

SUPPORTING INFORMATION

Replacing H⁺ by Na⁺ or K⁺ in phosphopeptide anions and cations prevents electron capture dissociation

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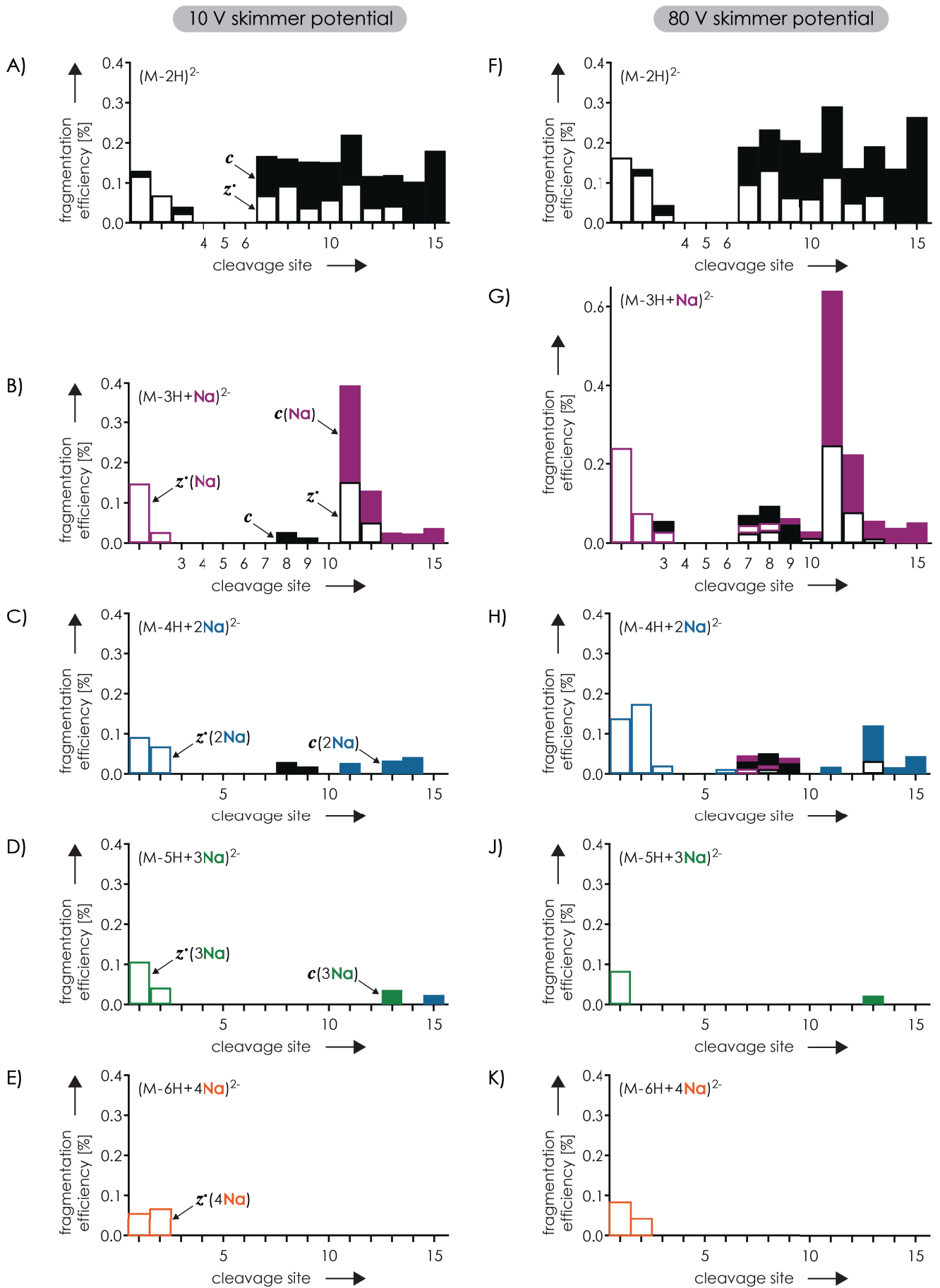


Figure S1. Site-specific fragmentation efficiency (c , z^* and c' , z fragments) in niECD of A) $(M-2H)^{2-}$, B) $(M-3H+Na)^{2-}$, C) $(M-4H+2Na)^{2-}$, D) $(M-5H+3Na)^{2-}$, and E) $(M-6H+4Na)^{2-}$ ions using a skimmer potential of 10 V, and of F) $(M-2H)^{2-}$, G) $(M-3H+Na)^{2-}$, H) $(M-4H+2Na)^{2-}$, J) $(M-5H+3Na)^{2-}$, and K) $(M-6H+4Na)^{2-}$ ions using a skimmer potential of 80 V.

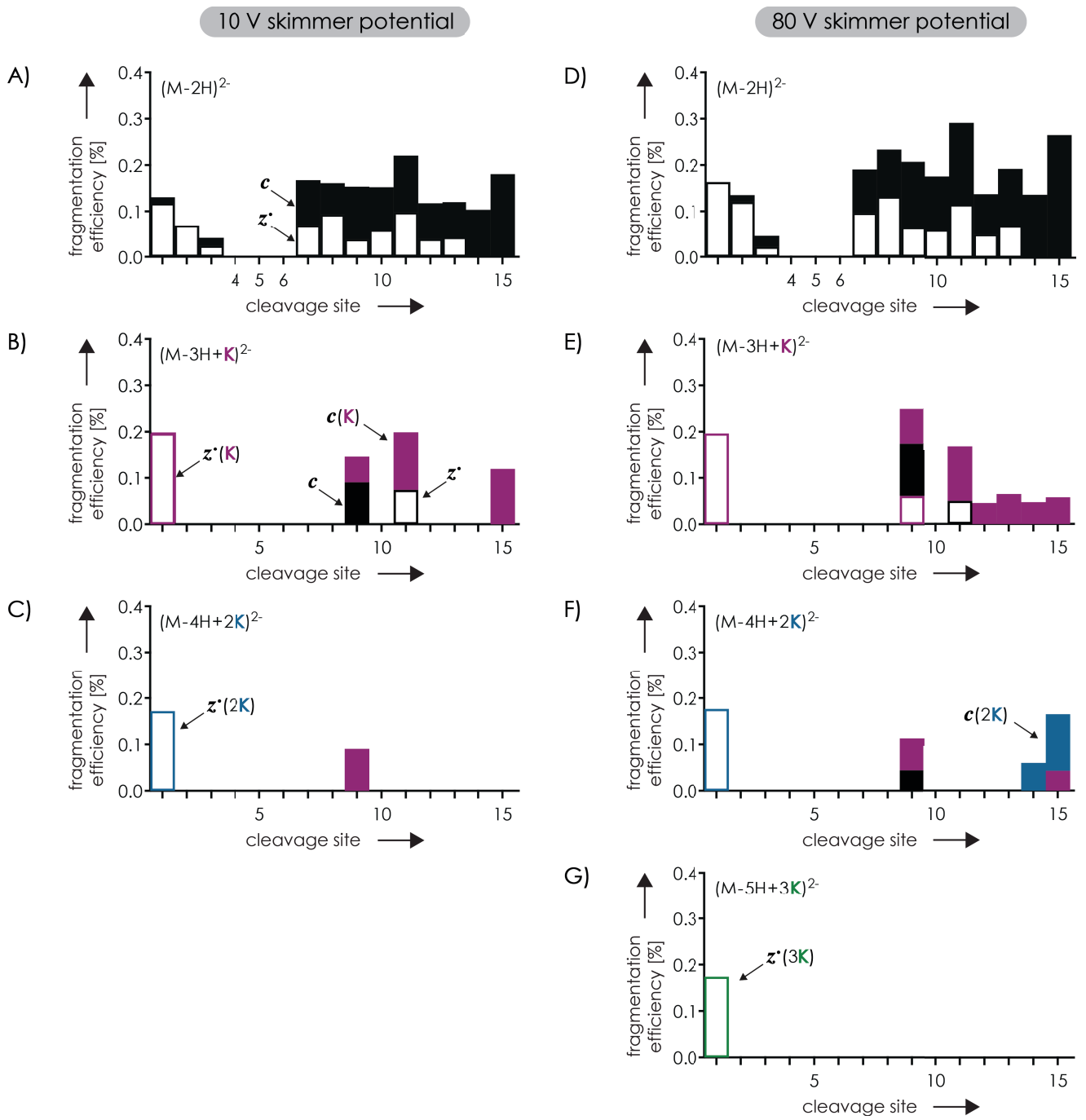


Figure S2. Site-specific fragmentation efficiency (c , z^* and c^* , z fragments) in niECD of A) $(M-2H)^{2-}$, B) $(M-3H+K)^{2-}$, and C) $(M-4H+2K)^{2-}$ ions using a skimmer potential of 10 V, and of D) $(M-2H)^{2-}$, E) $(M-3H+K)^{2-}$, F) $(M-4H+2K)^{2-}$, and G) $(M-5H+3K)^{2-}$ ions using a skimmer potential of 80 V.

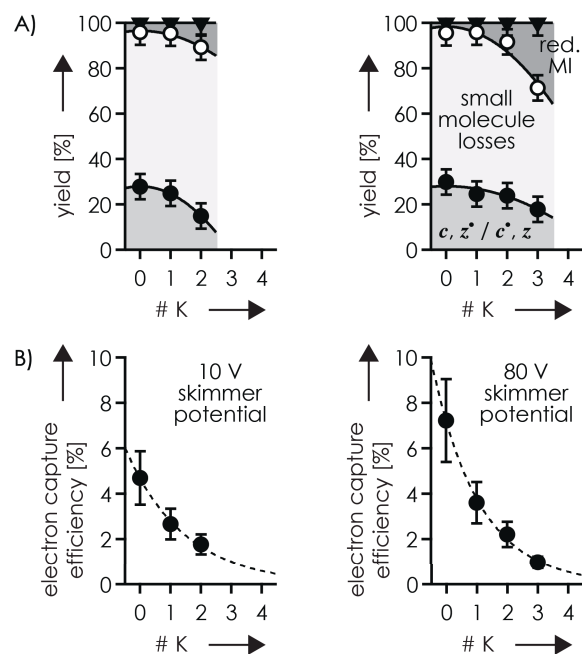


Figure S3. A) Percentage stacked area plots illustrating the yield of c, z^* and c^*, z fragments (filled circles), products from loss of small molecules (>70% NH_3 ; <30% CO , CONH_3 , $\text{C}_2\text{H}_6\text{O}$, $\text{C}_2\text{H}_4\text{O}_2$) from reduced molecular ions (open circles), and reduced molecular ions (triangles) of which ~20% showed loss of H^+ but not K^+ , from niECD of phosphopeptide anions with a net charge of 2- at 10 V (left) and 80 V (right) skimmer potential and B) corresponding electron capture efficiency versus the number of K^+ attached; solid lines in A are meant to guide the eye and dashed lines in B are exponential fit functions.

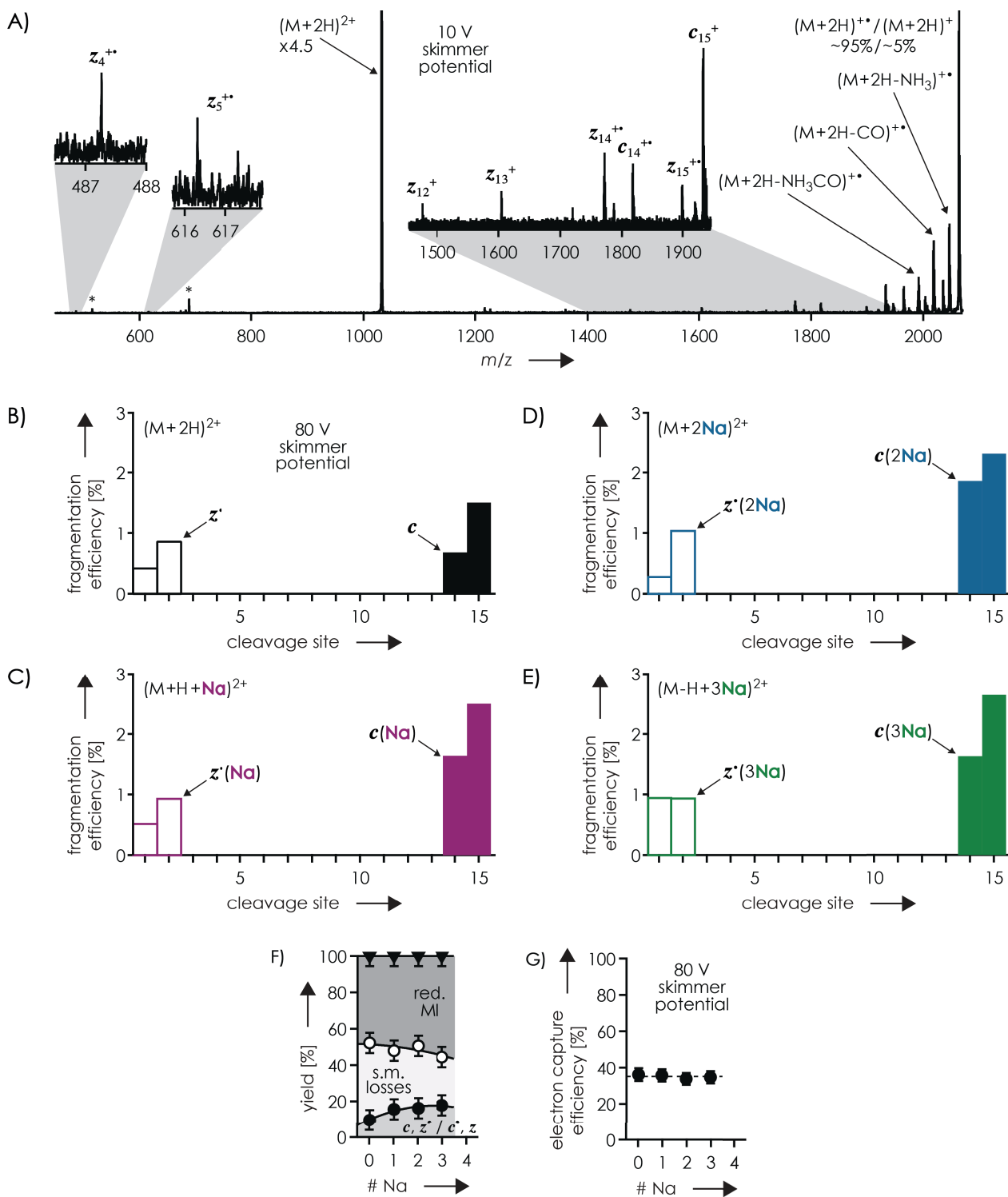


Figure S4. A) Spectrum from ECD of $(M+2H)^{2+}$ ions using a skimmer potential of 10 V (asterisks indicate harmonic signals), and site-specific fragmentation efficiency (c , z' and c' , z fragments) in ECD of B) $(M+2H)^{2+}$, C) $(M+H+Na)^{2+}$, D) $(M+2Na)^{2+}$, and E) $(M-H+3Na)^{2+}$ ions at 80 V skimmer potential; F) yield of c and z' fragments, products from loss of small molecules from reduced molecular ions, and reduced molecular ions from ECD of phosphopeptide ions with a net charge of 2+ at 80 V skimmer potential and G) corresponding electron capture efficiency versus the number of Na^+ attached, lines in F and G are meant to guide the eye.

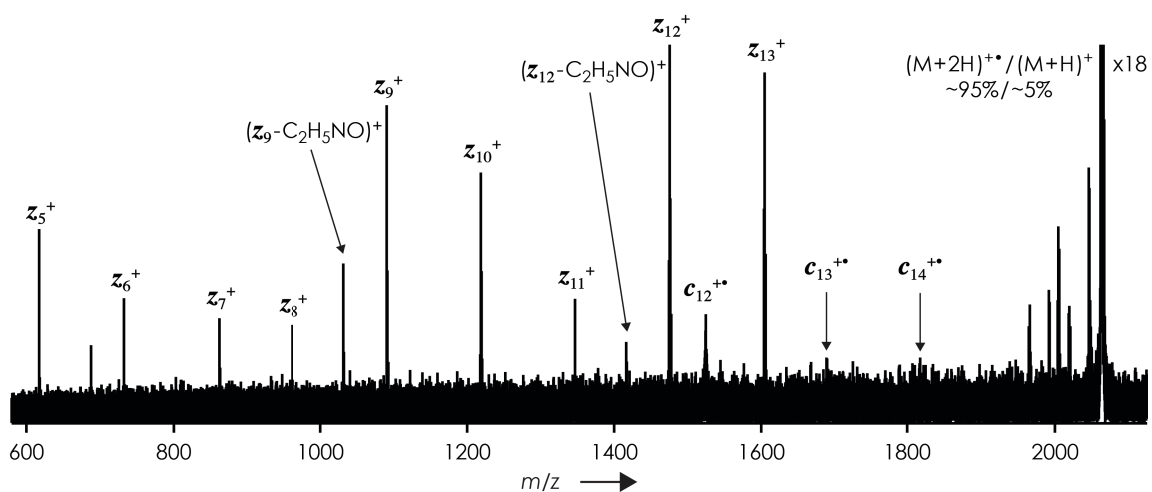


Figure S5. Spectrum from IRMPD (25% laser power, 180 ms irradiation time) of $(M+2H)^{++}$ (~95%) and $(M+H)^+$ (~5%) ions formed by ECD of $(M+2H)^{2+}$ ions at 80 V skimmer potential.

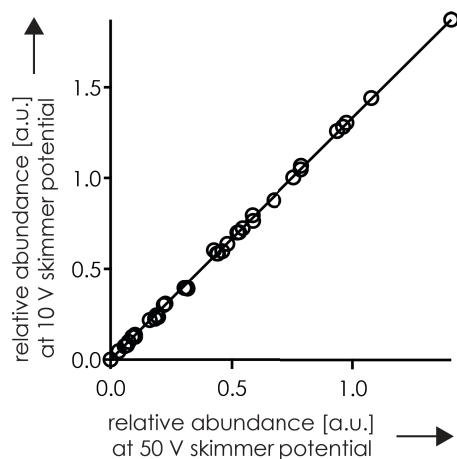


Figure S6. Relative abundance of c , z^* and c^* , z fragments from ECD of $(M+3H)^{3+}$ ions at 10 V versus 50 V skimmer potential, the solid line shows a linear fit with a correlation coefficient of 0.999807.

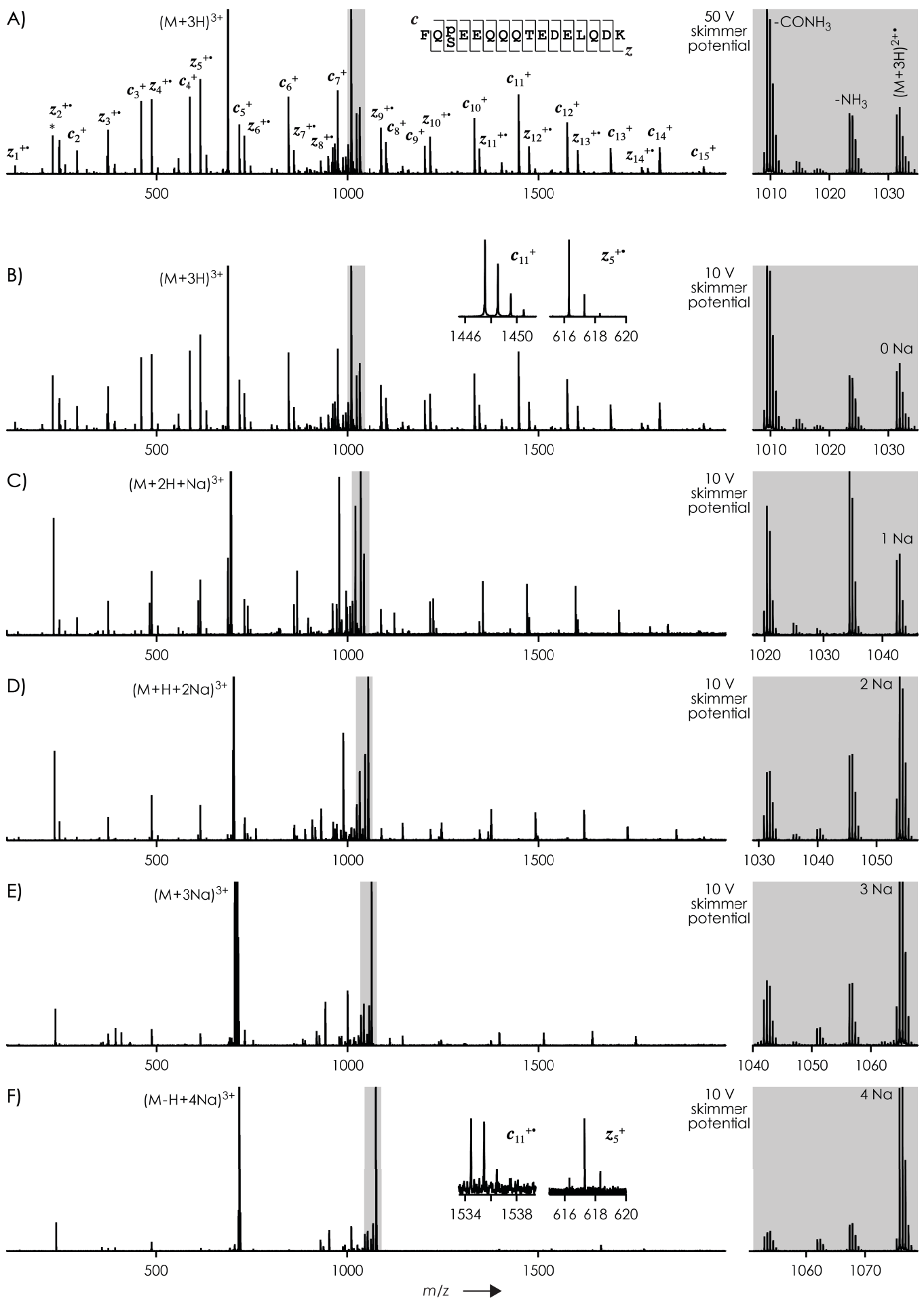


Figure S7. ECD spectra of phosphopeptide ions with a net charge of 3+ and up to four Na⁺ attached as indicated.

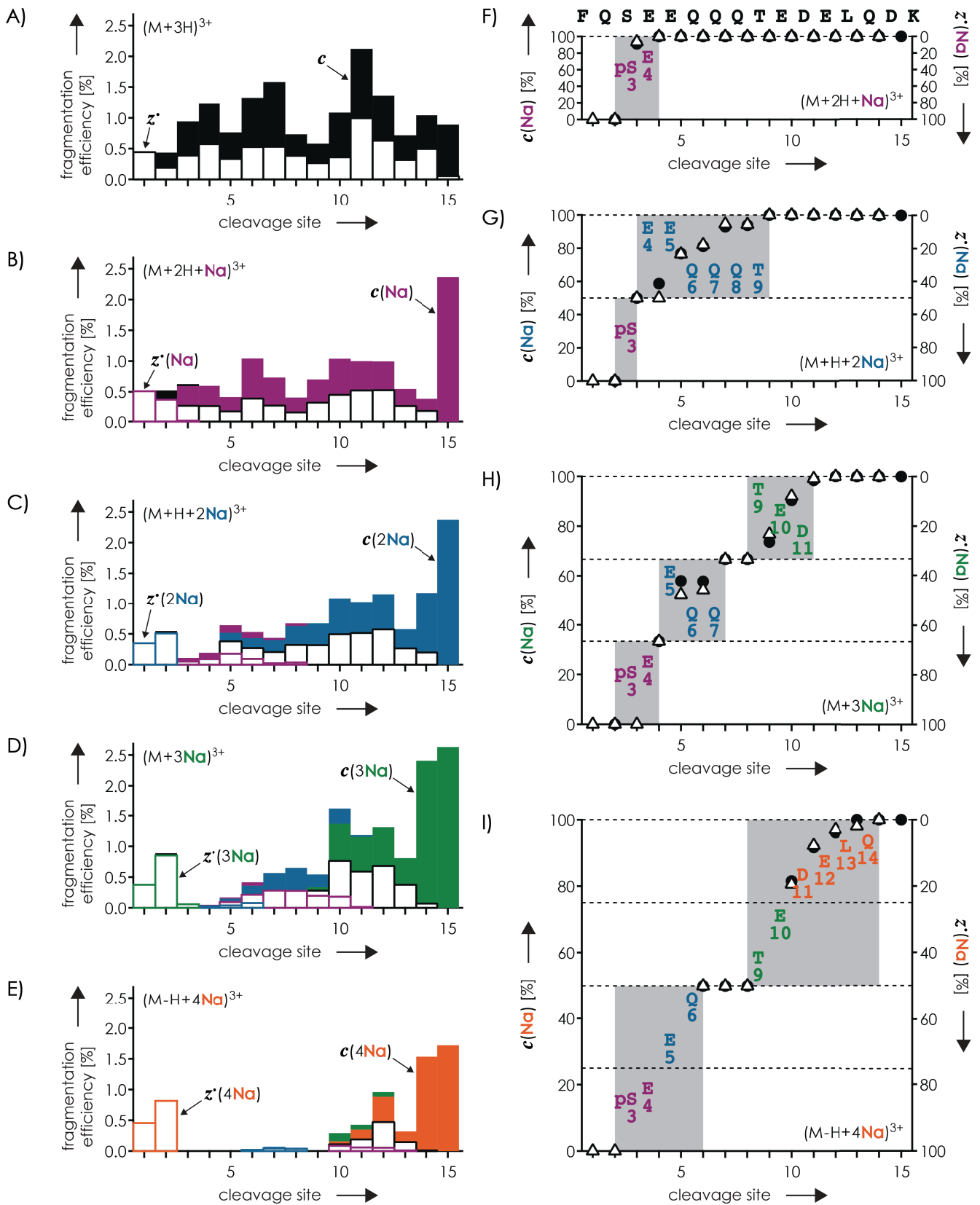


Figure S8. Site-specific fragmentation efficiency (c , z' and c' , z fragments) in ECD of A) (M+3H)³⁺, B) (M+2H+Na)³⁺, C) (M+H+2Na)³⁺, D) (M+3Na)³⁺, and E) (M-H+4Na)³⁺ ions at 10 V skimmer potential and F) - I) corresponding Na⁺ occupancy of c or c' (left axes) and z' or z (right axes) fragments with up to four Na⁺ attached.

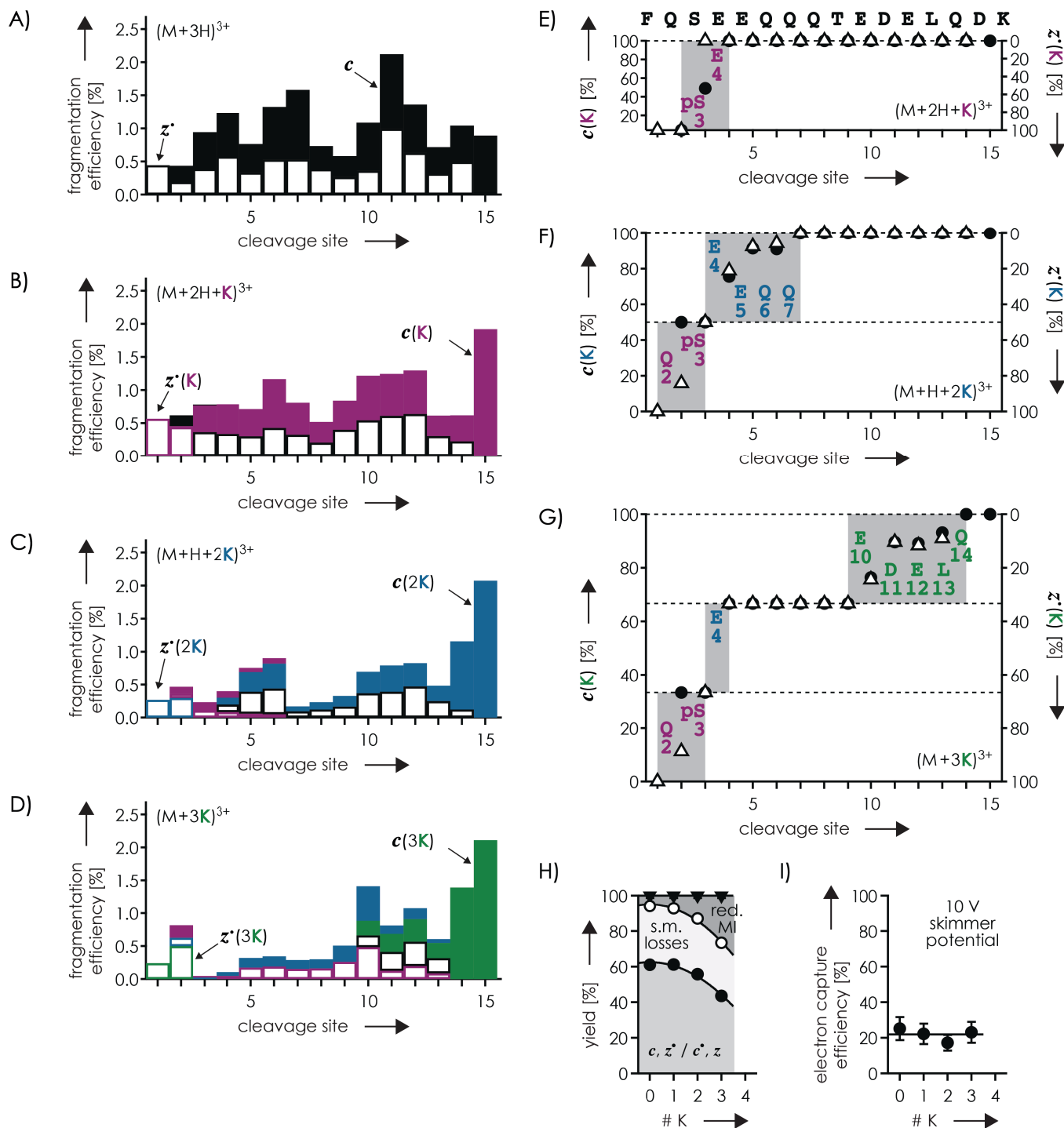


Figure S9. Site-specific fragmentation efficiency (c , z' and c' , z fragments) in ECD of A) $(M+3H)^{3+}$, B) $(M+2H+K)^{3+}$, C) $(M+H+2K)^{3+}$, and D) $(M+3K)^{3+}$ ions at 10 V skimmer potential and E)-G) corresponding K^+ occupancy of c or c' (left axes) and z' or z (right axes) fragments with up to three K^+ attached; H) yield of c , z' and c' , z fragments, products from loss of small molecules from reduced molecular ions, and reduced molecular ions from ECD of phosphopeptide ions with a net charge of $3+$ at 10 V skimmer potential and I) corresponding electron capture efficiency versus the number of K^+ attached

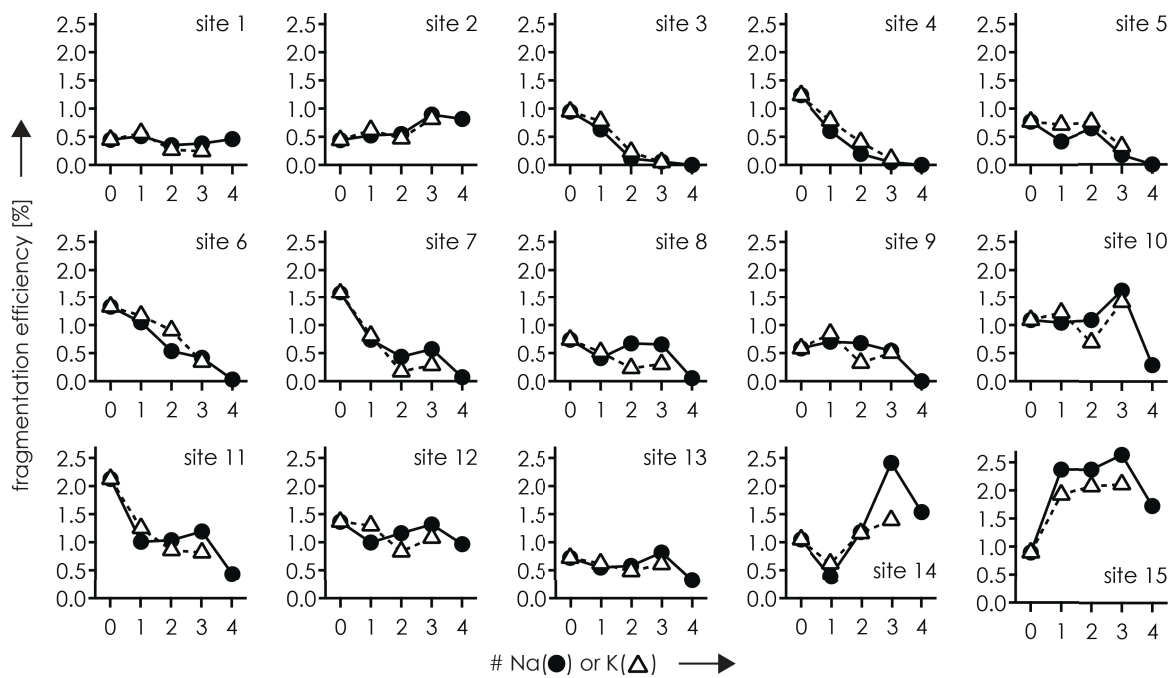


Figure S10. Site-specific fragmentation efficiency (c , z^* and c^* , z fragments) in ECD of phosphopeptide ions with a net charge of 3+ versus the number of Na⁺ (circles) or K⁺ (triangles) attached.

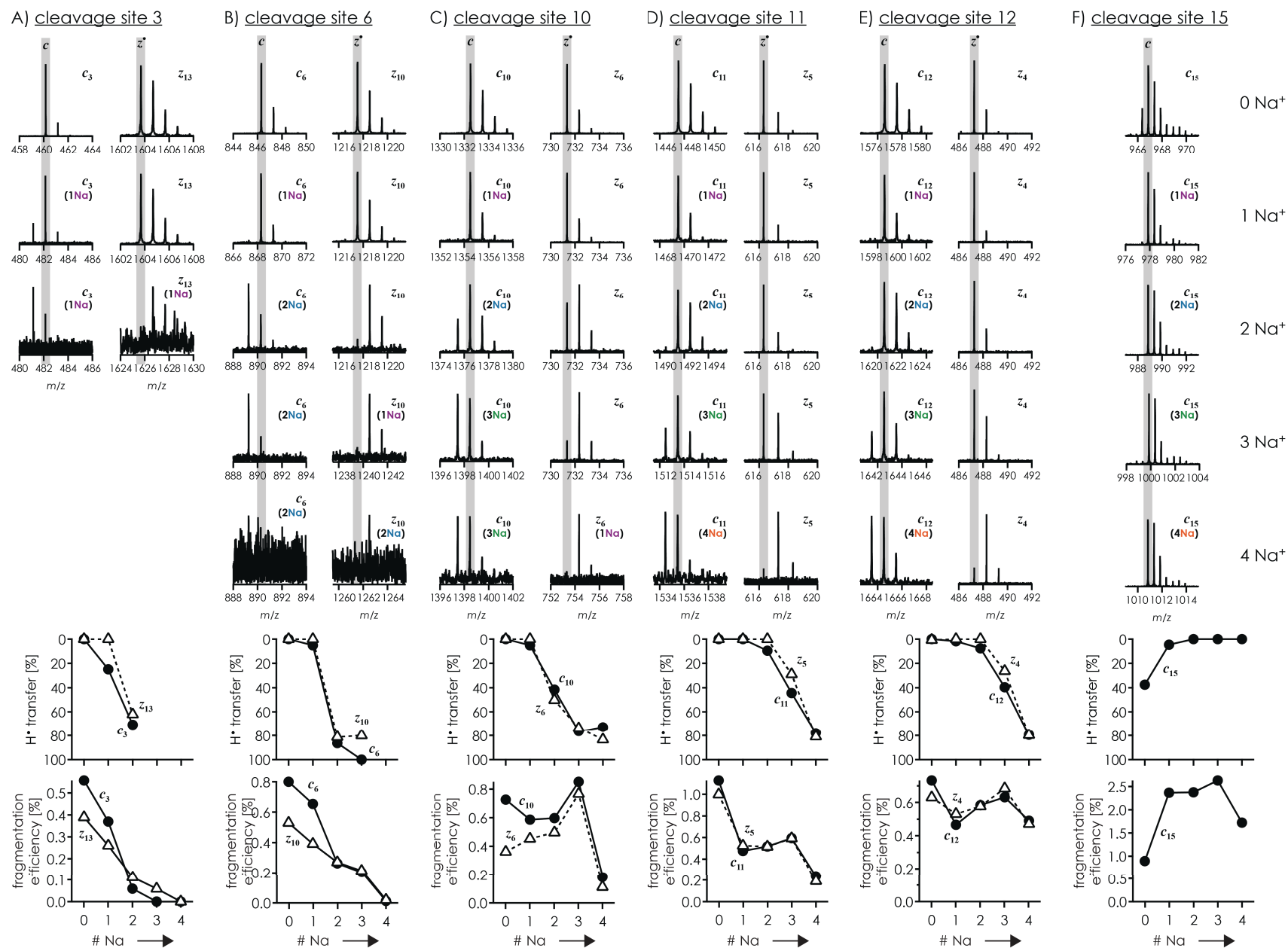


Figure S11. Hydrogen atom transfer between c and z' fragments and fragmentation efficiency in ECD of phosphopeptide ions with a net charge of 3+ and up to four Na⁺ attached, illustrated for cleavage sites A) 3, B) 6, C) 10, D) 11, E) 12, and F) 15.

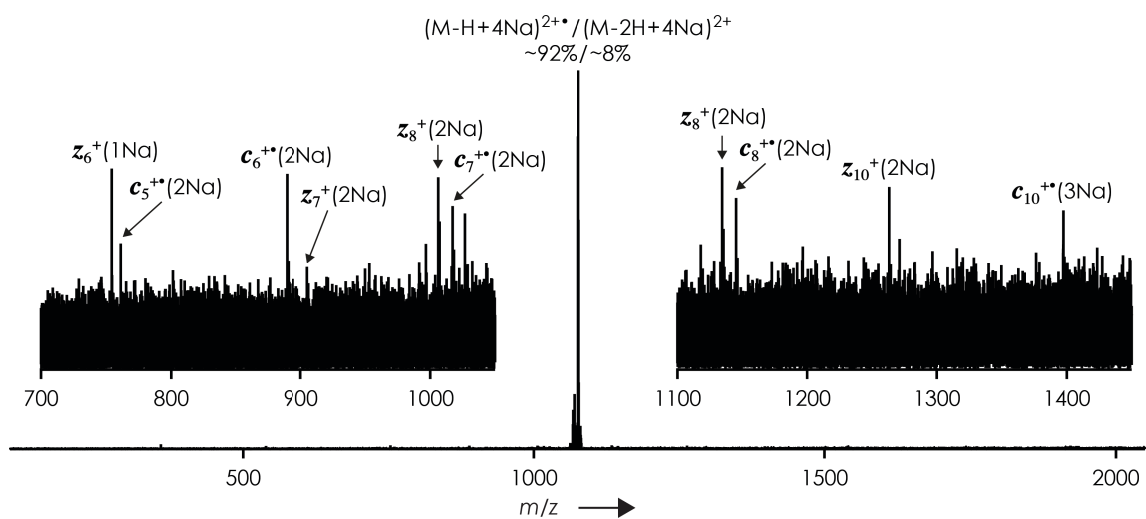


Figure S12. Spectrum from IRMPD (25% laser power, 180 ms irradiation time) of $(M-H+4Na)^{2+\bullet}$ ($\sim 92\%$) and $(M-2H+4Na)^{2+}$ ($\sim 8\%$) ions formed by ECD of $(M-H+4Na)^{3+}$ ions at 10 V skimmer potential.