

Supplementary Information for

**Consequences of point mutations in melanoma-associated antigen 4 (MAGE-A4)
protein: Insights from structural and biophysical studies**

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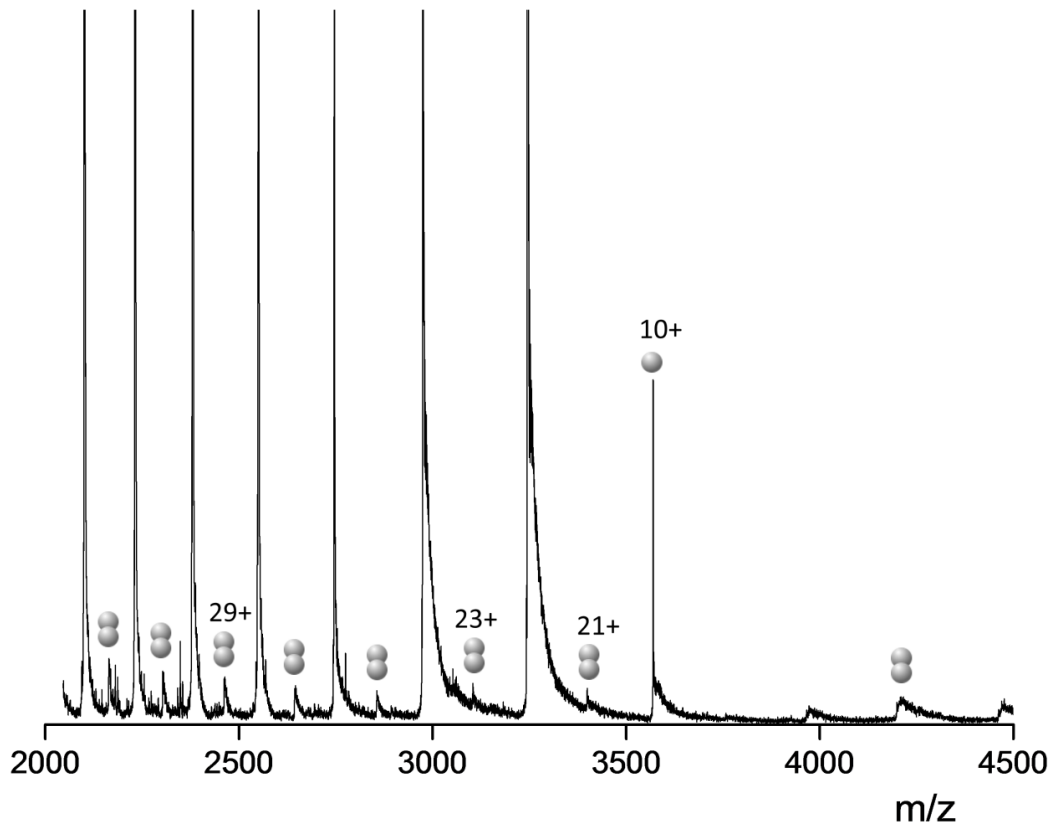
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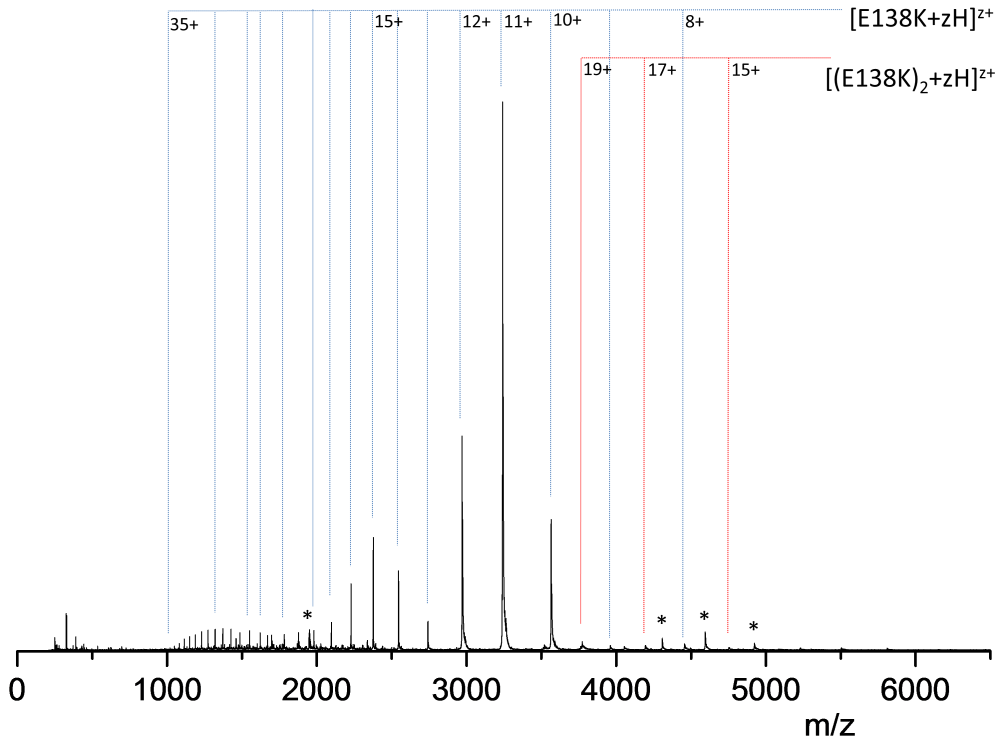
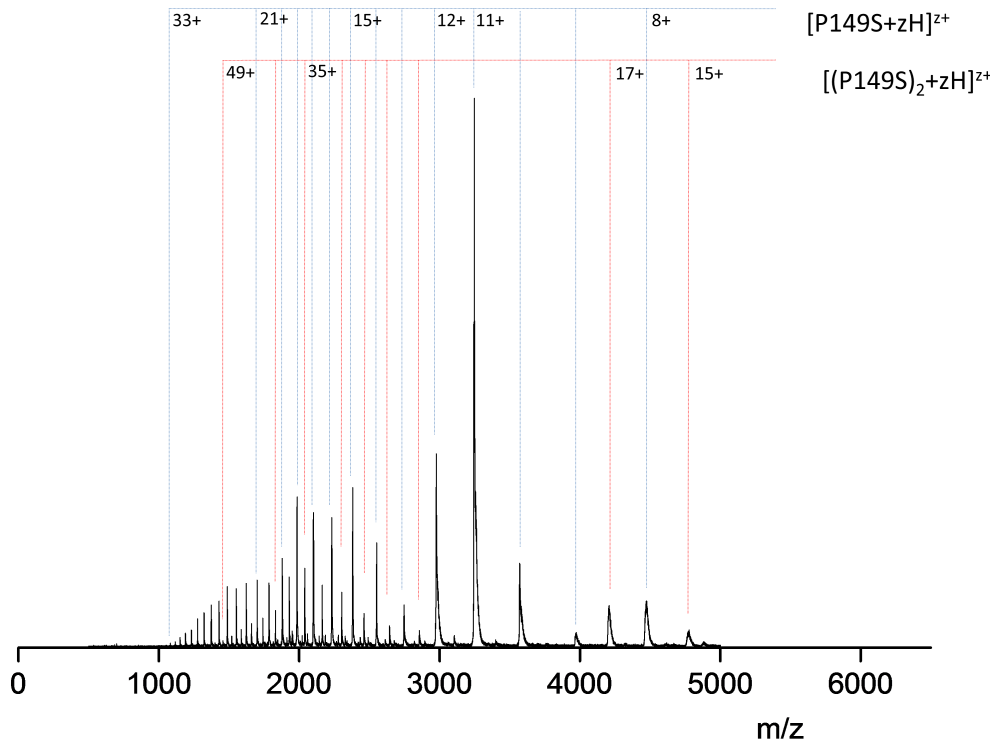
Dr. Penka V. Nikolova

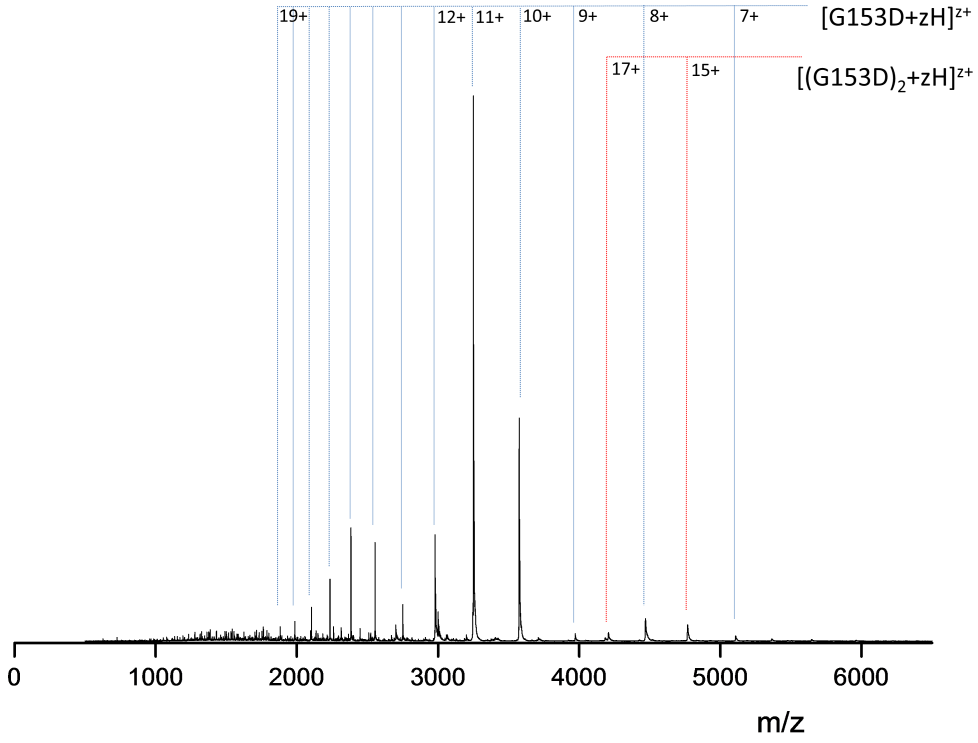
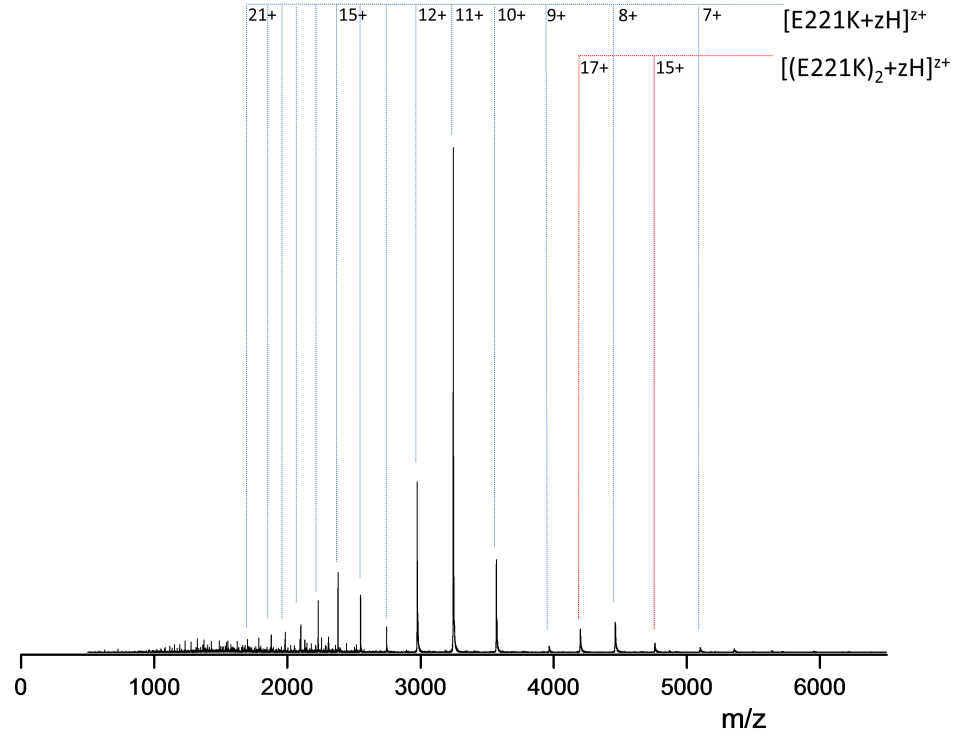
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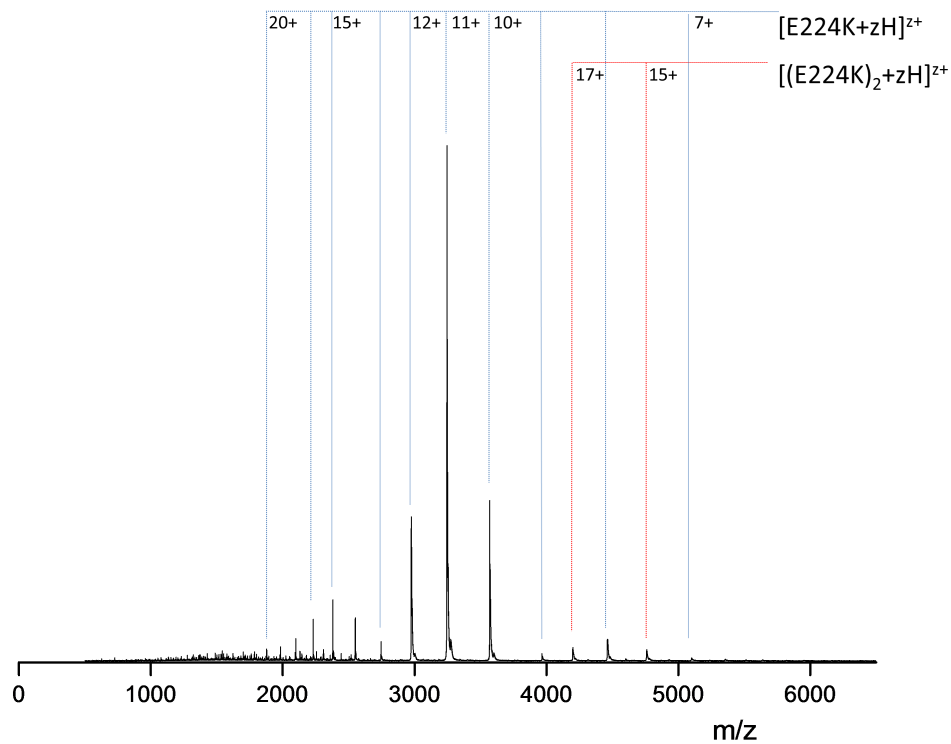
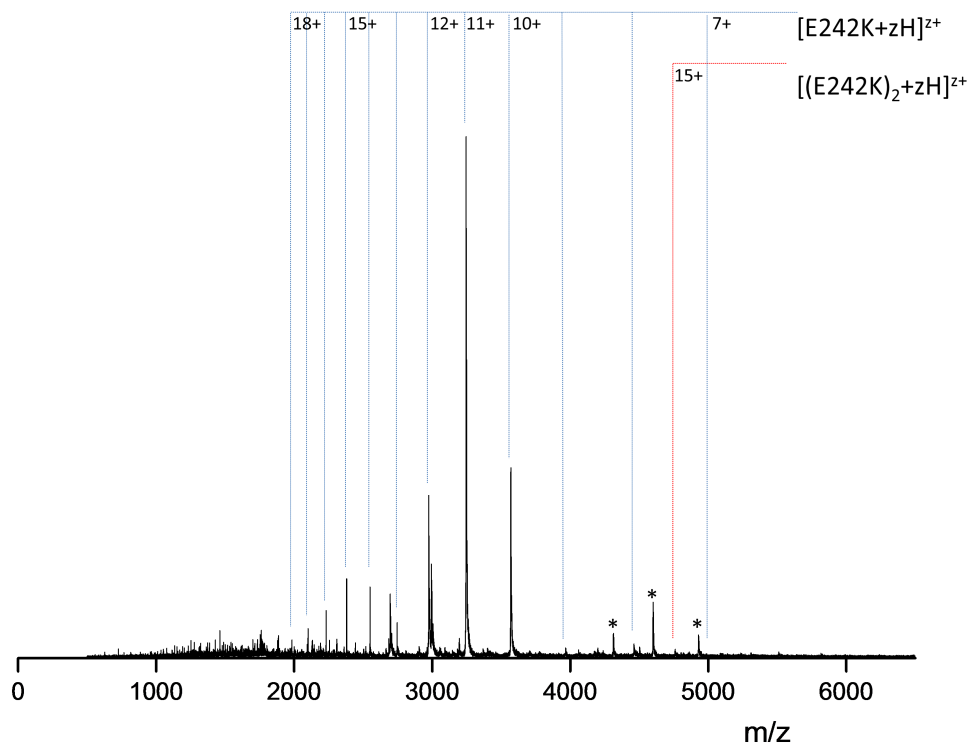
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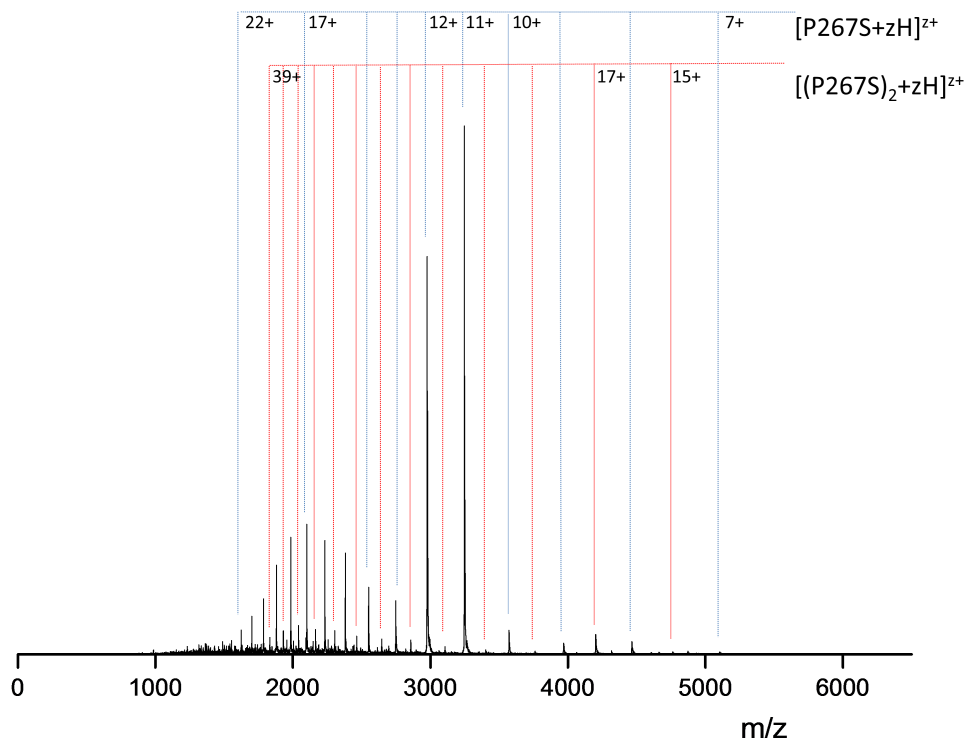
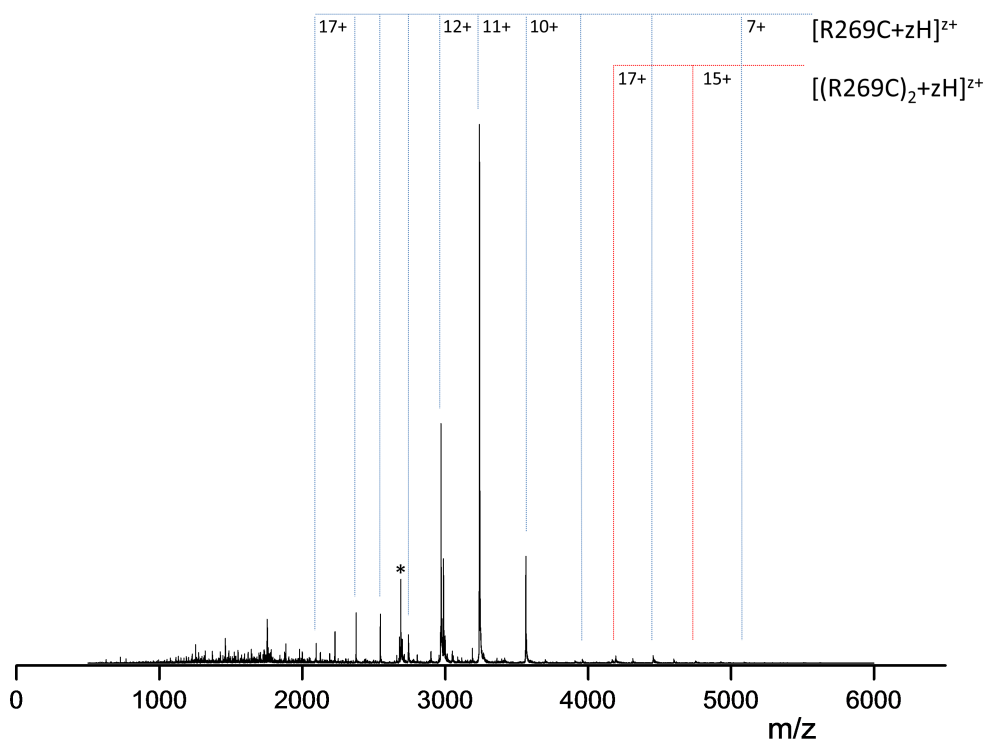


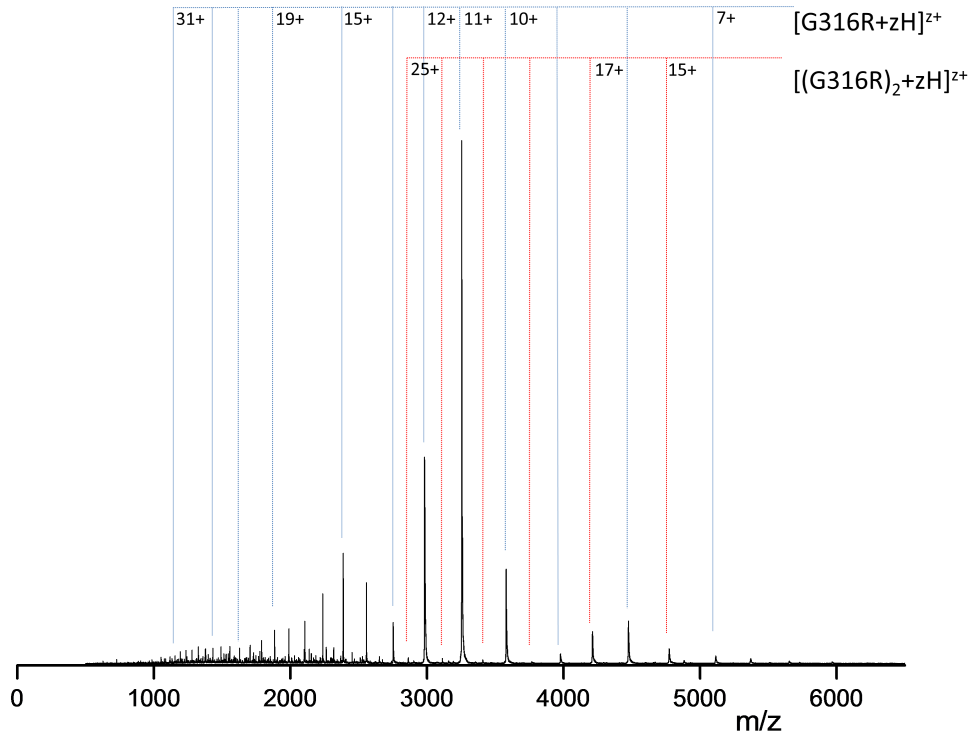
Supplementary Figure S1 nESI spectra adapted from Figure 7, showing a zoomed in section of 20 μ M WT MAGE-A4 sprayed from buffered conditions 50 mM ammonium acetate. Low intensity unique m/z dimeric species are denoted by double spheres. The $[M+10H]^{10+}$ monomeric species is labelled for intensity comparison.

a**b**

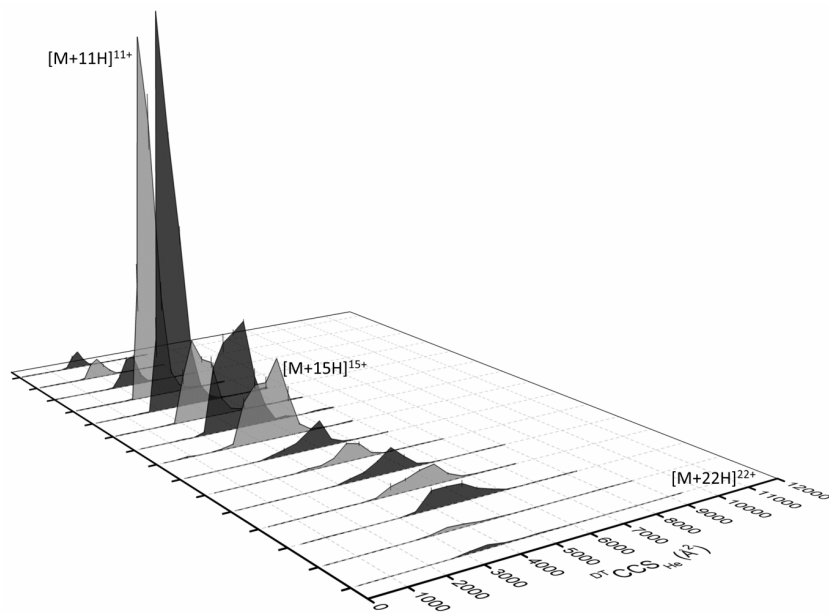
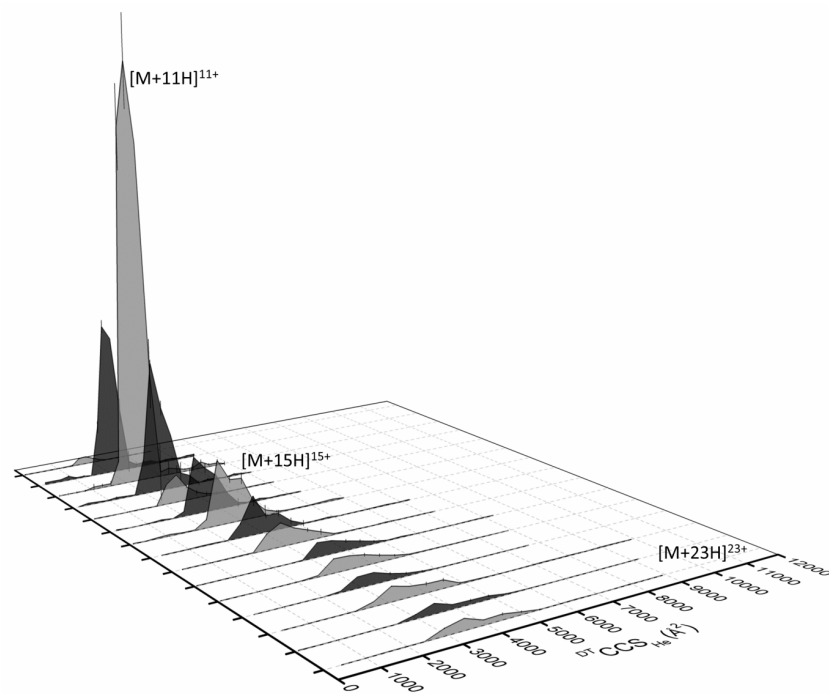
c**d**

e**f**

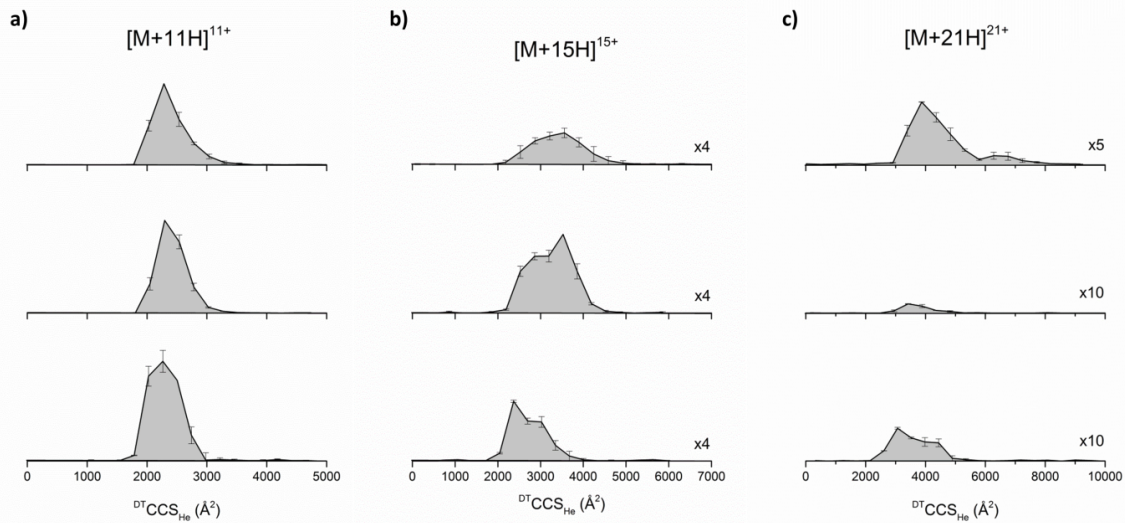
g**h**

i

Supplementary Figure S2 nESI mass spectra of 20 μ M MAGE-A4 mutants sprayed from buffered conditions of 50 mM Ammonium Acetate pH 6.8. Monomeric and dimeric species are depicted by blue and red dotted lines, respectively. Asterisks denote contaminant peaks in the spectra. The tested mutants are (a) E138K, (b) P149S, (c) G153D, (d) E221K, (e) E224K, (f) E242K, (g) P267S, (h) R269C and (i) G316R.

a**b**

Supplementary Figure S3 Collision cross section distributions (CCSDs) derived from arrival time distributions (ATDs) for **(a)** E221K MAGE-A4 and **(b)** E242K MAGE-A4 at drift voltage 35 V. The x-, y- and z-axes represent collision cross section (CCS, Å²), intensity directly acquired from IM-MS experiment, and charge state, respectively. Significant charge states have been labelled.



Supplementary Figure S4 Stacked collision cross section distributions (CCSDs) derived from arrival time distributions (ATDs) for wild-type MAGE-A4 (top panel), E221K (middle panel) and E242K (bottom panels) for (a) $[M+11H]^{11+}$, (b) $[M+15H]^{15+}$ and (c) $[M+21H]^{21+}$ at drift voltage 35 V. The x-, y- and z-axes represent collision cross section (CCS, \AA^2), intensity directly acquired from IM-MS experiment, and charge state, respectively. Magnification of some CCSDs for visibility is noted.