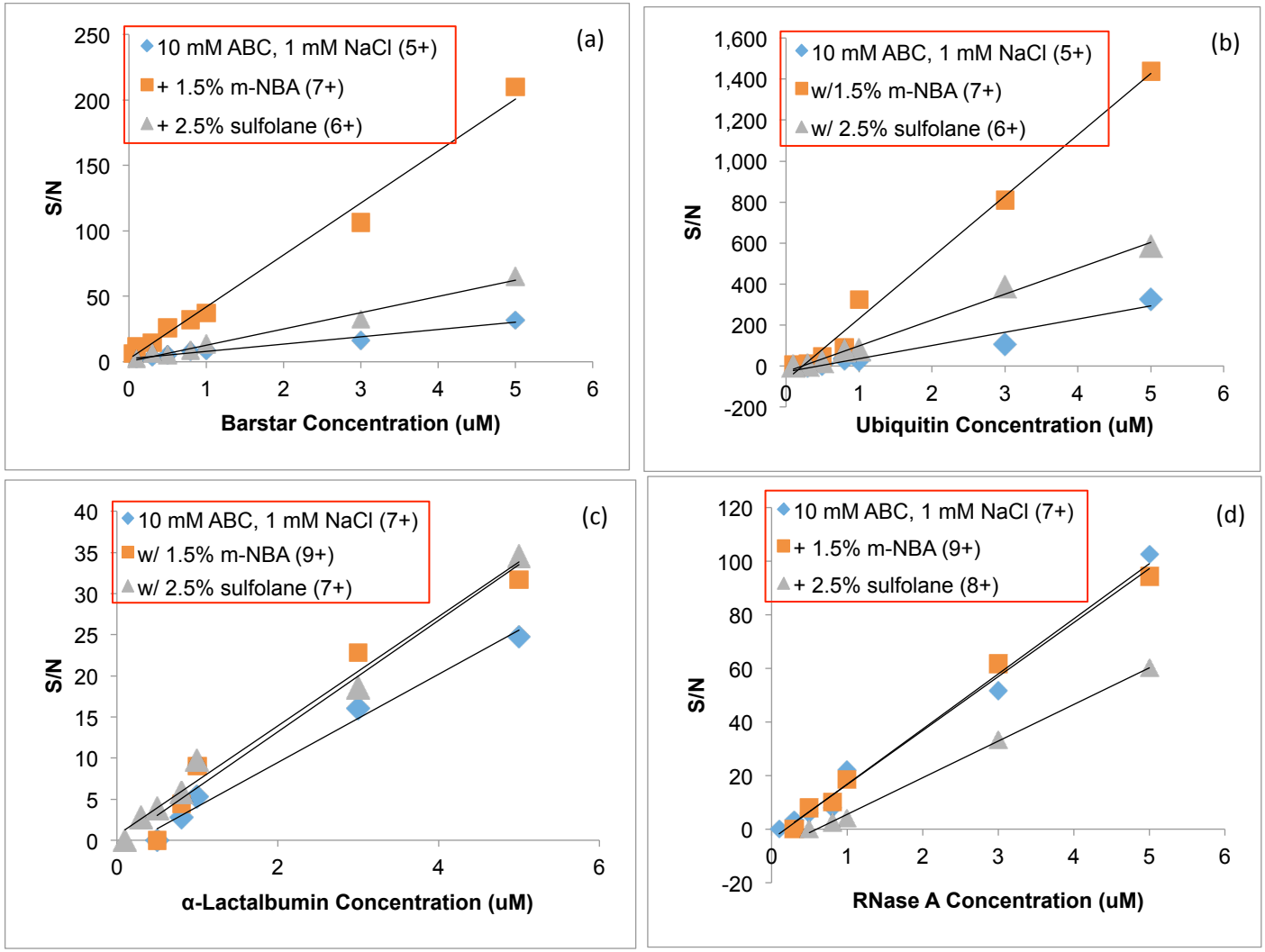

Figure S-3. Concentration dependence of the S/N of the most abundant protein ion for (a) barstar, (b) ubiquitin, (c) α-lactalbumin, and (d) ribonuclease A in 10 mM ammonium bicarbonate and 1 mM NaCl with no supercharging reagent, with 1.5% *m*-NBA, and with 2.5% sulfolane.