

Supporting Information

Surface Activity of Amines Provides Evidence for Sequential ESI Mechanism

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Description of the CC-FEM

The Rayleigh limit of charges in a droplet is described as the point at which the repulsion amongst charged species contained within a droplet exceeds the surface tension of the droplet, causing the droplet to undergo a fission event. While droplet fissioning is an important driving force for ESI, researchers have sought a more specifically defined mechanism for the generation of ions from electrospray droplets. Consta et al. have proposed that charged droplets do not necessarily have to be spherical and that they may actually deform as a way to reduce the repulsive forces that closely packed like-charged ions generate.^{1, 2} However, the droplet charge can be considered dynamic, and it is entirely possible that charged droplets will emit hydrated ions,³ for the purpose of preserving the integrity of the droplet, by pathways other than coulombic explosion. Thompson proposed a theory for generation of ions through what is now referred to as the IEM.⁴⁻⁶ **Equation 1** provides a framework for understanding the generative process of small ions from nanodroplets; n is the charge of the droplet, ϵ_0 is the permittivity of free space, E^* is critical charge of the droplet surface, e is elemental charge, and D is the diameter of the droplet.

$$n = \frac{\pi\epsilon_0 E^*}{e} D^2 \quad \text{Equation 1}$$

E^* essentially governs the rate of IEM-generated ions from nanodroplets and is an innate factor for charge-carrying species in ESI droplets. Hogan et al. proposed that charge carriers with lower E^* thresholds will be emitted from the surface of the droplet more preferentially than ions that have higher E^* thresholds.^{7, 8} As the droplet shrinks via solvent evaporation, the density of the charges in the nanodroplet increases; this increase in repulsive forces causes ions on the surface to be emitted from the droplet to reduce the energy needed to keep the droplet shape, as illustrated by Kim et al.³ Once the necessary critical surface charge is reached, ions on the surface of the droplet are ejected and the evaporation of the solvent continues until this step is repeated again. This process is considered separate from that of fissioning or coulombic explosion as the IEM process is concerned with the emission of charged species that may or may not be solvated.⁹

Table S1. Relevant experimental conditions and values for CCS measurements of CRP; ammonium acetate (AmAc), triethylammonium acetate (TEAA)

Buffer Conditions	Emitter Bias (V)	Mass (kDa)	He Pressure (Torr)	Z (+)	α	PF-DT CCS (nm ²)	Field Strength (V/cm-torr)
200 mM AmAc	0	115.2	1.725	24	0.90	71.9	5.71
				23	0.90	71.5	5.71
				22	0.90	70.8	5.71
				21	0.90		
				20	0.90		
				19	0.90		
				18	0.90		
200 mM AmAc	200	115.2	1.725	24	0.90	72.1	5.71
				23	0.90	71.5	5.71
				22	0.90	71.0	5.71
				21	0.90	68.8	5.71
				20	0.90		
				19	0.90		
				18	0.90		
160 mM AmAc 5 mM TEAA	0	115.2	1.725	24	0.90	72.2	5.71
				23	0.90	71.5	5.71
				22	0.90	71.0	5.71
				21	0.90	70.4	5.71
				20	0.90		
				19	0.90		
				18	0.90		
160 mM AmAc 5 mM TEAA	200	115.2	1.725	24	0.90	72.1	5.71
				23	0.90	71.7	5.71
				22	0.90	71.2	5.71
				21	0.90	70.8	5.71
				20	0.90	70.4	5.71
				19	0.90	70.2	5.71
				18	0.90	69.1	5.71
160 mM AmAc 10 mM TEAA	0	115.2	1.725	24	0.90	72.0	5.71
				23	0.90	71.5	5.71
				22	0.90	71.0	5.71
				21	0.90	70.5	5.71
				20	0.90	70.5	5.71
				19	0.90	70.1	5.71
				18	0.90	69.9	5.71
160 mM AmAc 10 mM TEAA	200	115.2	1.725	24	0.90	72.3	5.71
				23	0.90	71.4	5.71
				22	0.90	70.9	5.71
				21	0.90	70.9	5.71

				20	0.90	70.4	5.71
				19	0.90	70.0	5.71
				18	0.90	69.8	5.71
160 mM AmAc 10 mM TEAA	400	115.2	1.725	24	0.90	72.4	5.71
				23	0.90	71.5	5.71
				22	0.90	71.2	5.71
				21	0.90	70.6	5.71
				20	0.90	70.6	5.71
				19	0.90	70.0	5.71
				18	0.90	69.6	5.71
				160 mM AmAc 15 mM TEAA	0	115.2	1.725
23	0.90	71.8	5.71				
22	0.90	71.2	5.71				
21	0.90	70.0	5.71				
20	0.90	70.4	5.71				
19	0.90	70.1	5.71				
18	0.90	69.6	5.71				
160 mM AmAc 15 mM TEAA	200	115.2	1.725	24	0.90		
				23	0.90		
				22	0.90		
				21	0.90		
				20	0.90		
				19	0.90	70.5	5.71
				18	0.90	69.8	5.71

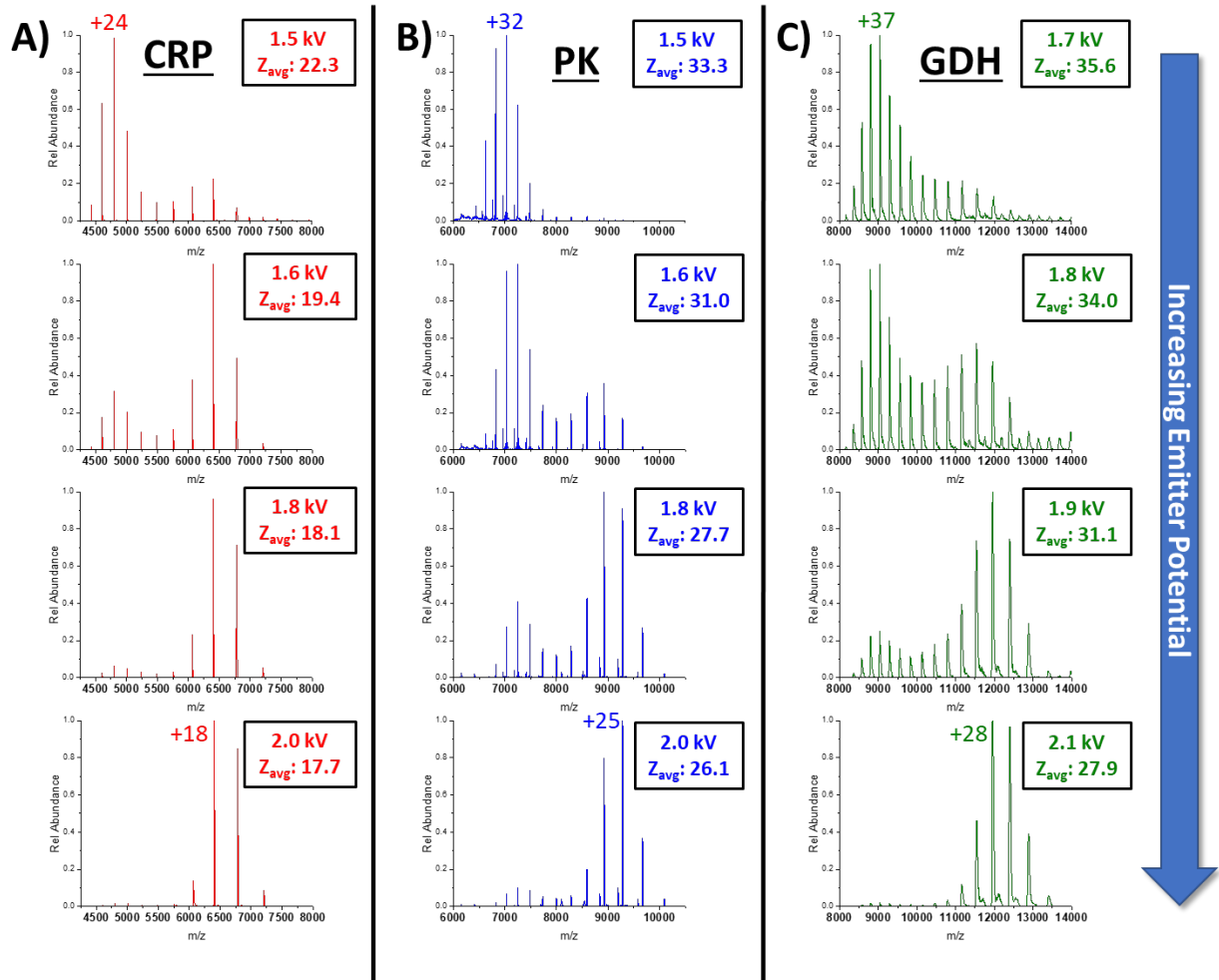


Figure S1. Spectra of CRP, PK, and GDH displaying the effect of emitter potential on the observed charge state distributions of these protein complexes. The applied emitter potential (relative to ground) along with the weighted average charge state is displayed with each spectrum. The solution conditions are 750 nM, 2 μ M, and 2 μ M of protein complex (CRP, PK, and GDH, respectively) in 160 mM AmAc and 10 mM TEAA. As the emitter potential is increased, the relative concentration of surface-active charge carriers on the surface of the droplet is increased. Increased concentrations of surface-active charge carriers allow for more charges to be relinquished by the droplet, resulting in lower average charge states.

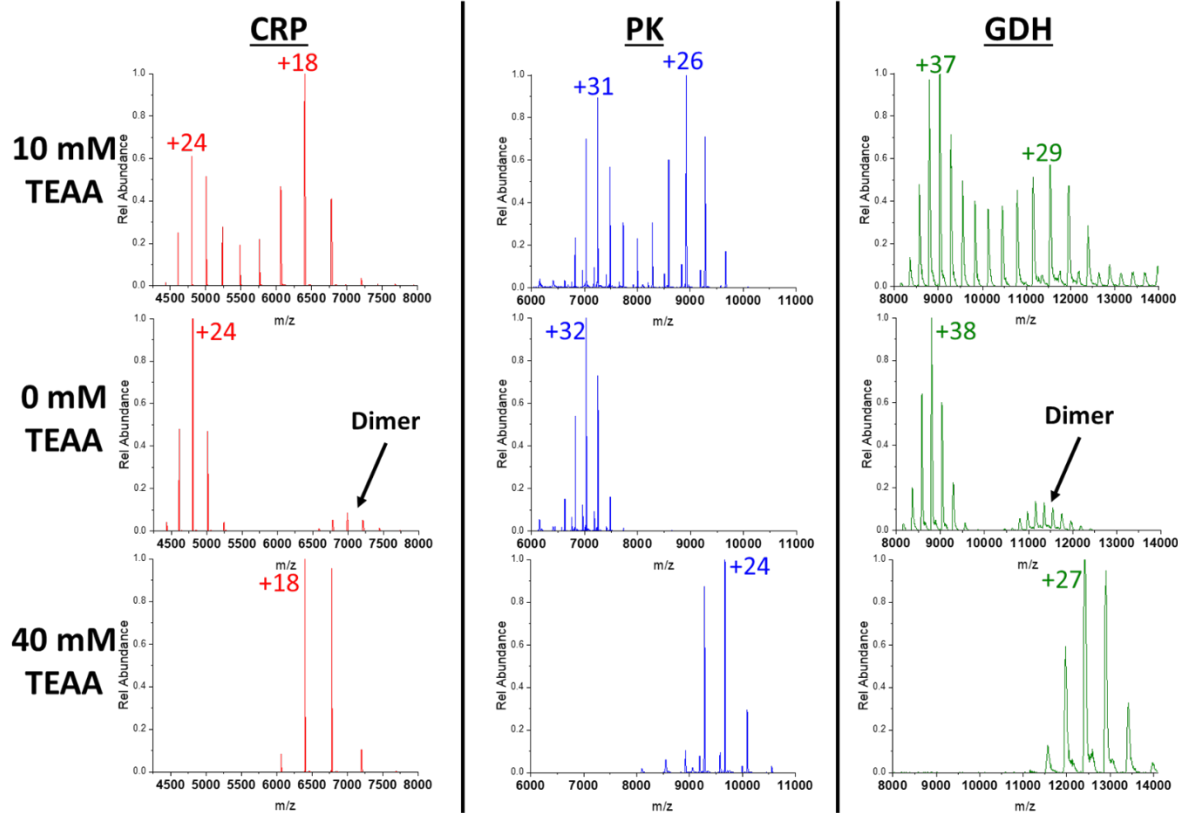


Figure S2. Spectra of CRP, PK, and GDH displaying the effect of TEAA concentration on the observed charge state distributions of these protein complexes. In the 10 mM TEAA solution conditions the bimodal distribution of charge states resembles a combination of and AmAc distribution and a 40 mM TEAA distribution (each of which are shown for the requisite protein complex). Dimers of the protein complexes are observed in some of the spectra and are labeled as not to confuse the dimer signals with those of the charge reduced protein complex.

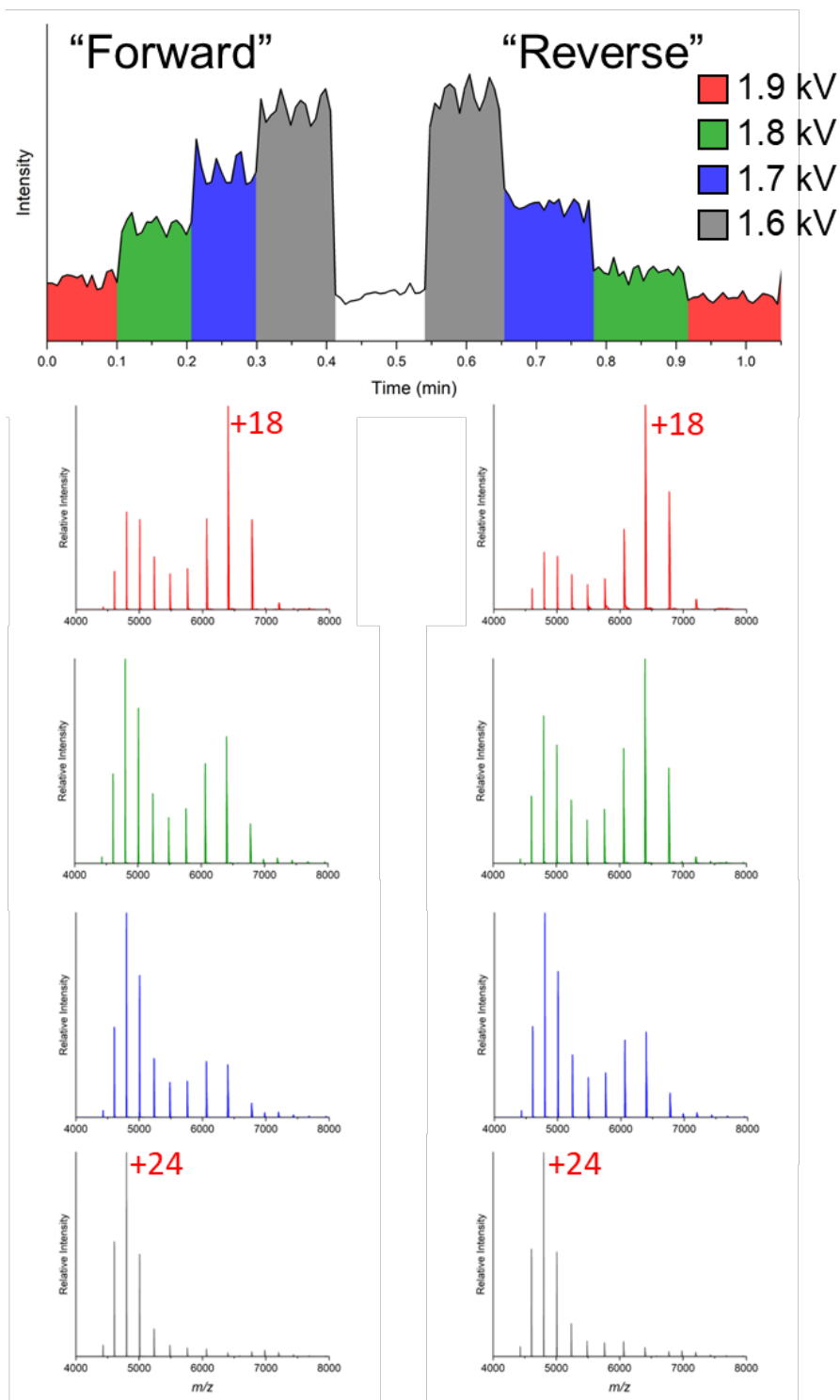


Figure S3. Demonstrates the reversibility of the charge reduction phenomenon. The top plot is the total signal intensity as a function of time. The stepwise pattern of the plot corresponds to changes in the emitter voltage applied to the sample. The various time regions are colored and correlate to the spectra represented below. As the voltage is decreased the charge reduction effect becomes less dominant but can be reversed by applying more voltage again. The solution conditions are 750 nM CRP, 10 mM TEAA, and 160 mM AmAc.

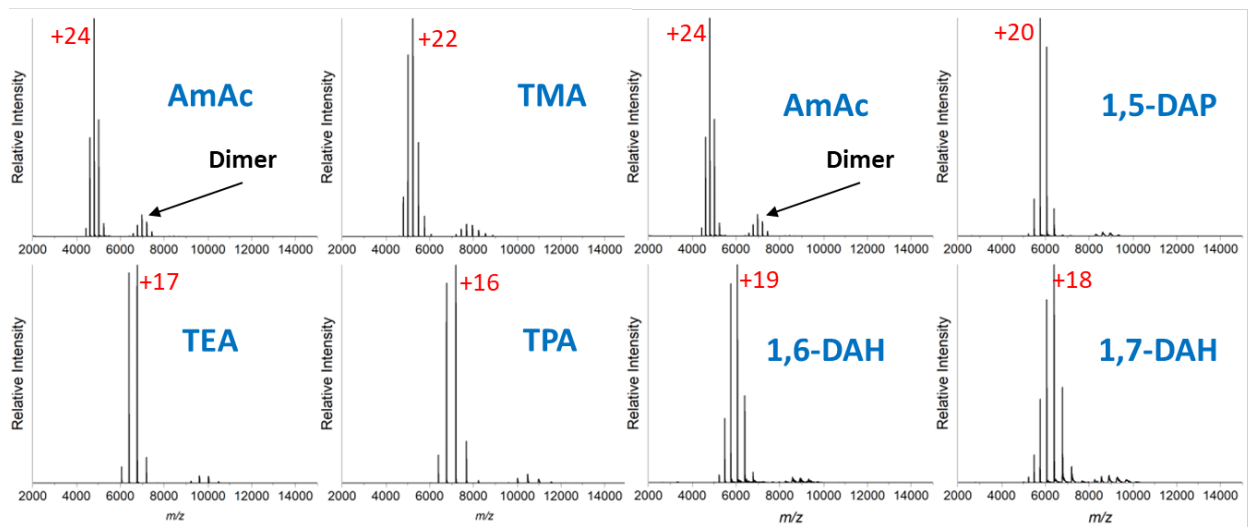


Figure S4. Full mass spectral data of CRP for all solution conditions. The lack of observed dissociated monomer (subunits of the native pentamer complex) confirms that CRP remains stable even in the presence of various amines.

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