

SUPPORTING INFORMATION

**Exploring the Mechanism of Salt-Induced Signal Suppression in
Protein Electrospray Mass Spectrometry Using Experiments and
Molecular Dynamics Simulations**

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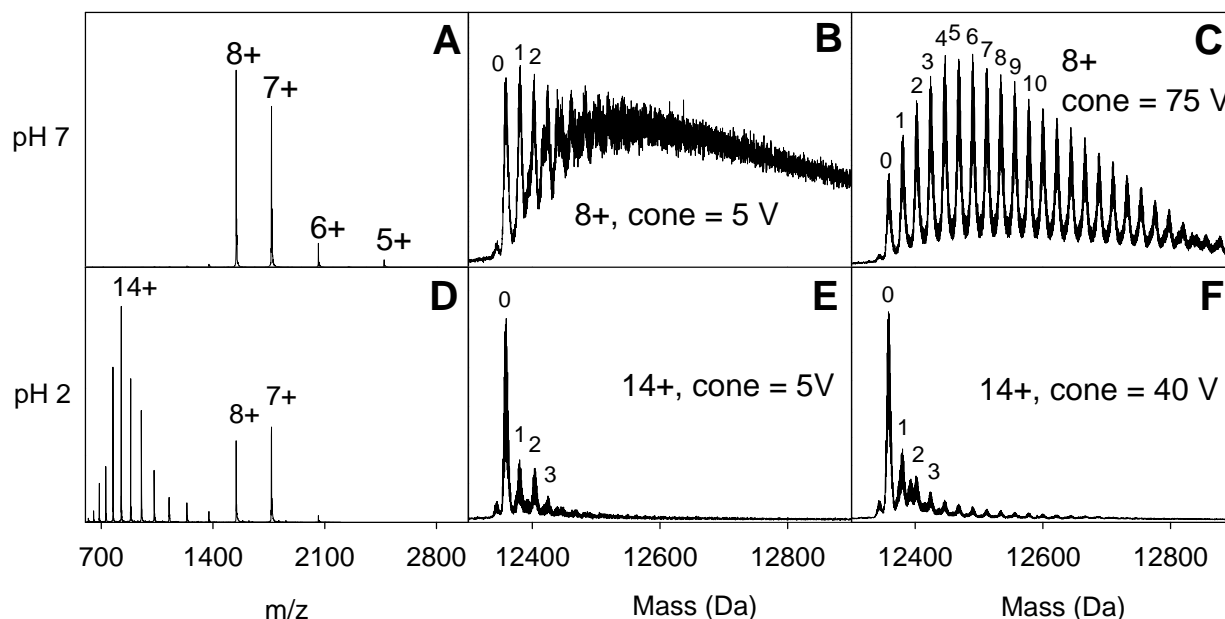
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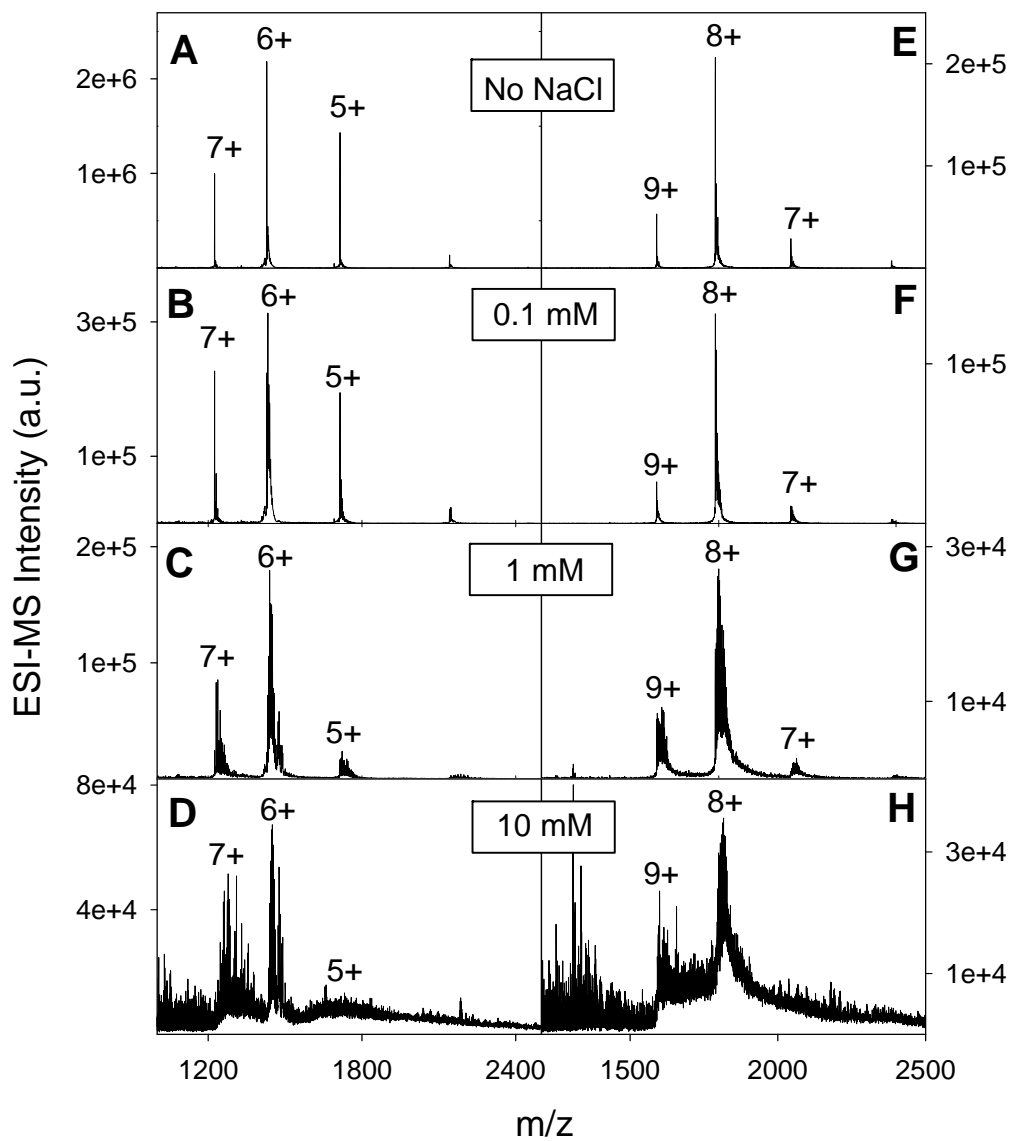
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Molecular Dynamics Simulation Details. All-atom MD simulations of salt-containing droplets were carried out using Gromacs 4.6.5^{1,2} with the CHARMM36 force field³ and the TIP3P water model.⁴ Production runs employed trajectory stitching as described previously⁵ with Nosé-Hoover temperature coupling.⁶ The structure of NBu_4^+ was obtained from the ZINC database,⁷ and it was parameterized using the ParamChem server.⁸ All bonds were constrained, thereby allowing for a 2 fs integration time step. To take advantage of GPU acceleration the simulations were carried out in a 1 μm box using a potential-shift non-bonded interaction cut-off of 333.3 nm without relying on particle-mesh Ewald summation. Initial system configurations consisted of spherical water droplets with radius 3 nm containing 10 Na/Cl pairs. Each droplet also contained a 12+ excess charge consisting of either 12 Na^+ or 12 NBu_4^+ . These systems were subjected to energy minimization followed by 10 ps of equilibration at 330 K using a modified Berendsen thermostat.⁹ Production runs employed trajectory stitching as described previously.⁵ Individual MD segments consisted of 500 ps windows at 330 K, between which any molecules that had drifted more than 10 nm from the droplet center were removed from the system. The simulated droplets are highly dynamic and undergo occasional distortions into non-spherical shapes. For reporting the droplet size we report an “effective” radius r corresponding to that of a sphere with the equivalent number of molecules. Calculations involving the surface tension of water employed the experimental value of $\gamma = 0.06624 \text{ Nm}^{-1}$ at 330 K,¹⁰ although standard MD water models yield values that are somewhat lower.^{11,12} All simulations were carried out in triplicate with different starting configurations and random seeds for initial velocity assignments.

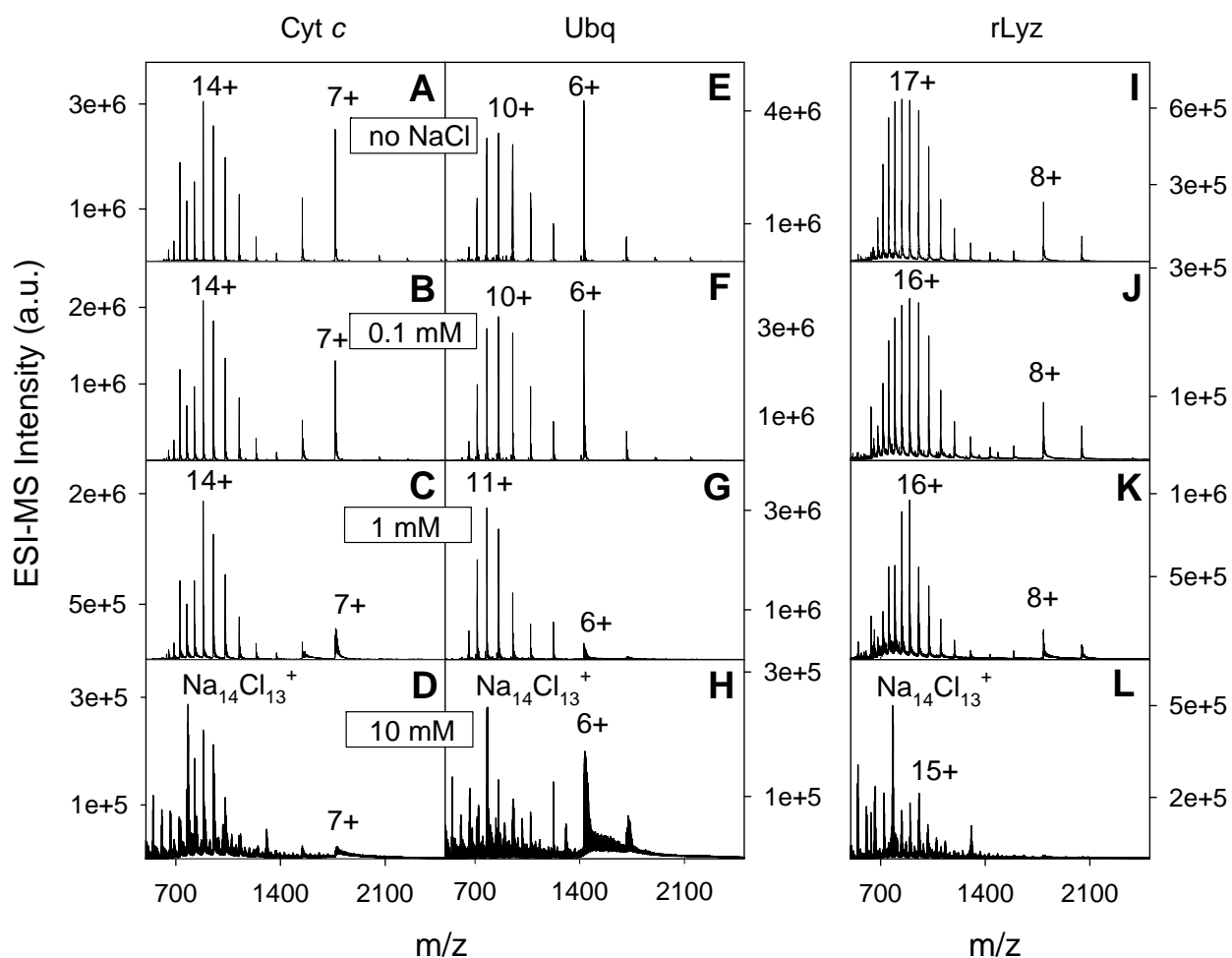
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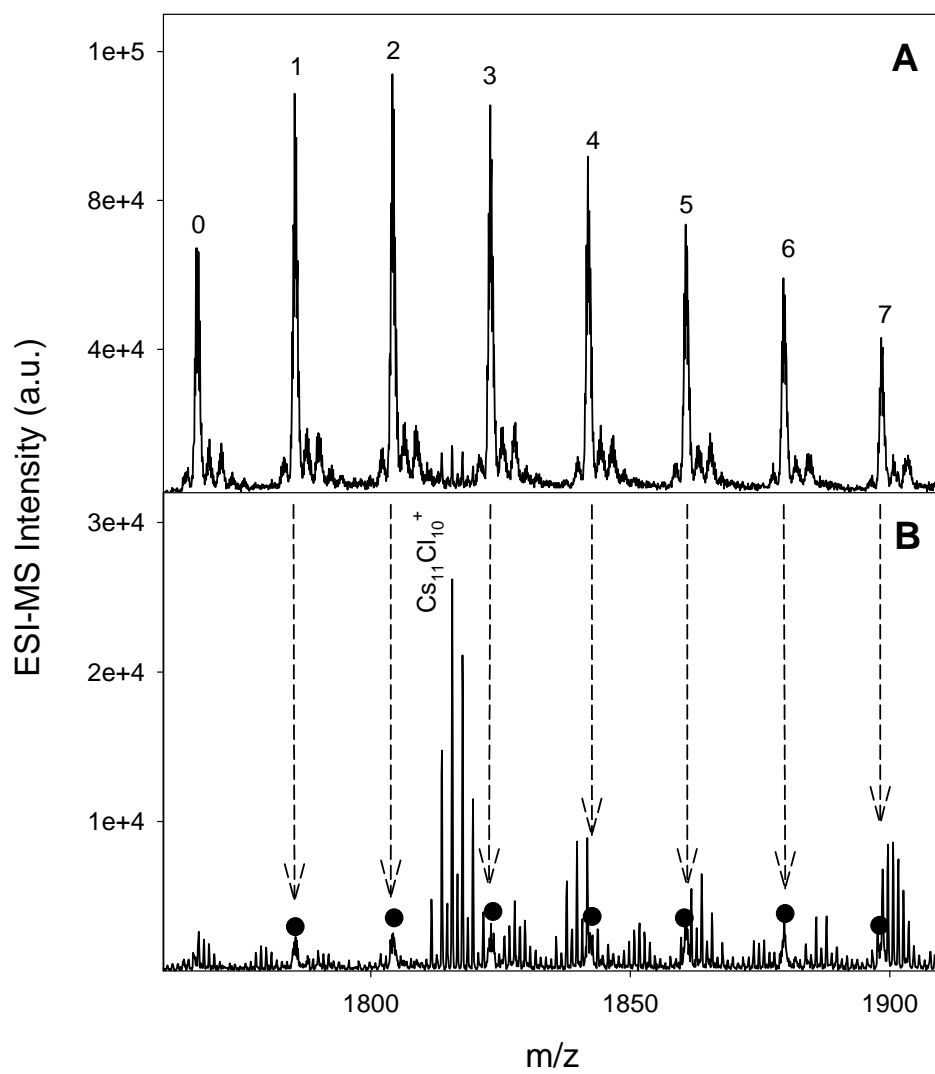
Supporting Figure S1. Cone voltage effects on ESI mass spectra of *cyt c*. Panels A-C refer to data acquired at pH 7 in aqueous solution, spectra in panels D-F were recorded at pH 2 in 50:50 (v/v) water/methanol. (A) Complete spectrum of native *Cyt c* electrosprayed in the absence of NaCl. (B) Close-up view of the 8+ charge state in the presence of 1 mM NaCl at a cone voltage of 5 V. (C) Same as in panel B, but for a cone voltage of 75 V. Note that the m/z axes were converted to *Mass* in panels B, C, E, and F. The “cleaner” appearance of the spectrum in panel C is due to the collisional loss of chloride adducts (as HCl). (D) Complete spectrum of denatured *Cyt c* electrosprayed in the absence of NaCl. (E) Close-up view of the 14+ charge state in the presence of 1 mM NaCl at a cone voltage of 5 V. (F) Same as in panel E, but for a cone voltage of 40 V. Numbers 0, 1, 2, ... indicate how many adducted sodium ions can be resolved in the spectra.



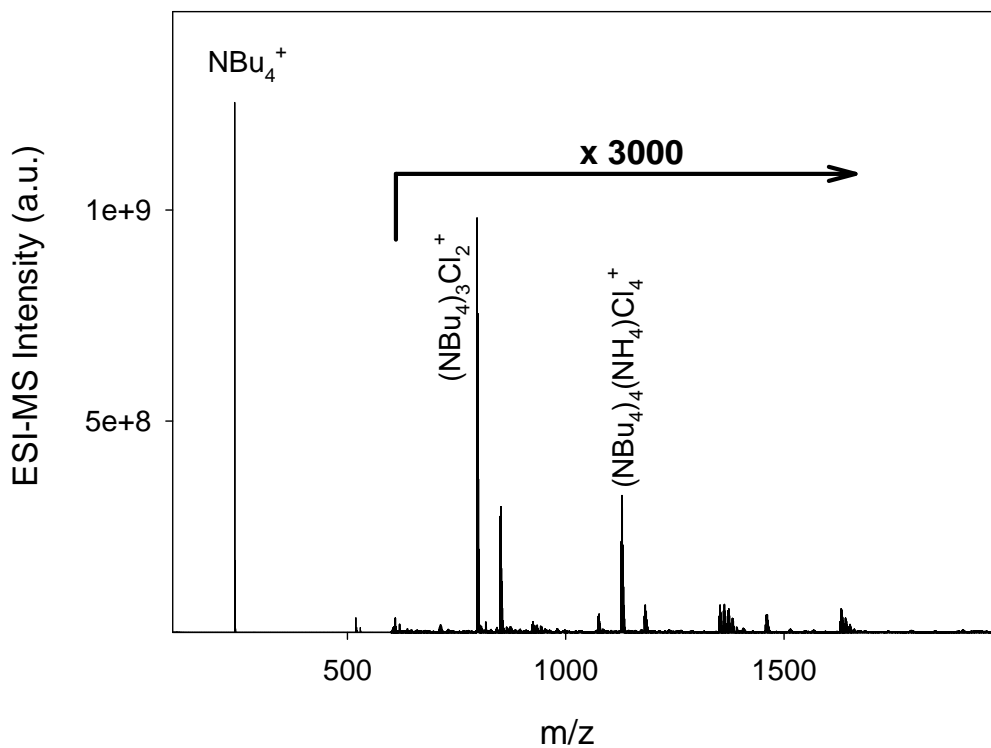
Supporting Figure S2. ESI mass spectra of Ubq (A-D) and Lyz (E-H) acquired at pH 7 in the presence of different NaCl concentrations (zero to 10 mM NaCl, as indicated in the individual panels).



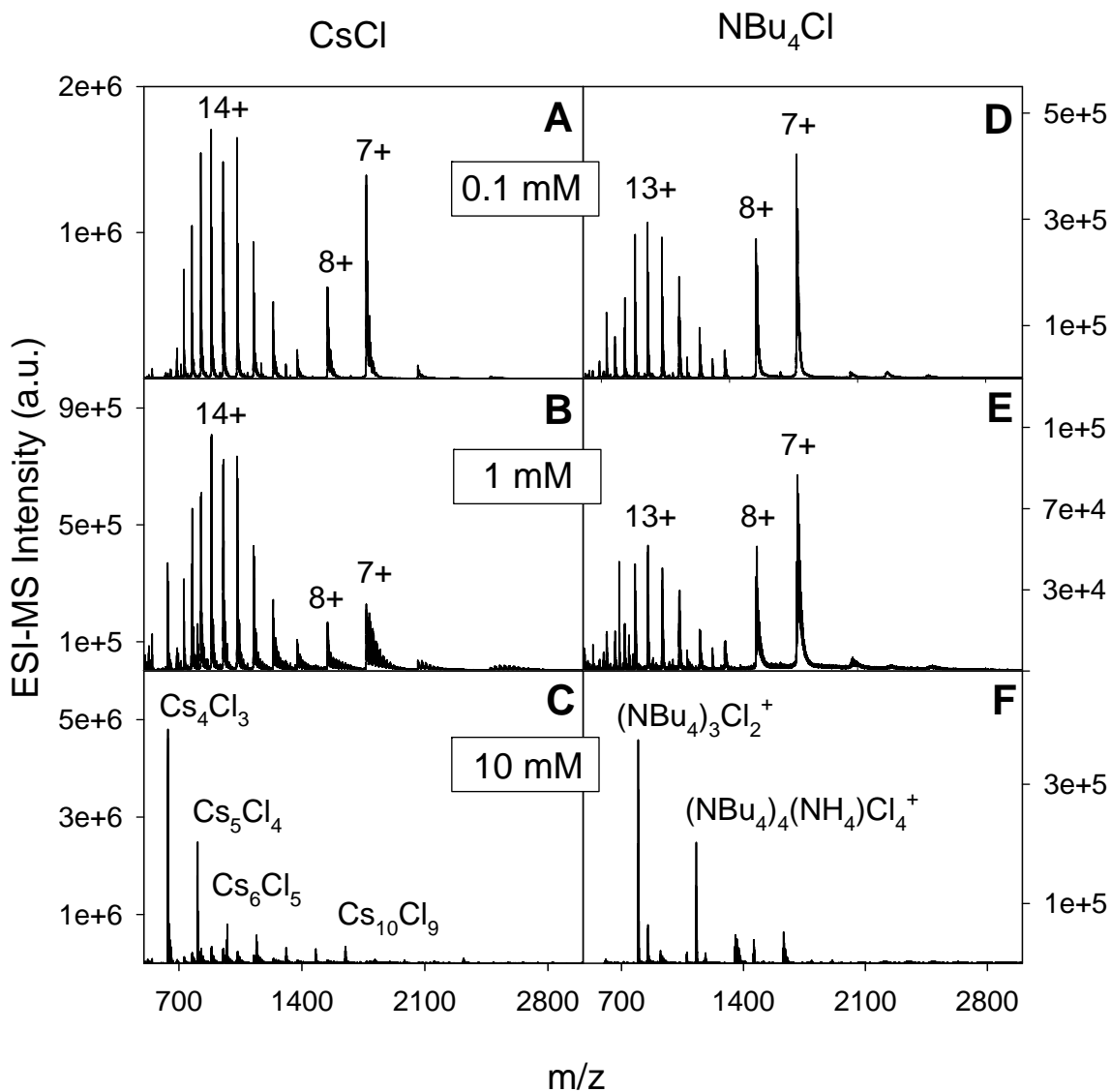
Supporting Figure S3. ESI mass spectra of Cyt *c* (A-D), Ubq (E-H) and rLyz (I-L) acquired under denaturing solvent conditions (pH 2 in water/methanol) in the presence of different NaCl concentrations (zero to 10 mM NaCl, as indicated in the individual panels).



Supporting Figure S4. Close-up views of the Cyt *c* the 7+ charge state acquired at pH 7 in the presence of (A) 1 mM CsCl and (B) 10 mM CsCl. Numbers $n = 0, 1, 2, \dots$ in panel A denote how many Cs^+ ions are bound to the $[\text{M} + 7\text{H} + n(\text{Cs} - \text{H})]^{7+}$ ions. Dashed arrows in panel B emphasize that protein signals are still observable in 10 mM CsCl, despite the dominance of salt cluster signals. Protein signals in panel B are further highlighted by filled circles.



Supporting Figure S5. ESI mass spectrum of Cyt *c* acquired at pH 7 in the presence of 10 mM NBu_4Cl . Protein signals are almost completely suppressed; instead the spectrum is dominated by NBu_4^+ . Note that the signal intensity has been magnified by a factor of 3000 for $m/z > 600$.



Supporting Figure S6. ESI mass spectra of Cyt *c* acquired at pH 2 in water/methanol and in the presence of different concentrations of CsCl (A-C) and NBu₄Cl (D-F). Protein charge states as well as the composition of selected salt cluster signals are indicated.