Supporting Information for:

Imidazole Derivatives Improve Charge Reduction and Stabilization for Native Mass Spectrometry

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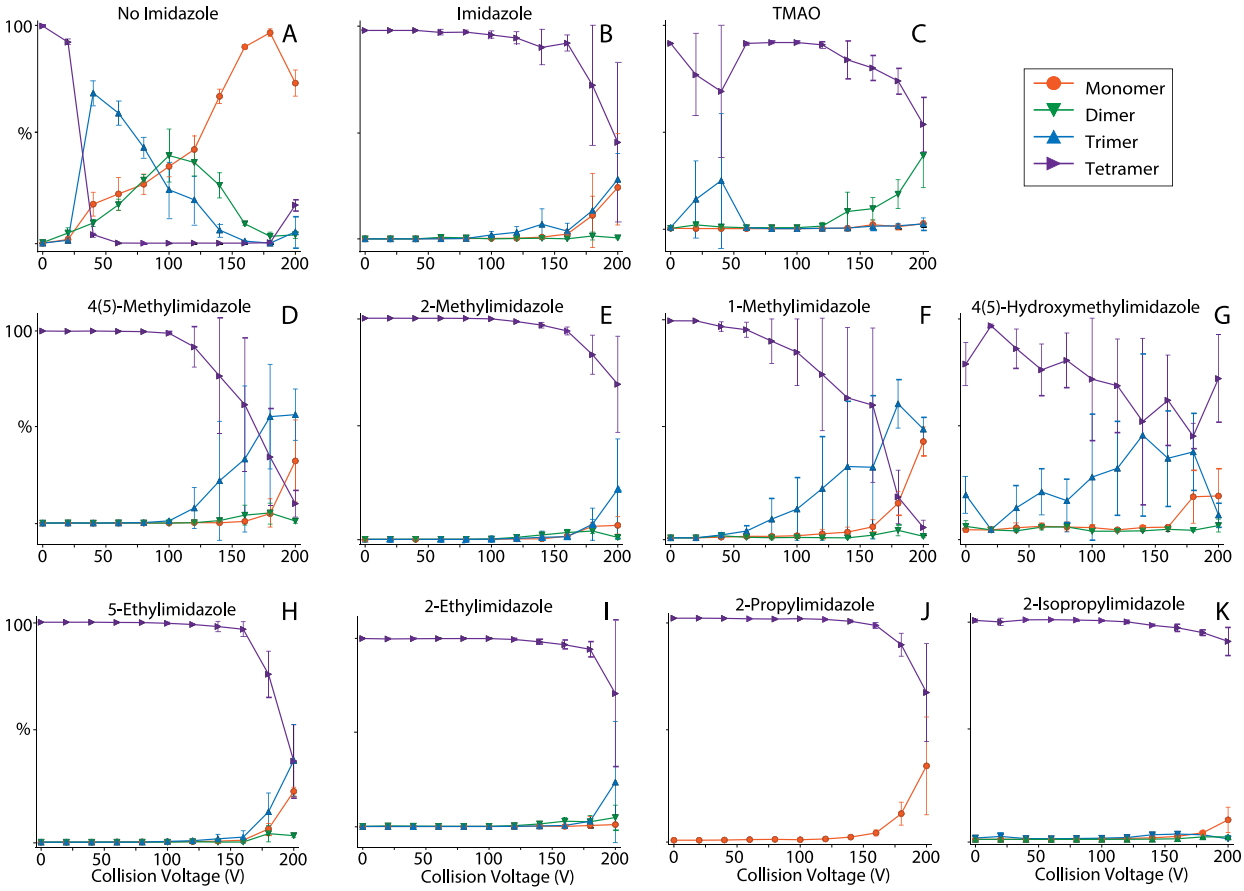

Figure S1. Streptavidin in the presence of (A) no additive or 40 mM (B) imidazole, (C) TMAO, (D) 4(5)-methylimidazole, (E) 2-methylimidazole, (F) 1-methylimidazole, (G) 4(5)-hydroxy methylimidazole, (H) 5-ethylimidazole, (I) 2-ethylimidazole, (J) 2-propylimidazole, and (K) 2-isopropylimidazole. The collision voltage was increased from 0-200 V in 20 V increments.

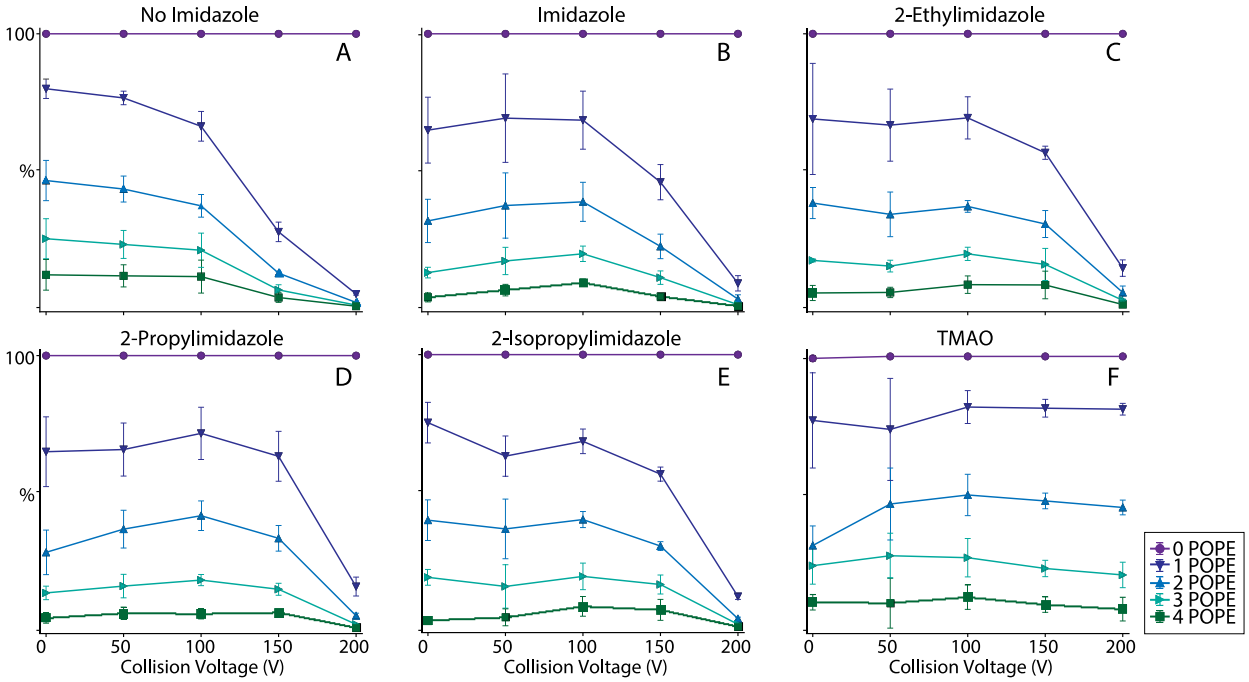

Figure S2. The retention of POPE lipids on AmtB in C8E4 as the collision voltage was increased from 0-200 V in 50 V increments with the addition of (A) no additive, or 40 mM of (B) imidazole, (C) 2-ethylimidazole, (D) 2-propylimidazole, (E) 2-isopropylimidazole, and (F) TMAO.

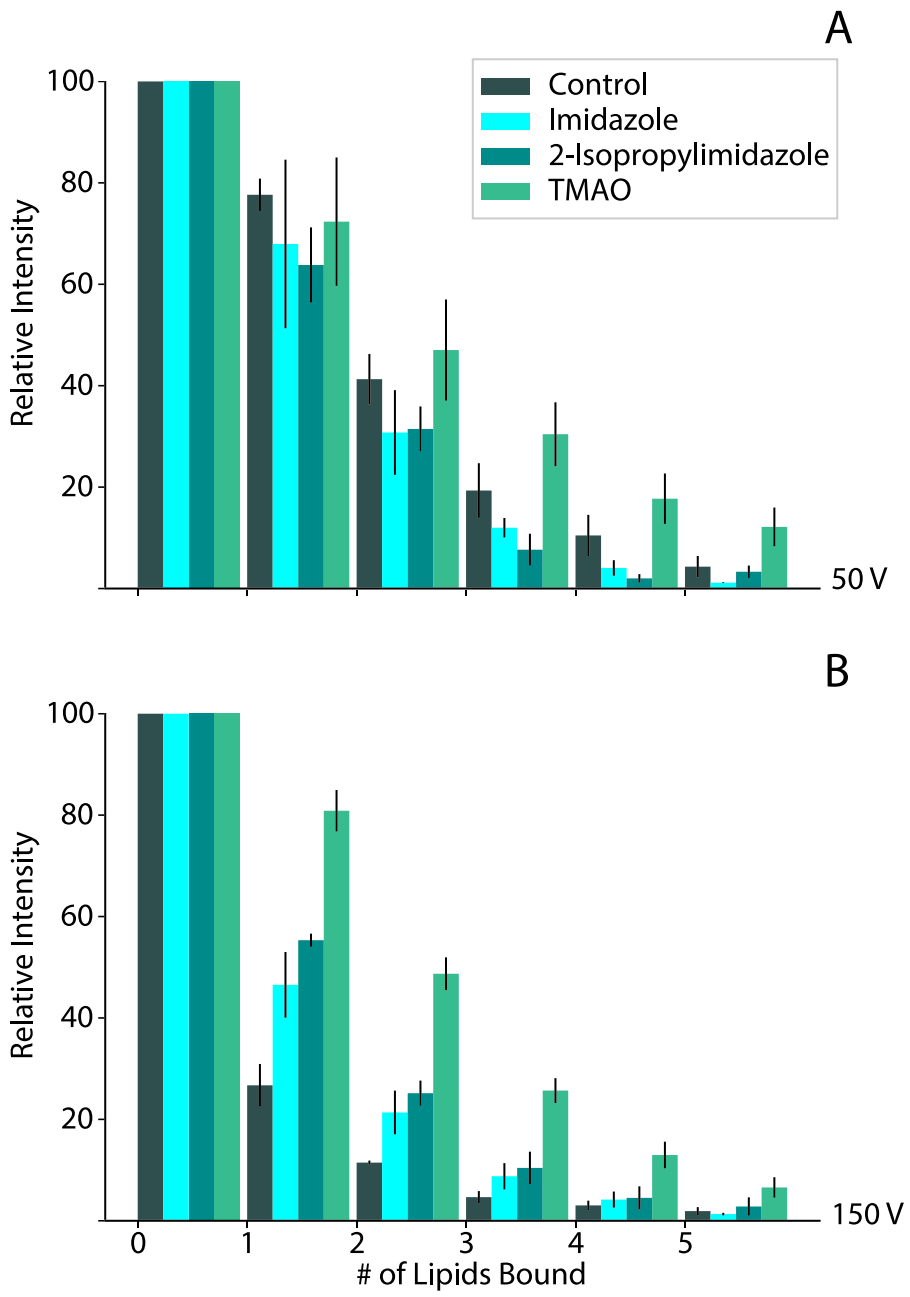

Figure S3. The binding of POPE lipids under A) 50 V and B) 150 V collisional activation in the presence of no additive or 40 mM imidazole, 2-isopropylimidazole, and TMAO.

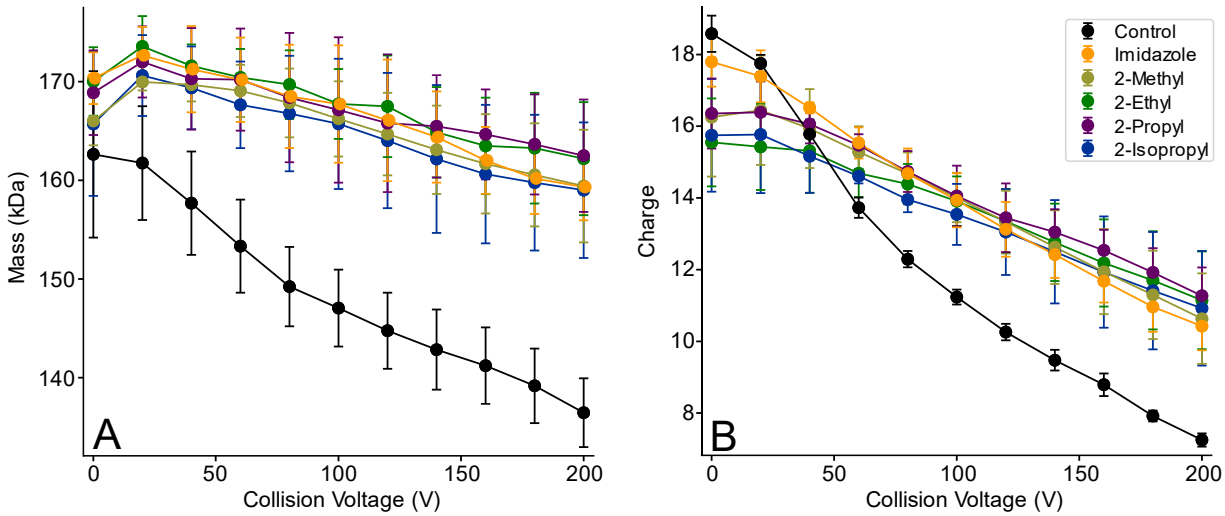

Figure S4. The addition of 10 mM imidazole derivatives to empty DMPC nanodiscs (A) stabilizes the average mass towards increasing collision voltage and (B) reduces the initial average charge. Water was added as a control. The collision voltage was increased from 0-200 V in 20 V increments.

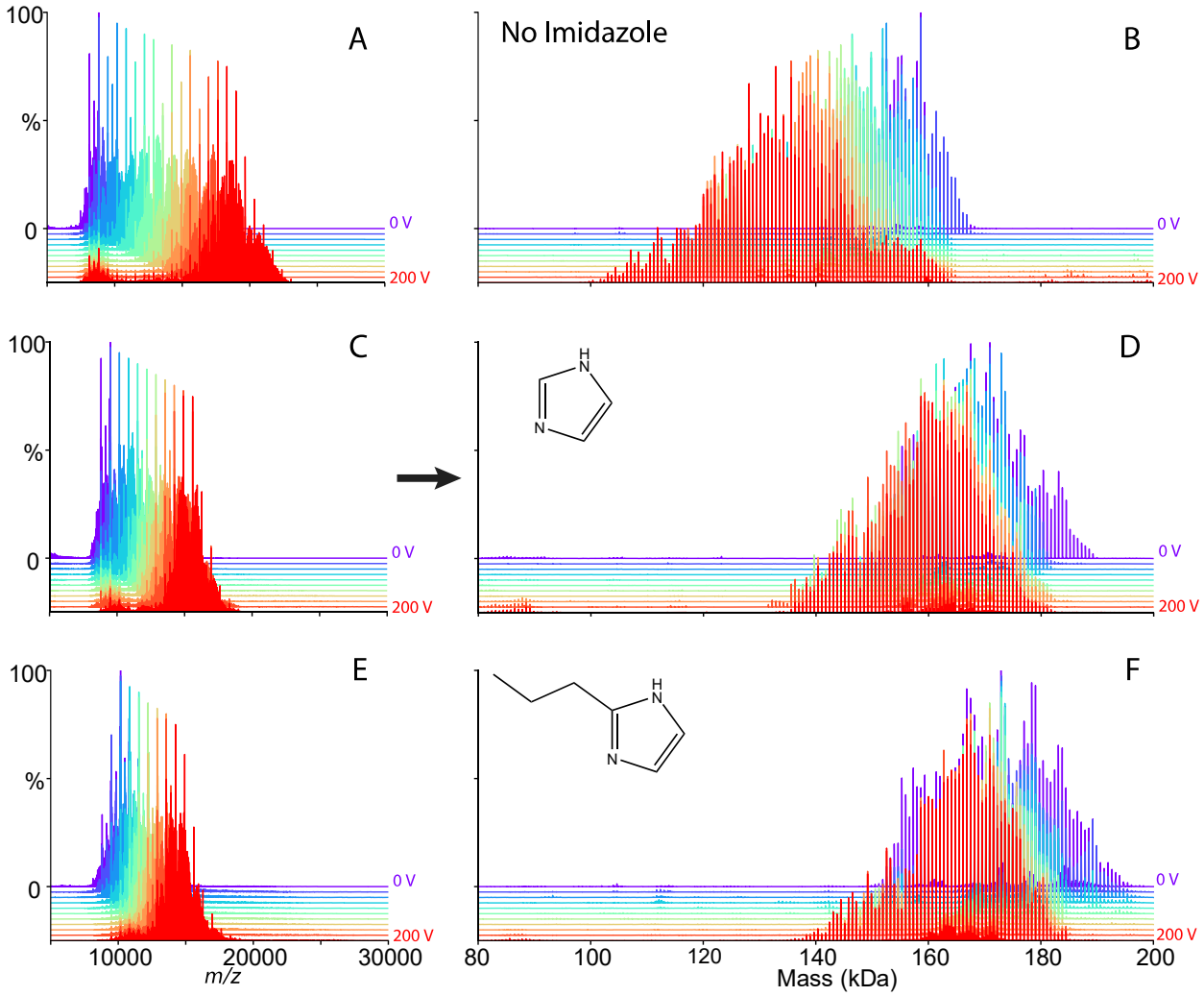

Figure S5. The mass spectra (A, C, E) and deconvolved mass distributions (B, D, F) of empty DMPC nanodiscs in the presence of (A) no additive, or 10 mM of (B) imidazole and (C) 2-propylimidazole. The in-source trapping collision voltage was increased in 0-200 V increments of 20 V.

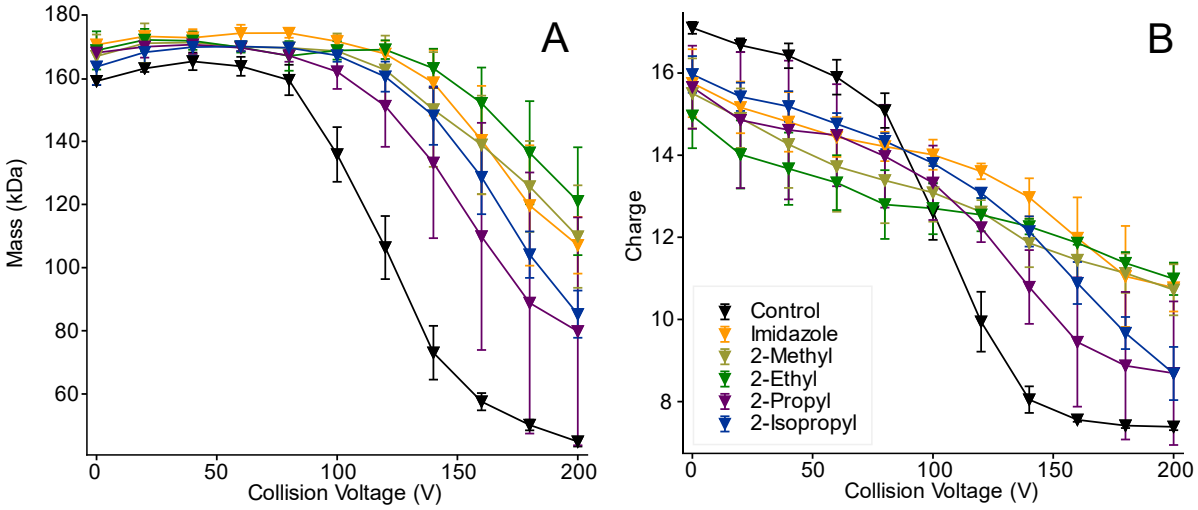

Figure S6. The effect of the addition of 10 mM charge reducing agent on the average (A) mass and (B) charge of empty DMPG nanodiscs with increasing collisional voltage. The collision voltage was increased from 0-200 V in 50 V increments.

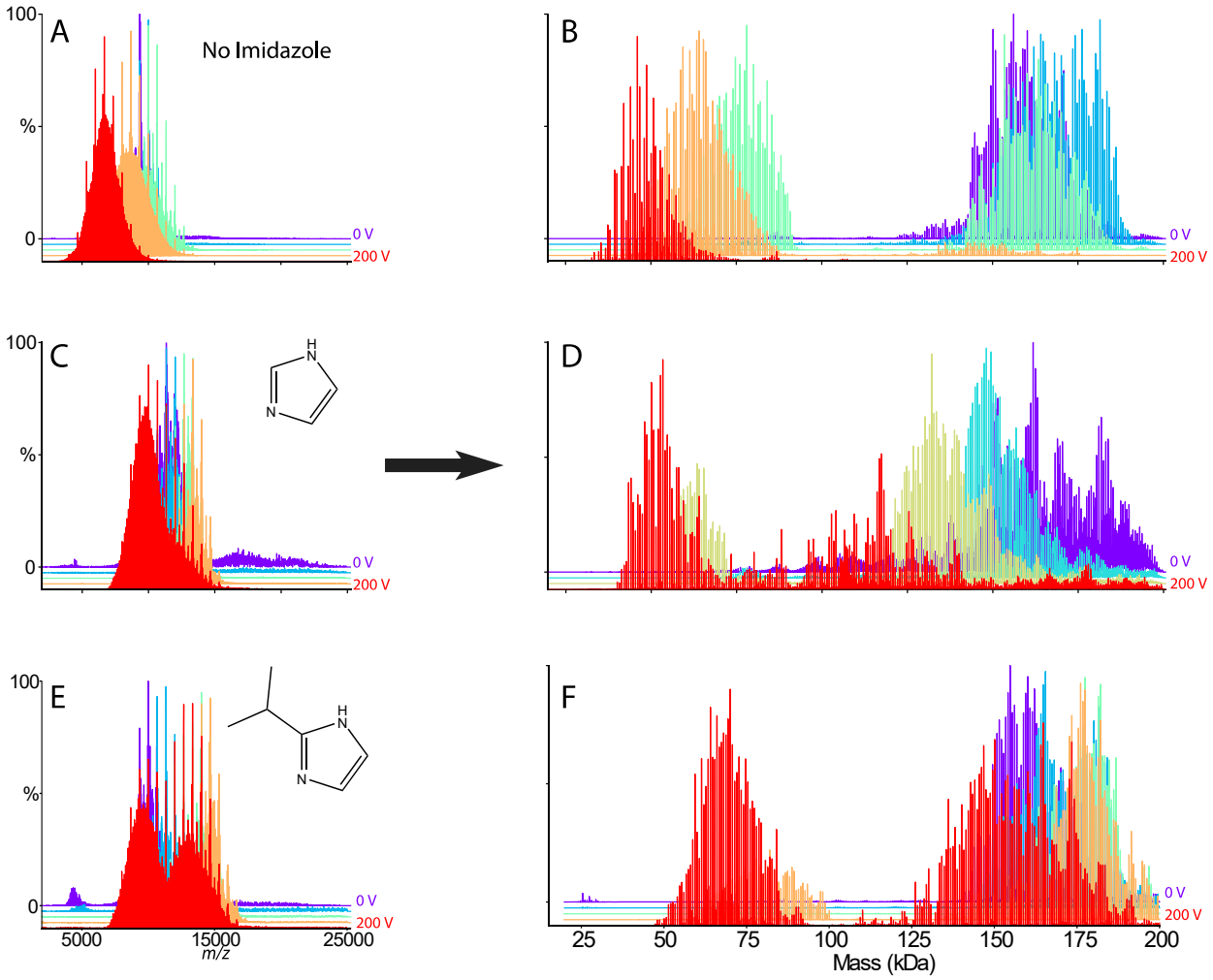

Figure S7. The mass spectra (A, C, E) and deconvolved mass distributions (B, D, F) of empty DMPG nanodiscs in the presence of (A, B) no additive, or 10 mM (C, D) imidazole and (E, F) 2-isopropylimidazole. The collision voltage was increased from 0-200 V in increments of 50 V.

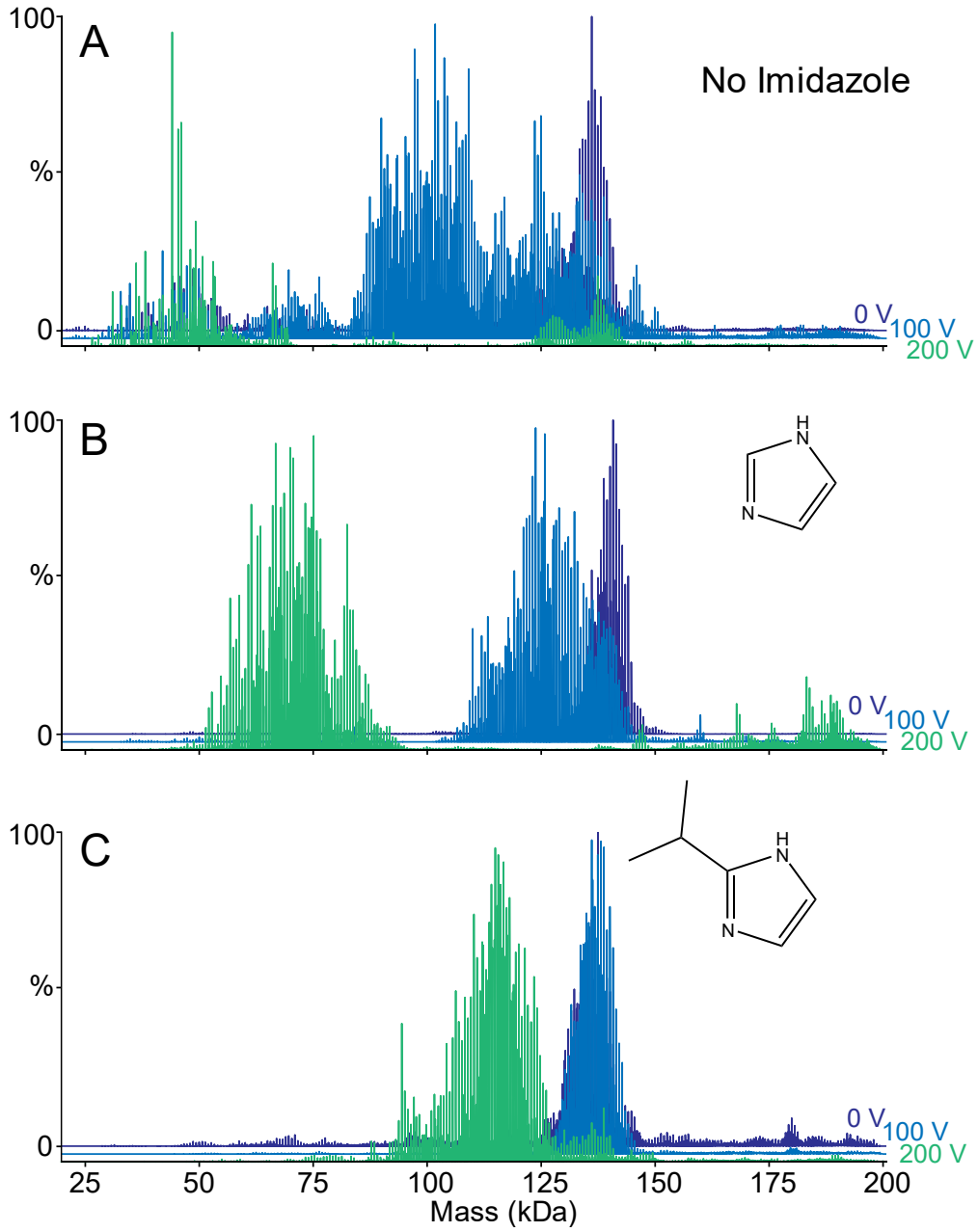

Figure S8. Mass spectra of DMPG nanodiscs and 6/1 ratio of LL-37 in the presence of (A) no additive, (B) 9 mM imidazole, and (C) 9 mM 2-isopropylimidazole. The collision voltage was increased from 0-200 V in increments of 100 V.

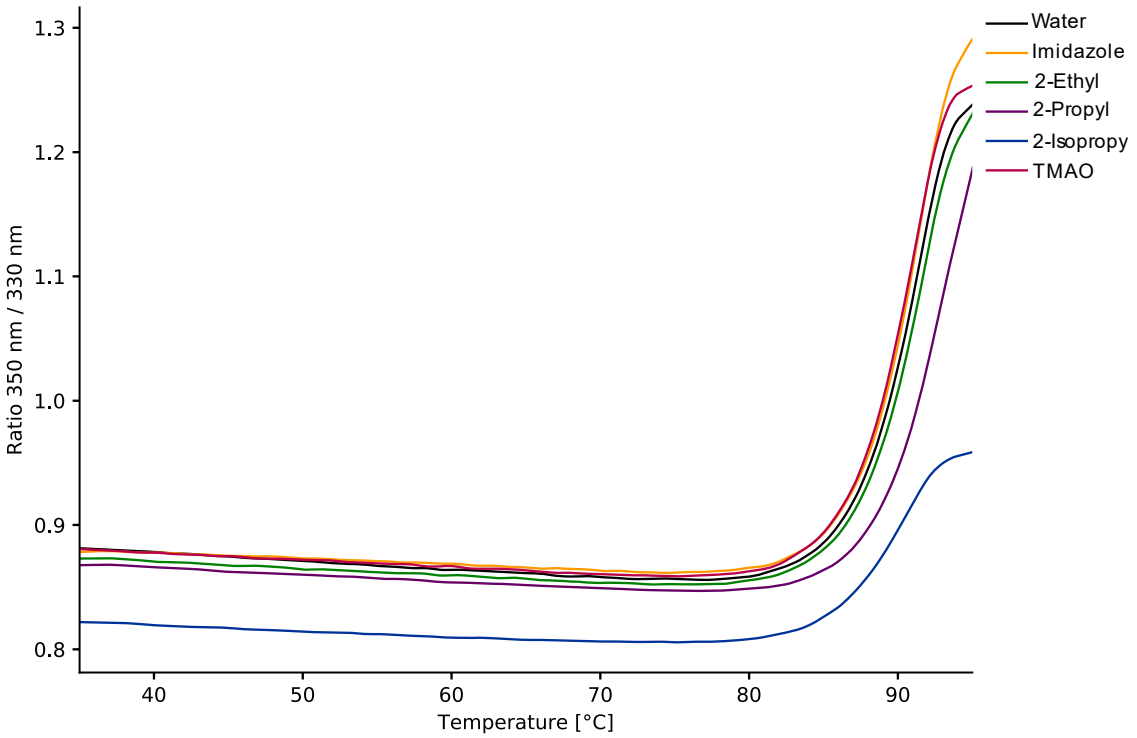

Figure S9. A representative nanoDSF data set of the three thermostability experiments for streptavidin. The charge reducing reagents were added at a concentration of 40 mM.

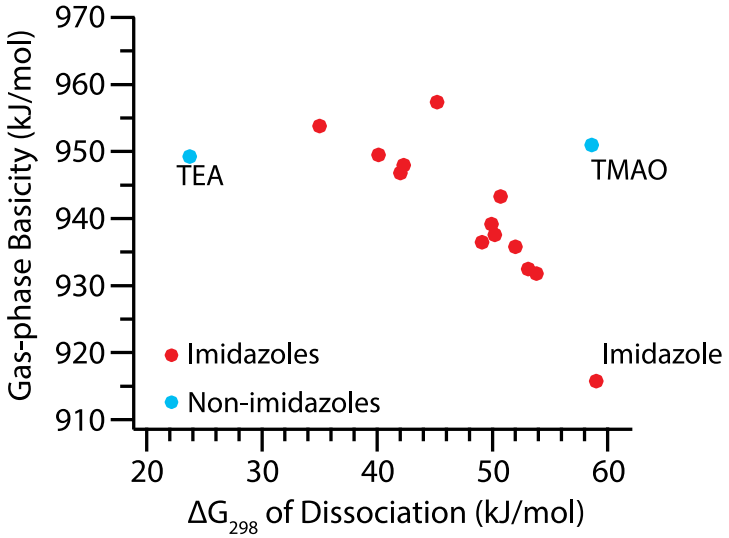

Figure S10. A comparison of computed gas-phase basicities against the ΔG_{298} of dissociation for proton-bound dimer with *n*-butylamine. Imidazole and its derivatives are shown in *red*. TMAO and TEA are shown in *blue*.

SUPPLEMENTAL TABLES

Table S1. The average unfolding temperatures, T_m (°C), for NanoDSF experiments with streptavidin, AmtB, and empty DMPC nanodiscs in the presence of charge reducing reagents. Each sample was measured with each of the charge reducing reagents in triplicate. The unfolding temperature for streptavidin in the presence of 2-propylimidazole was not detected because it was above of the upper limit of detection.

Reagent	Streptavidin	AmtB	Nanodiscs
Water	91.05±0.03	76.85±0.15	79.40±1.13
Imidazole	91.15±0.04	76.94±0.01	73.34±0.84
2-Ethylimidazole	91.27±0.04	76.98±0.07	75.17±0.92
2-Propylimidazole	ND	76.53±0.16	74.53±0.78
2-Isopropylimidazole	90.40±0.20	76.34±0.25	77.37±1.37
TMAO	90.64±0.11	76.90±0.05	80.12±3.85

Table S2. Calculated ΔG_{298} of dissociation for proton-bound dimer with *n*-butylamine, proton affinity (PA), and gas-phase basicity (GB) values for different charge reduction reagents. All values are reported in kJ/mol. PA and GB values reported in parentheses are experimental values from Hunter and Lias, 1998. Our values are systematically higher than those from Hunter and Lias, due to the difficulty of establishing an accurate experimental anchor value for PA/GB measurements as well as computational error, thus we expect the computed differences in PA/GB between different bases are more reliable than their absolute values. Dimer dissociation energies were not computed for *n*-butylamine and methylguanidine.

Reagent	**ΔG_{298} Diss.**	**PA**	**GB**
imidazole	59.0	947.8 (942.8)	915.5 (909.2)
1-methylimidazole	52.0	968.2 (959.6)	935.5 (927.7)
2-methylimidazole	50.2	973.0 (963.4)	937.3 (929.6)
4-methylimidazole	53.1	964.4 (952.8)	932.2 (920.9)
4-hydroxymethylimidazole	53.8	963.8	931.5
1-ethylimidazole	50.7	975.1	943.0
2-ethylimidazole	49.9	978.3	938.9
4-ethylimidazole	49.1	967.8	936.2
2-propylimidazole	42.3	979.6	947.7
1-isopropylimidazole	40.1	981.6	949.2
2-isopropylimidazole	42.0	979.9	946.5
1-*t*-butylimidazole	45.2	989.6	957.1
2-*t*-butylimidazole	35.0	984.5	953.5
TMAO	58.4	980.5	950.7
TEA	22.0	981.5 (981.8)	949.0 (951.)
n-butylamine	--	923.0 (921.5)	891.6 (886.6)
methylguanidine	--	1012.6	982.2