Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Corona discharge electrospray ionization of formate-containing solutions enables in-source reduction of disulfide bonds

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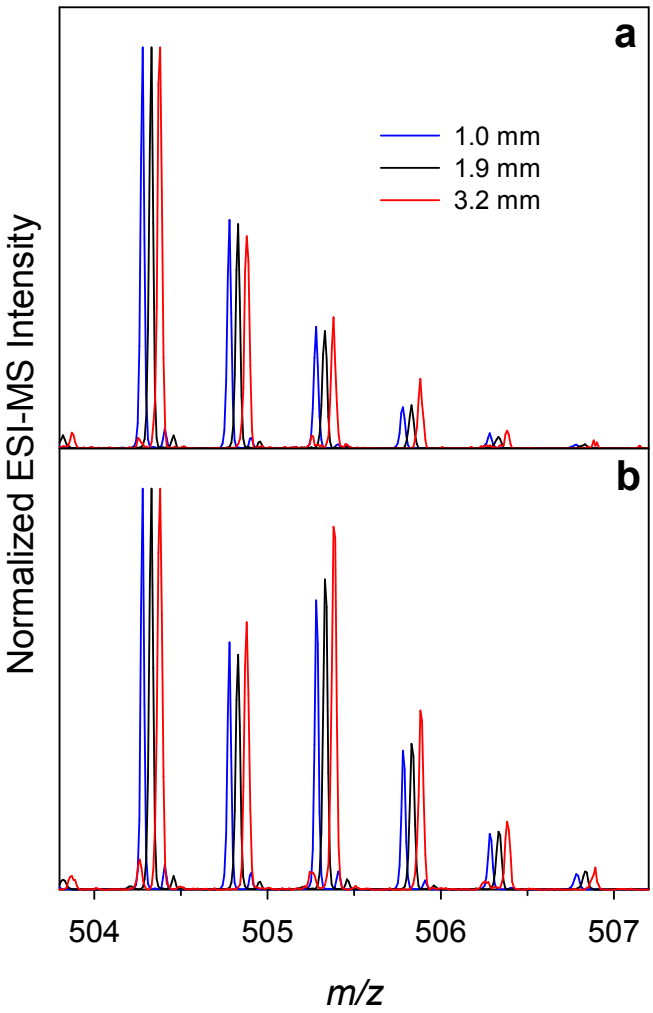

Fig. S1 Mass spectra of $[OT + 2H]^{2+}$ acquired at 4 kV at varying capillary-to-MS distance. Indicated values refer to capillary lengths extruding from ESI probe, therefore smaller lengths correspond to larger capillary-to-MS distances. OT dissolved in: (a) 100 mM ammonium acetate, pH 6.7 or (b) 100 mM ammonium formate, pH 6.7. Lengths were 1.0 mm (blue line), 1.9 mm (red, offset by 0.05 Da), or 3.2 mm (black, offset by 0.1 Da). Spectra were offset for clarity

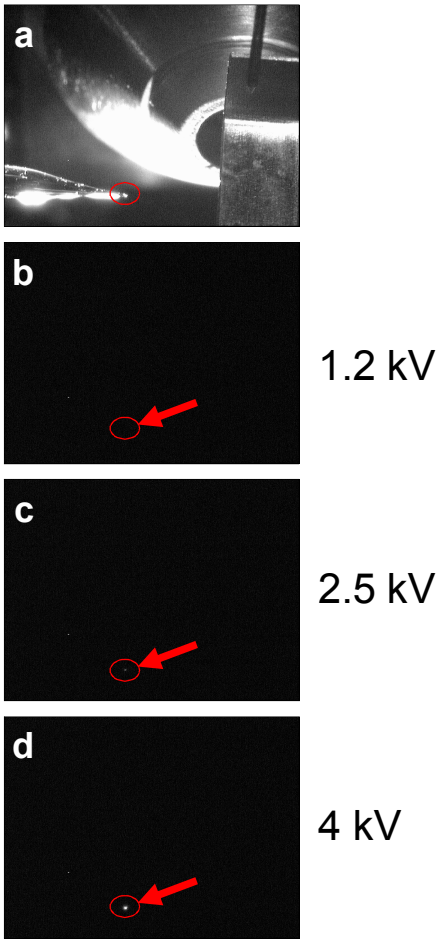

Fig. S2 Close-up images of nanospray capillary and MS sample cone cover taken with camera embedded in NanoLockspray ionization source. Red circles locate ESI capillary tip. Source illumination turned off for panels b-d. ESI capillary voltage is (b) 1.2 kV, (c) 2.5 kV, or (d) 4 kV

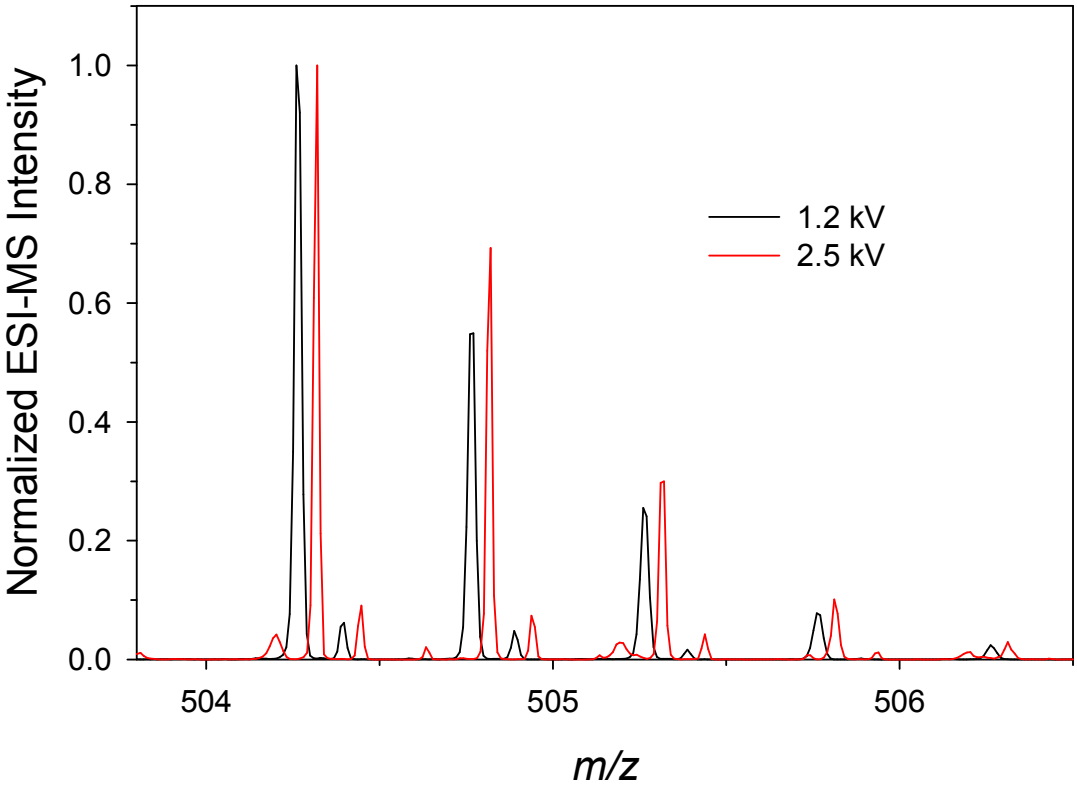

Fig. S3 Oxytocin mass spectra obtained on the Synapt G2 using the NanoLockspray ionization source. Peptide was dissolved in solution **2** (10 mM ammonium acetate, titrated to pH 2.7 with formic acid) and electrosprayed at 1.2 kV (black line) and 2.5 kV (red line). Spectrum acquired at 2.5 kV is offset by 0.05 Da to enhance clarity

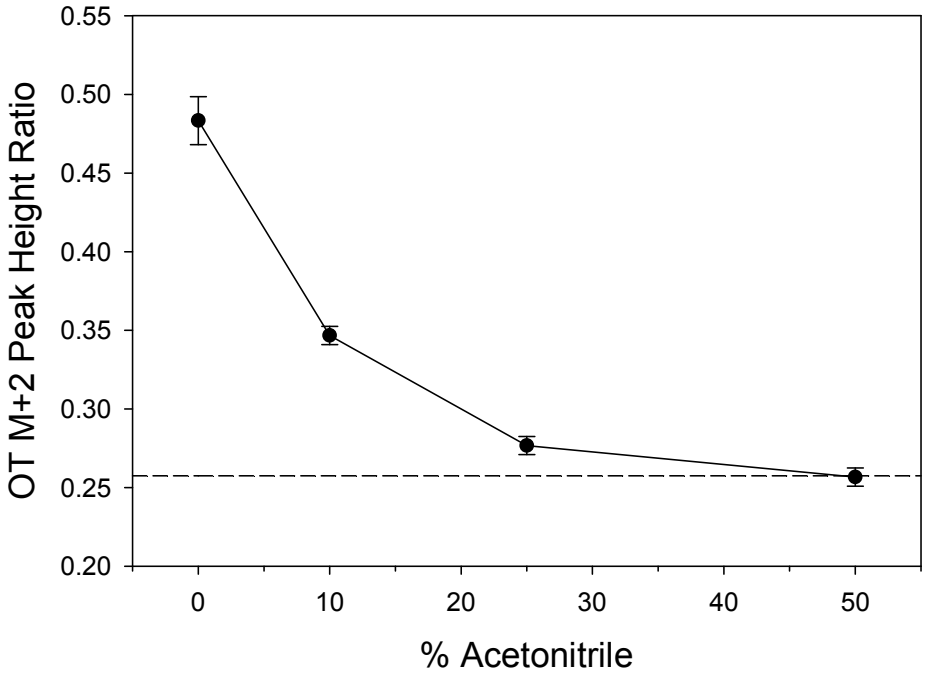

Fig. S4 Peak height ratio of OT_{M+2} measured with increasing percentage of acetonitrile. Peptide was prepared at 40 µM peptide in 0.1 % formic acid, and was teed into the LC flow from a Thermo Vanquish UHPLC. Resulting mixture was directed into an Orbitrap Fusion Lumos at 100 µL min^{-1}. Depictied acetonitrile percentages represent post-mixing values. Error bars represent standard deviations of triplicate measurements. Dashed line indicates the theoretical OT_{M+2} ratio

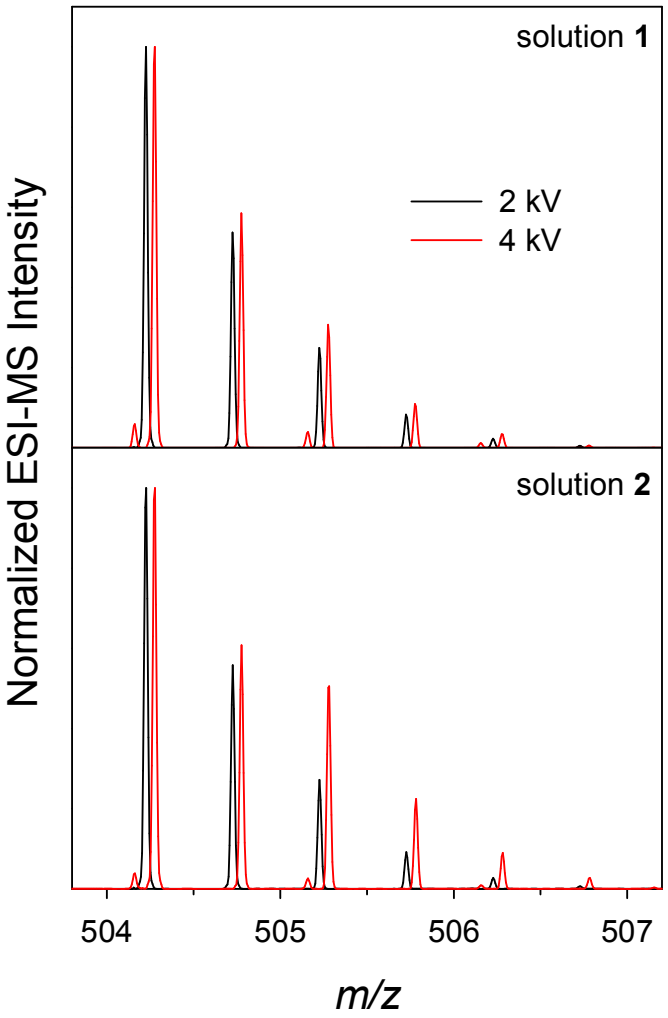

Fig. S5 Oxytocin ISR measured on an Orbitrap Fusion Lumos. Peptide was dissolved in 10 mM ammonium acetate at pH 6.7 (solution **1**, top panel) or pH 2.7 (solution **2**, bottom) and electrosprayed at 2 kV (black line) or 4 kV (red line). Spectra acquired at 4 kV were offset by 0.05 Da for clarity

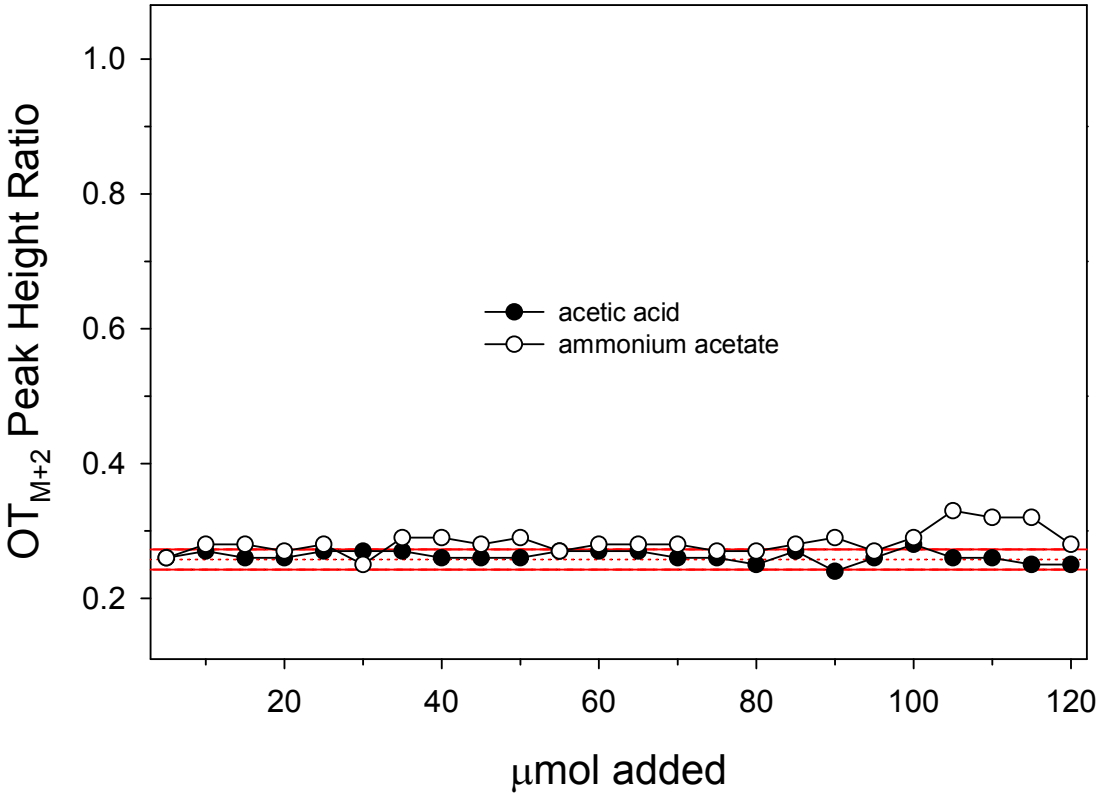

Fig. S6 Oxytocin ISR measured in the presence of acetate. Peak height ratio of OT_{M+2} as a function of amount of acetic acid (filled circles) or ammonium acetate (open circles). Red lines represent the theoretical ratio ± uncertainty

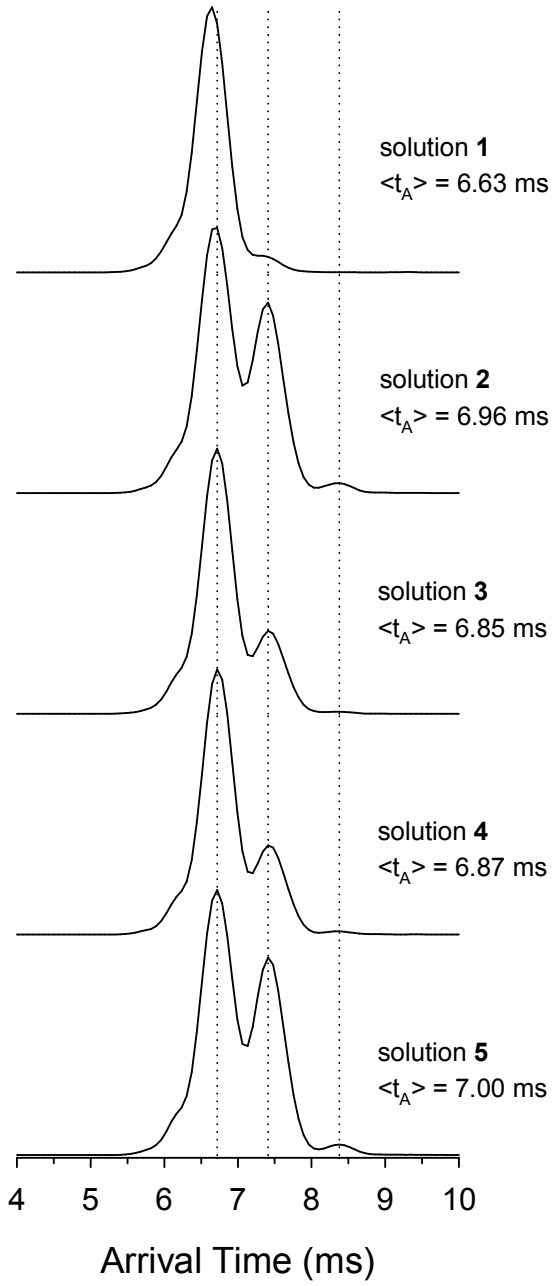

Fig. S7 Arrival time distributions of the insulin 5+ charge state from various solutions. Vertical dotted lines are for feature alignment at arrival times 6.72, 7.41, 8.38 ms. Average arrival times <tA>, were calculated as in ref. 21 from the main text. Distributions were acquired with an ESI capillary voltage of 4 kV

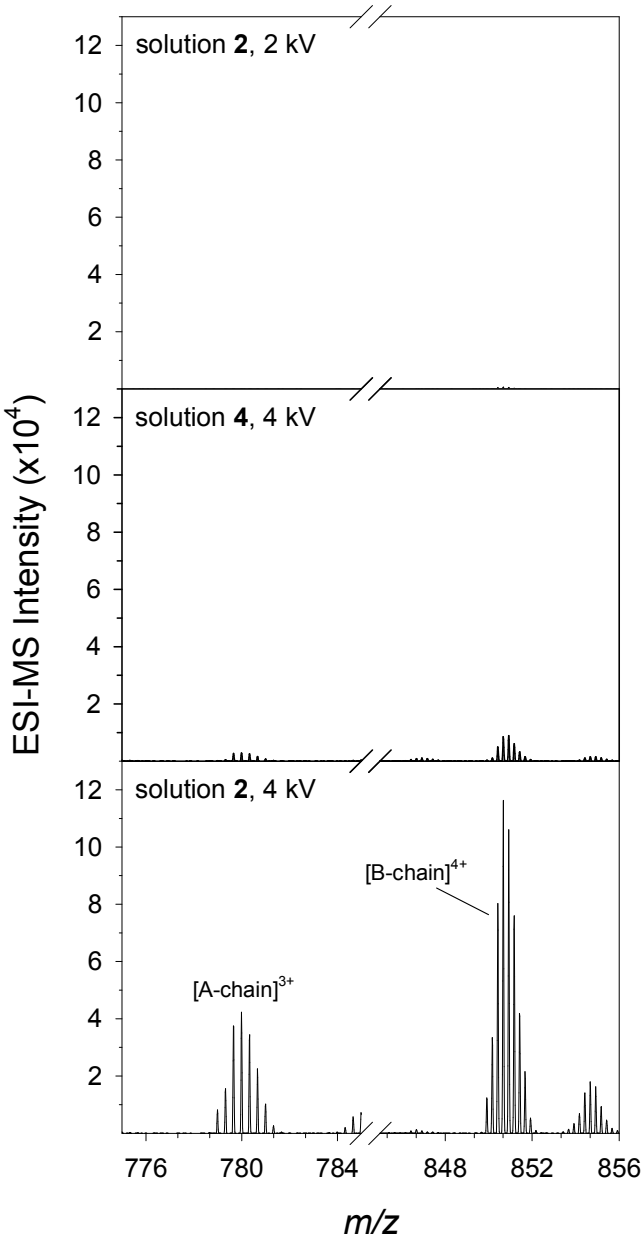

Fig. S8 Released A- and B- chains due to insulin in-source reduction. Data acquired on an Orbitrap Fusion Lumos. All panels are plotted on the same intensity scale. Insulin dissolved in: solution **2** and sprayed at 2 kV (*top*); solution **4**, 4 kV (*middle*); and solution **2**, 4 kV (bottom)

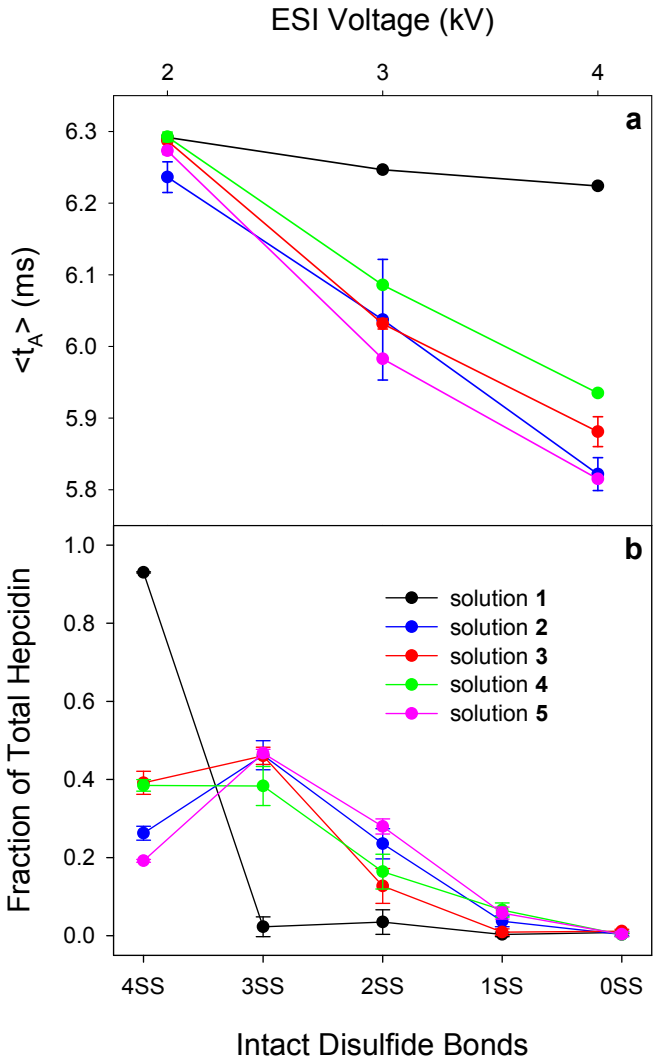

Fig. S9 (a) Average arrival time plots for [hepcidin + 3H]$^{3+}$ sprayed from various solution conditions as a function of electrospray capillary voltage. (b) Disulfide bond distributions for [hepcidin + 3H]$^{3+}$ from various solution conditions sprayed at 4 kV. Isotope modeling carried out as described in *Experimental*. Error bars represent the standard deviations of triplicate measurements

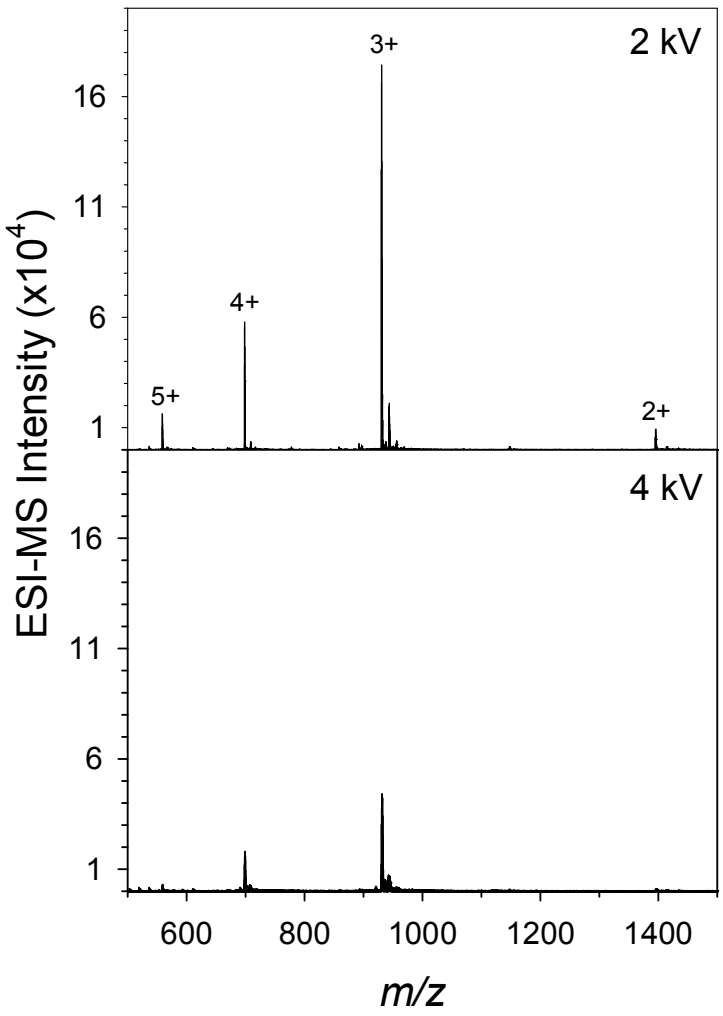

Fig. S10 Mass spectra of hepcidin infused from solution **2** at ESI voltages of 2 kV (*top*) and 4 kV (*bottom*). Both spectra are plotted on the same intensity scale. Observed charge states are indicated in top panel