Supporting Information for

Lipid Head Group Adduction to Soluble Proteins Follows Gas-Phase Basicity Predictions: Dissociation Barriers and Charge Abstraction

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Theory and Data Analysis

A linearized form of the Eyring equation was used to determine activation enthalpies and entropies:

$$\ln\left(\frac{-\ln\frac{[R]}{[R+P]}}{T}\right) = -\frac{\Delta H^{\ddagger}}{k_B T} + \ln\frac{k_B}{h} + \ln t + \frac{\Delta S^{\ddagger}}{k_B} \quad (1)$$

where R denotes the precursor ion, and P denotes the product ion, T is the effective temperature of the precursor ion, k_B is the Boltzmann constant, h is Planck's constant, t is the reaction time, $\Delta H^{\ddagger}$ is the activation enthalpy, and $\Delta S^{\ddagger}$ is the activation entropy.

These values were used to compute the left-hand side of Equation 1, which was plotted against the reciprocal of effective temperature multiplied by the Boltzmann constant. The slope and intercept were then used to determine $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, respectively, using Equation 1. $\Delta G^{\ddagger}$ was determined from $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and the average effective temperature over the range of data included in the fit.

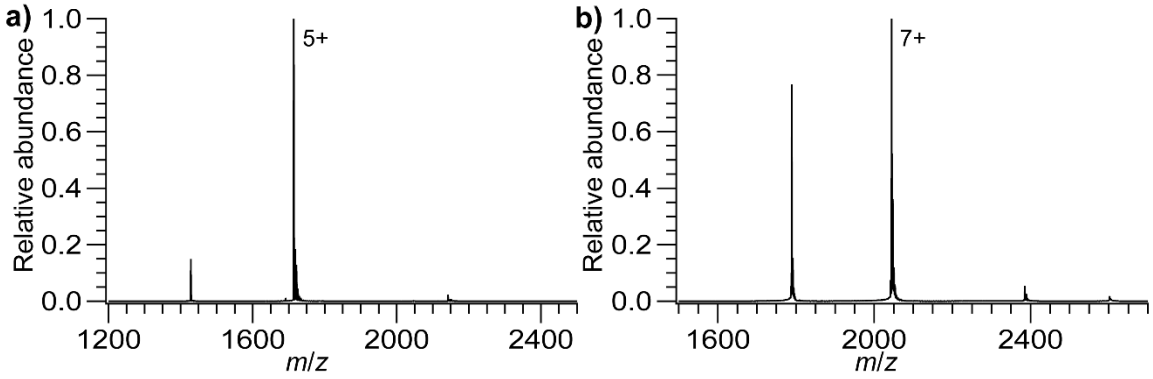

Figure S1. Mass spectra of **(a)** ubiquitin (Ubq) and **(b)** lysozyme (LZ). For both proteins, the most abundant charge state, which was used for CID experiments, is labeled.

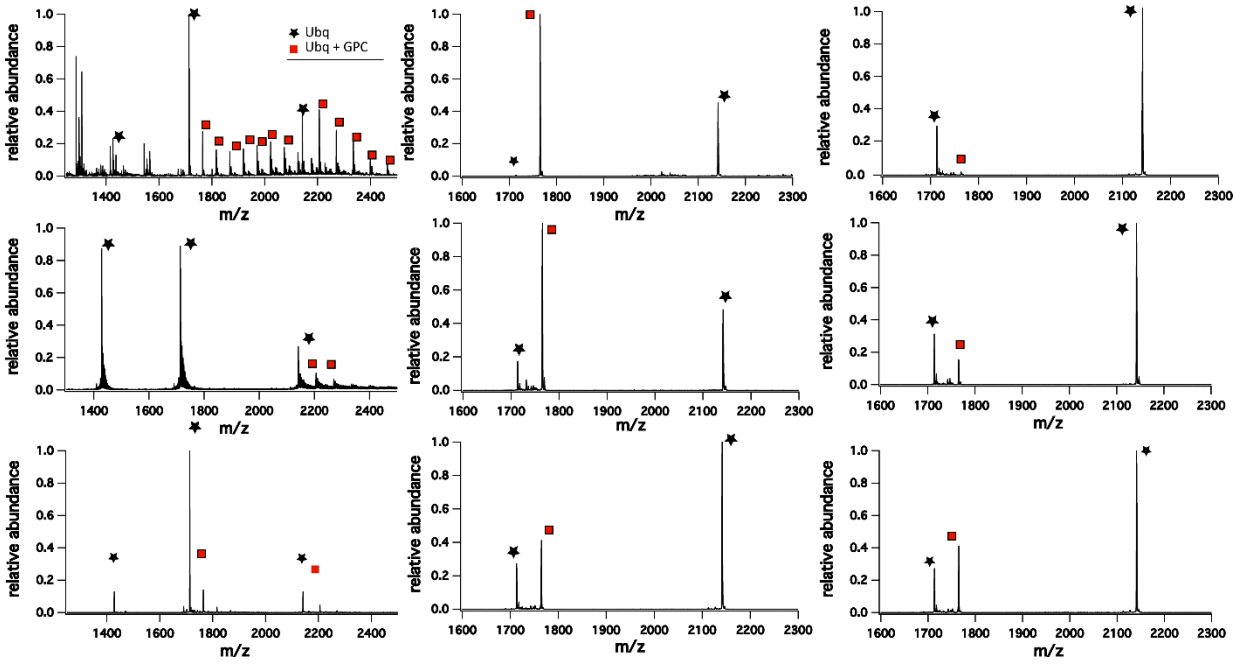

Figure S2: Mass spectra of Ubiquitin and GPC, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

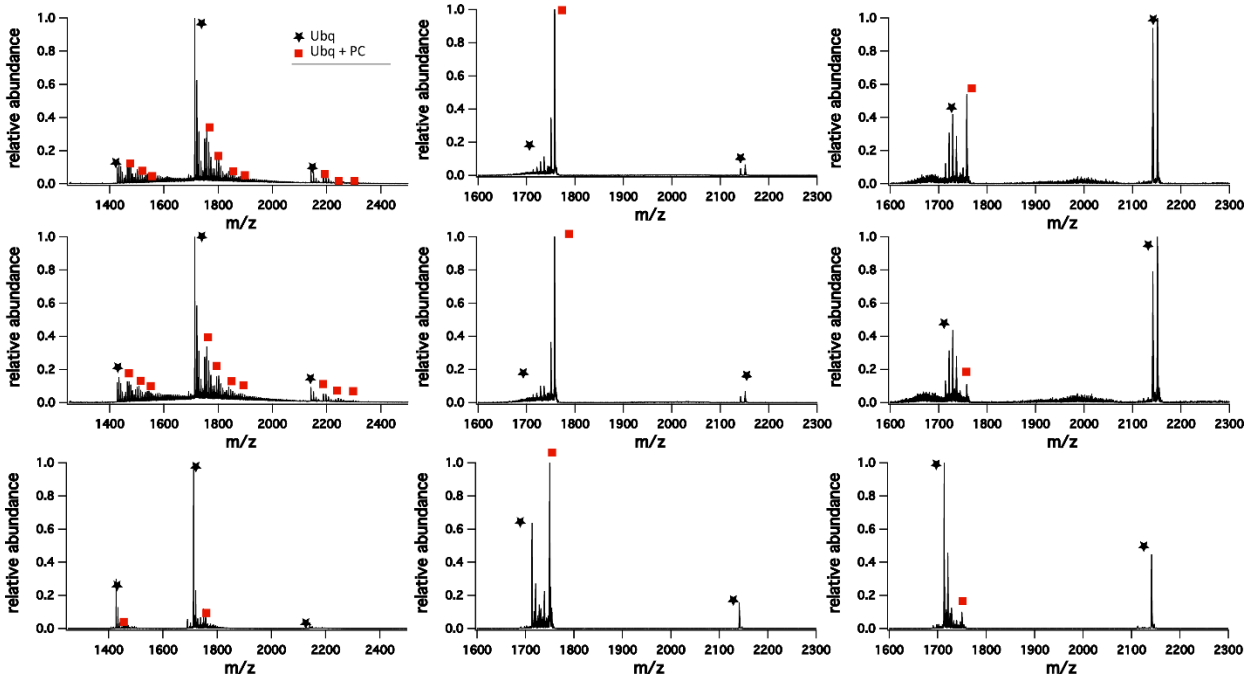

Figure S3: Mass spectra of Ubiquitin and PC, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

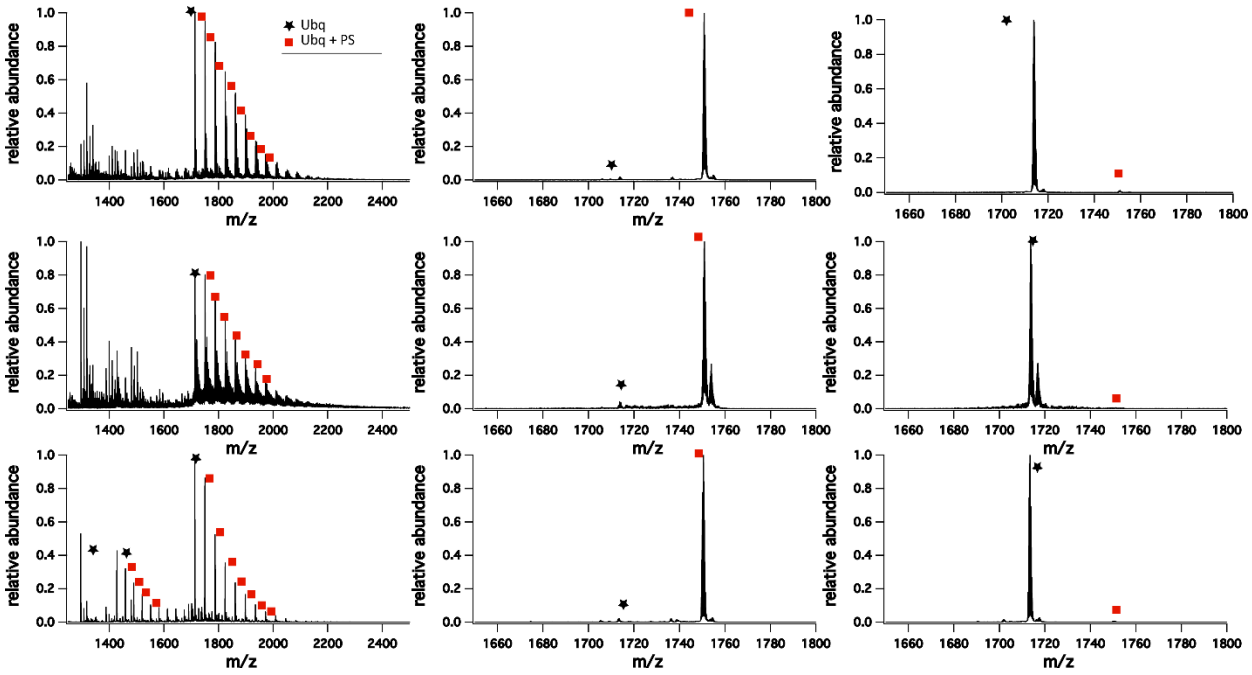

Figure S4: Mass spectra of Ubiquitin and PS, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

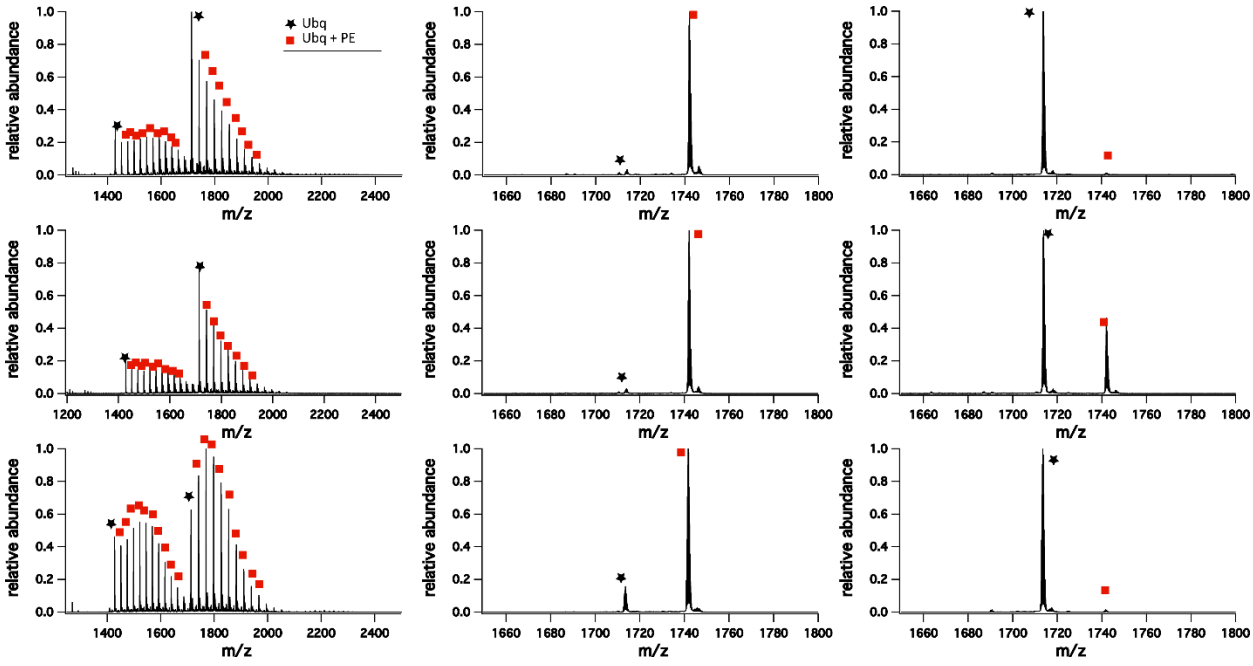

Figure S5: Mass spectra of Ubiquitin and PE, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

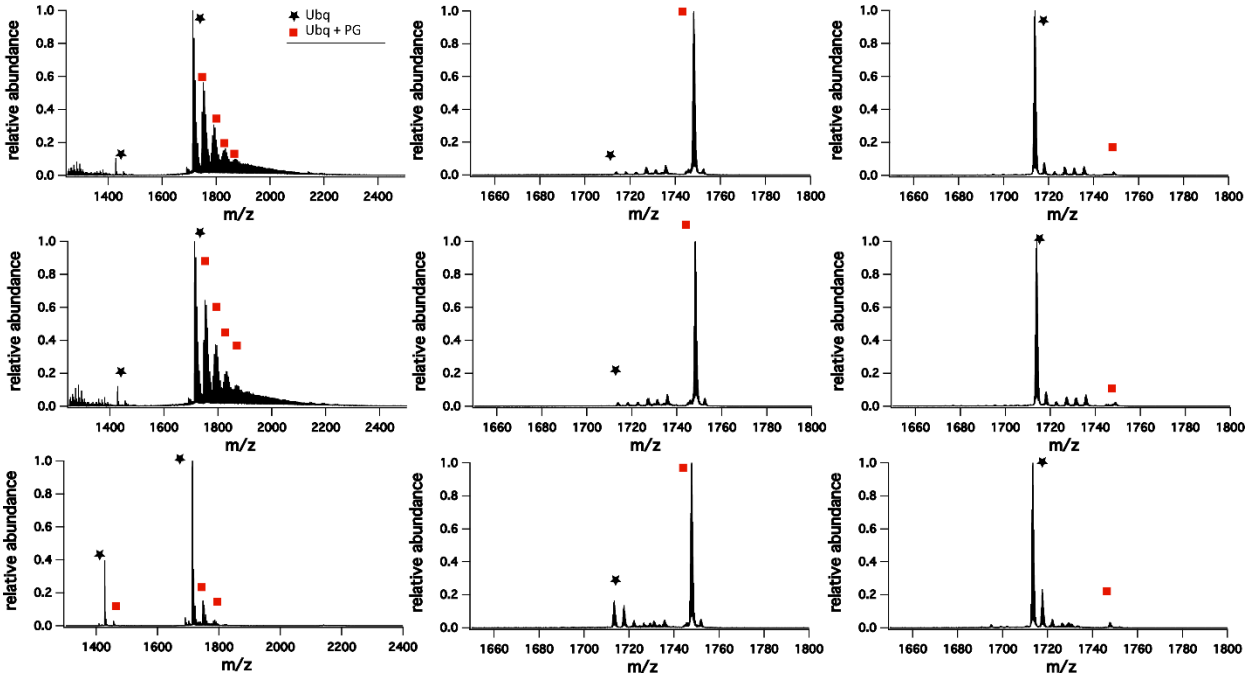

Figure S6: Mass spectra of Ubiquitin and PG, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

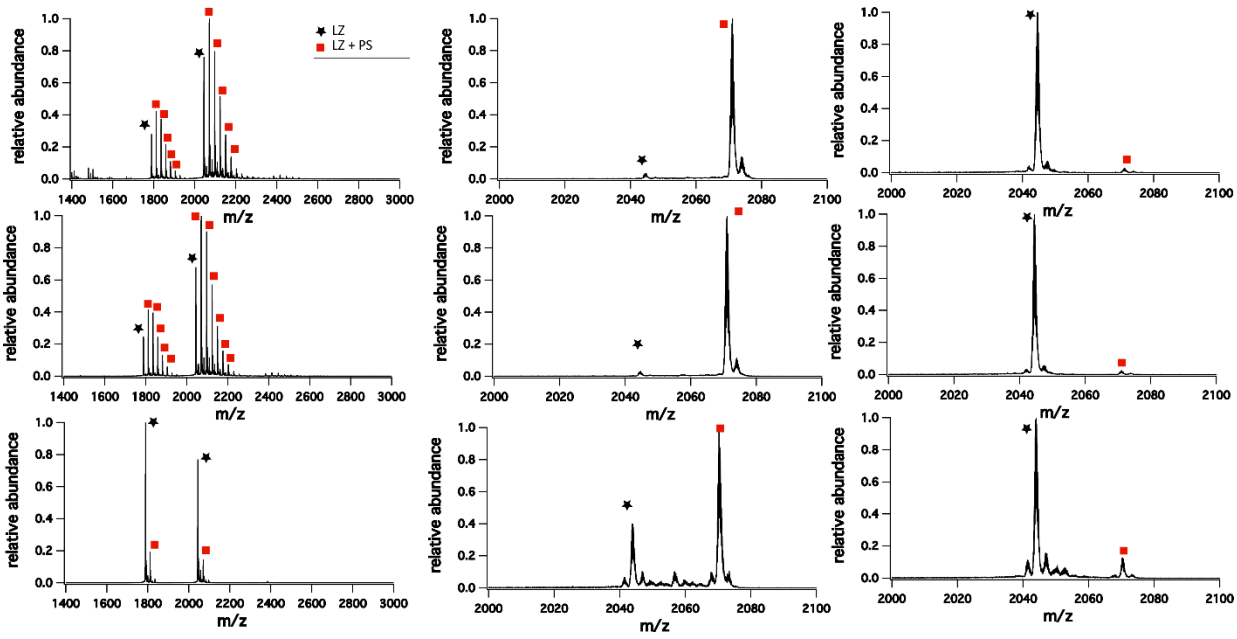

Figure S7: Mass spectra of Lysozyme and PS, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

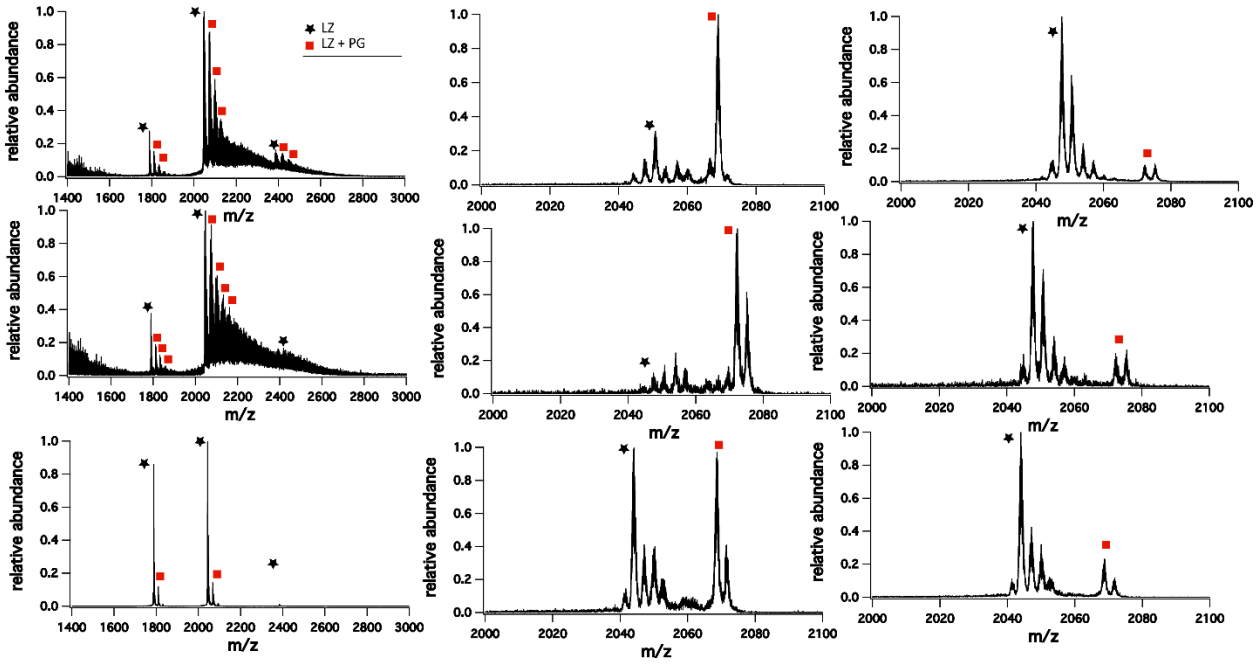

Figure S8: Mass spectra of Lysozyme and PG, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

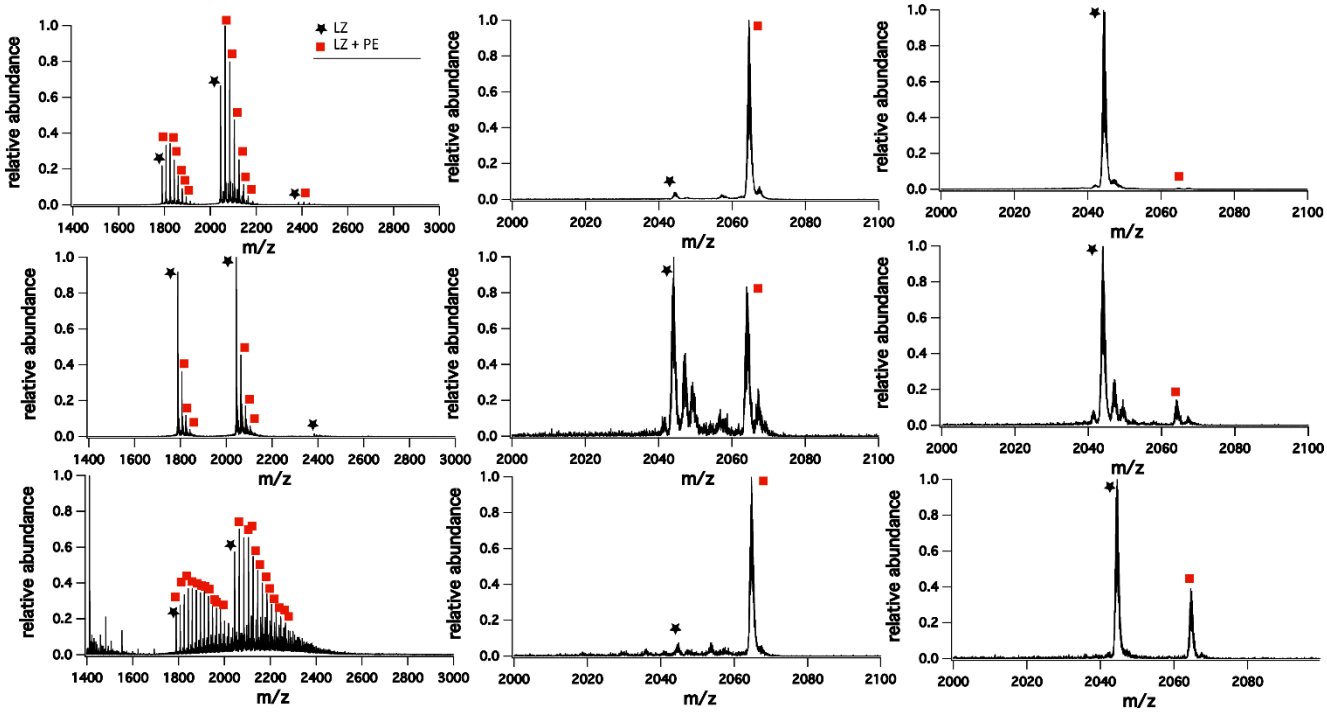

Figure S9: Mass spectra of Lysozyme and PE, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

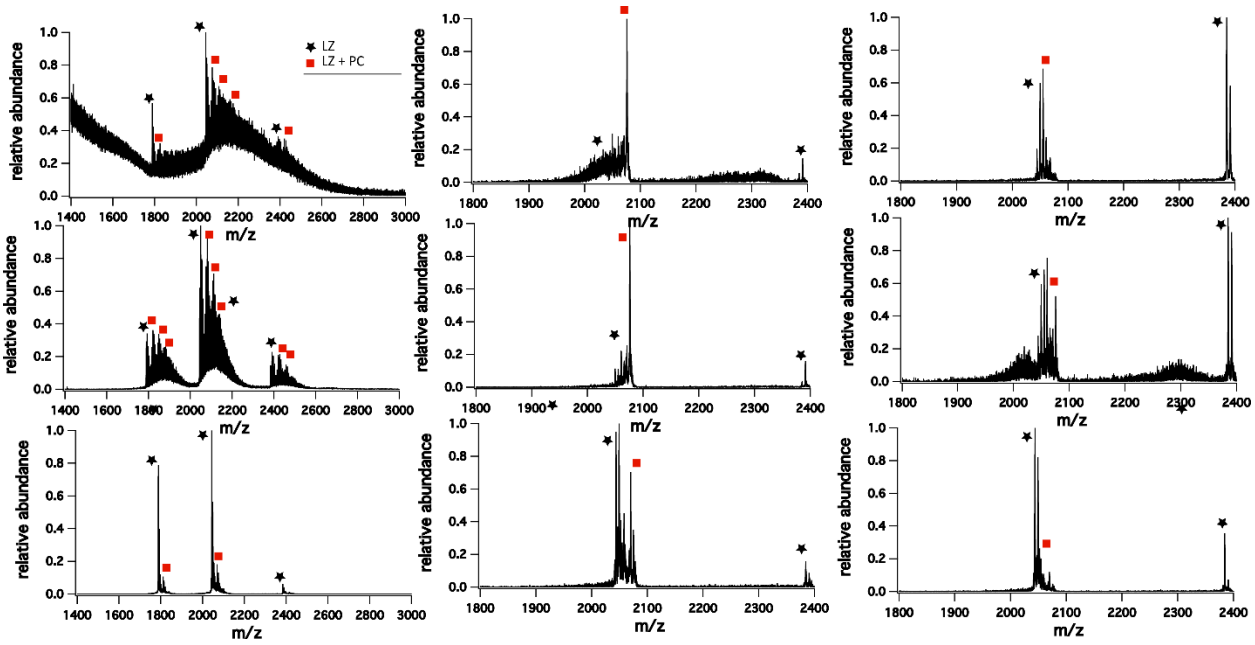

Figure S10: Mass spectra of Lysozyme and PC, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

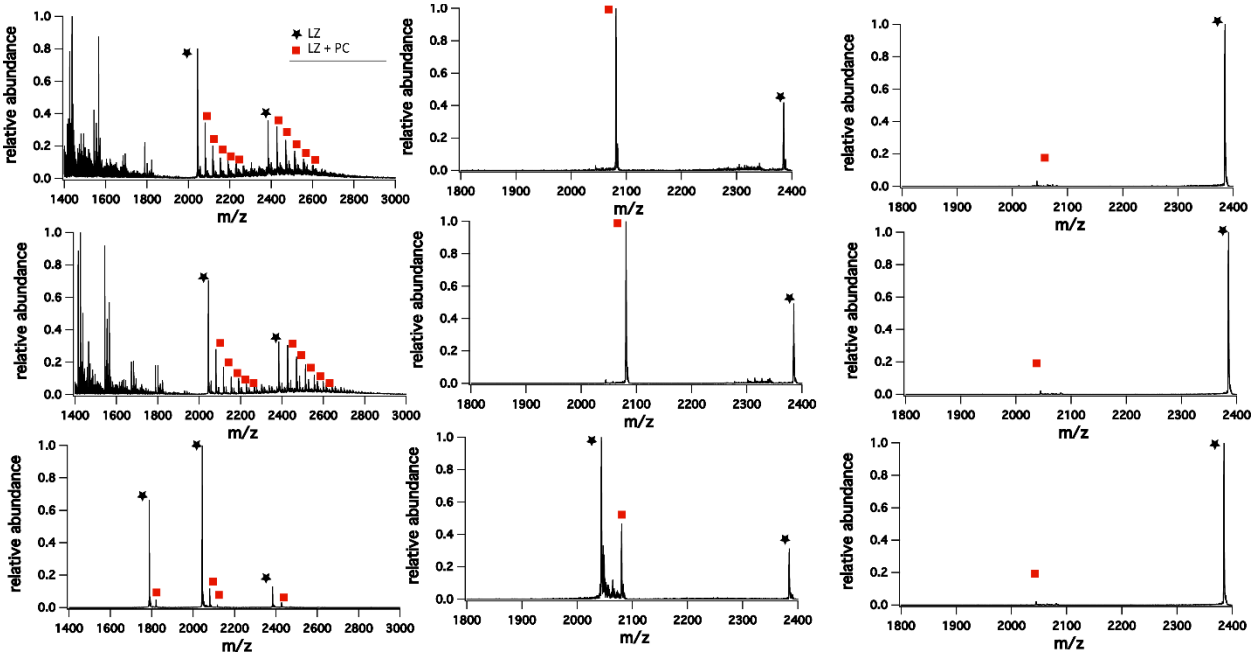

Figure S11: Mass spectra of Lysozyme and GPC, left column is no isolation, middle column is isolation and low activation, right column is isolation and high activation.

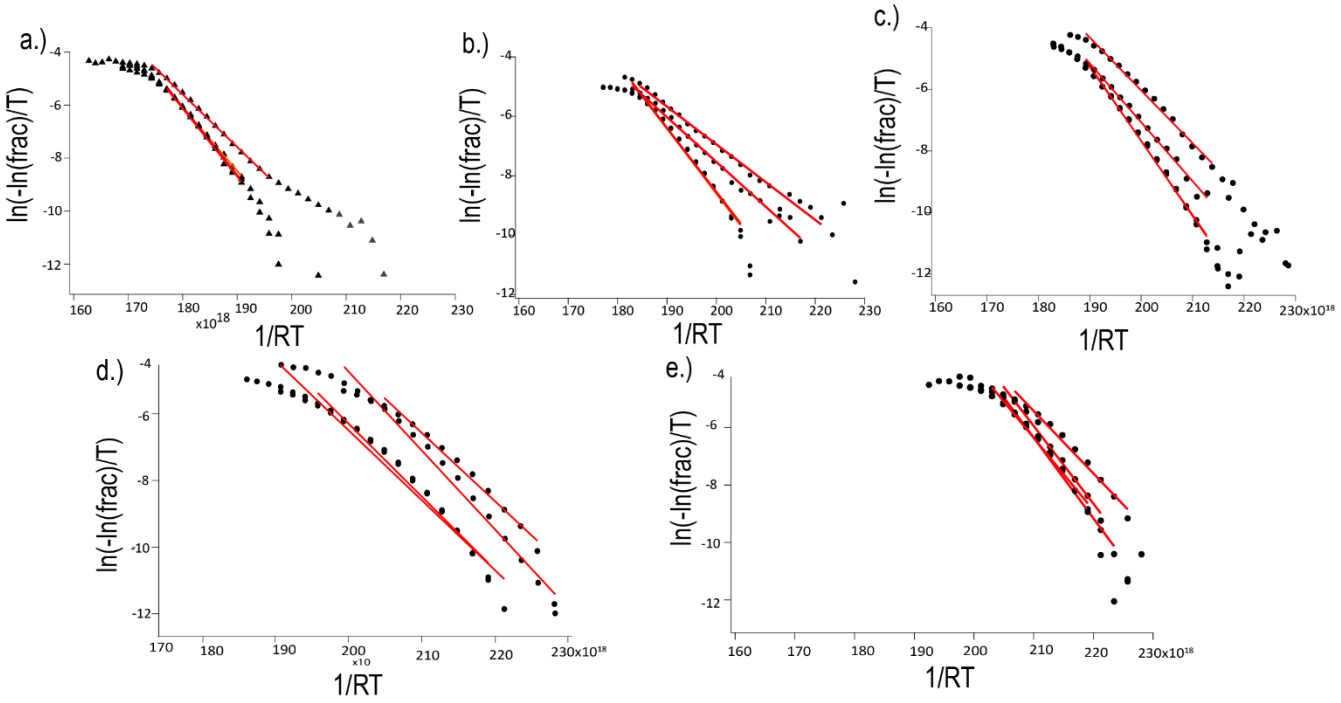

Figure S12. Eyring plots for CID of ubiquitin, 5+ losing bound lipid head groups **(a)** GPC **(b)** PC **(c)** PE **(d)** PG and **(e)** PS. The slope of the fit line is related to the activation enthalpy, and the y-intercept is related to the activation entropy. The data from 10% to 90% completed reaction was fit to the Eyring equation as explained in the theory and analysis portion.

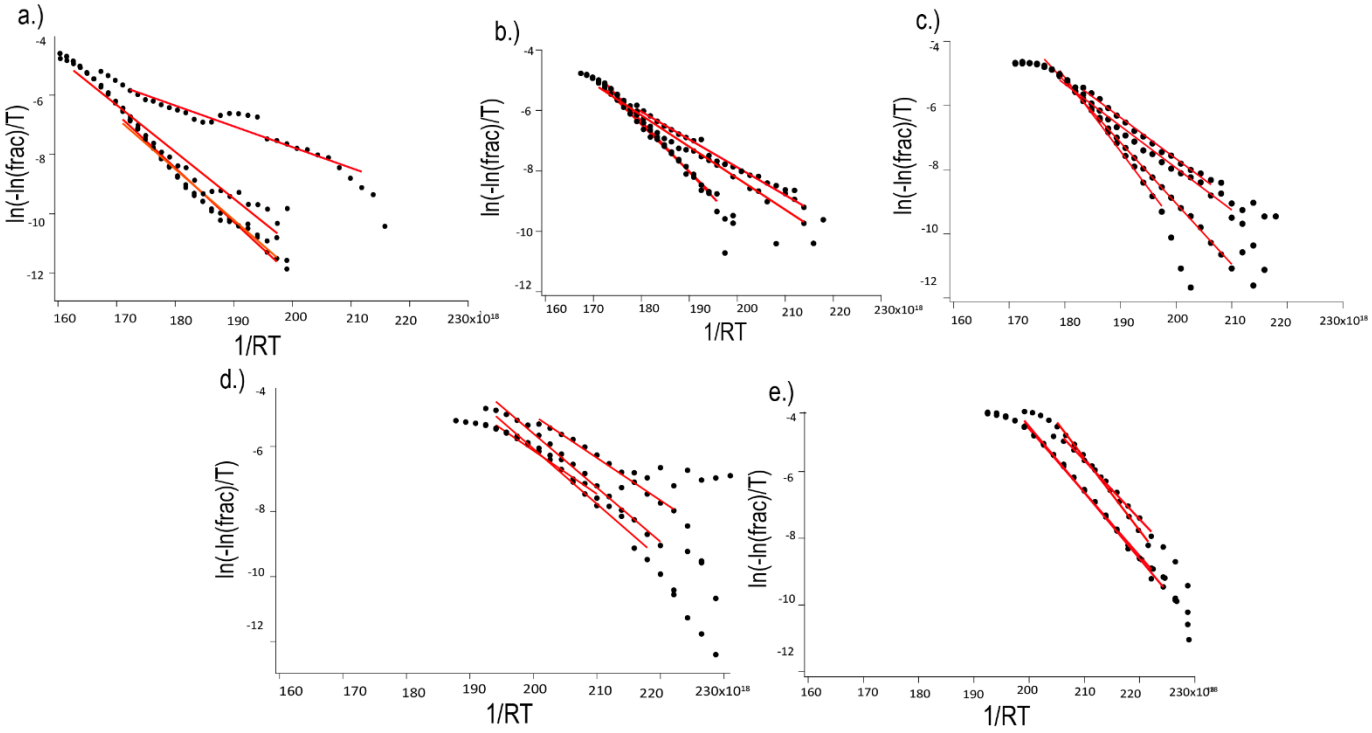

Figure S13. Eyring plots for CID of lysozyme, 7+ losing bound lipid head groups **(a)** GPC **(b)** PC **(c)** PE **(d)** PG and **(e)** PS. The slope of the fit line is related to the activation enthalpy, and the y-intercept is related to the activation entropy. In these experiments considerable drift was seen on the order of days and weeks. Because two sets of trials were taken on the same day with exceptionally low error (~1% difference), this is a long term drift and unlikely related to tip pulling.

Table S1. Activation enthalpies and entropies for lipid head group CID.

Head group	Ubiquitin, 5+ $\Delta S^{\ddagger}$ (kJ/mol)	Lysozyme, 7+ $\Delta S^{\ddagger}$ (kJ/mol)
GPC	275.0 ± 80.2	149.4 ± 47.0
PC	170.8 ± 26.5	63.4 ± 31.2
PE	150.8 ± 46.6	66.5 ± 71.6
PG	86.2 ± 68.7	17.2 ± 52.9
PS	160 ± 39.8	6.0 ± 68.3

Head group	Ubiquitin, 5+ $\Delta H^{\ddagger}$ (kJ/mol)	Lysozyme, 7+ $\Delta H^{\ddagger}$ (kJ/mol)
GPC	159.4 ± 27.8	116.9 ± 15.8
PC	128.4 ± 8.3	91.1 ± 10.7
PE	127.0 ± 17.8	99.8 ± 28.6
PG	105.0 ± 27.2	83.8 ± 21.9
PS	140.0 ± 16.8	84.0 ± 30.8

Head group	Ubiquitin, 5+ $\Delta G^{\ddagger}$ (kJ/mol)	Lysozyme, 7+ $\Delta G^{\ddagger}$ (kJ/mol)
GPC	64.6 ± 1.4	65.5 ± 2.0
PC	69.2 ± 3.0	69.1 ± 0.9
PE	73.4 ± 1.5	74.9 ± 2.5
PG	73.6 ± 2.3	76.7 ± 2.0
PS	79.4 ± 1.8	81.5 ± 3.0

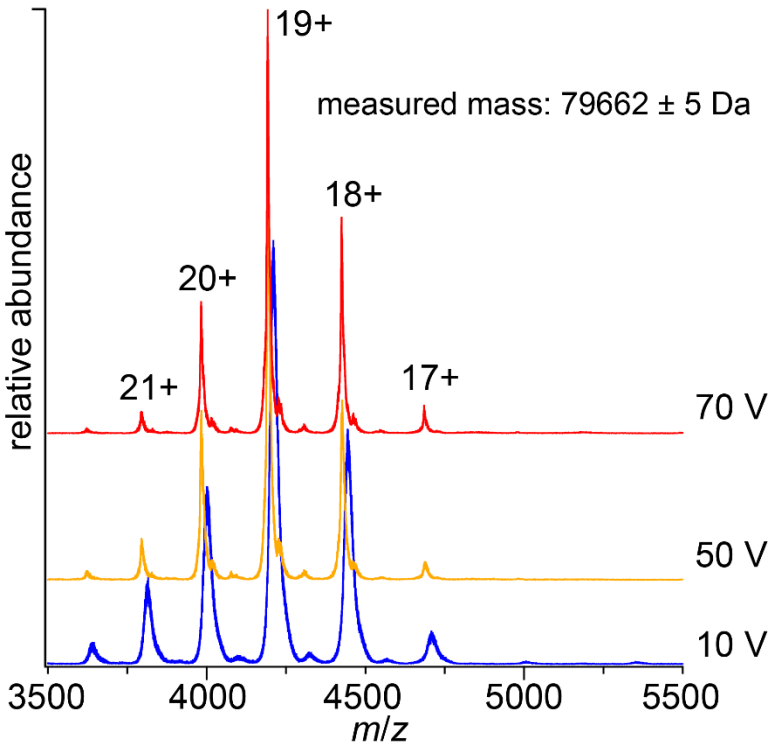

Figure S14. Native mass spectra of transferrin with no lipid head group present at Trap CE values of 10, 50, and 70 V. At all levels of activation transferrin produces clearly resolved spectra with a homogenous base mass.

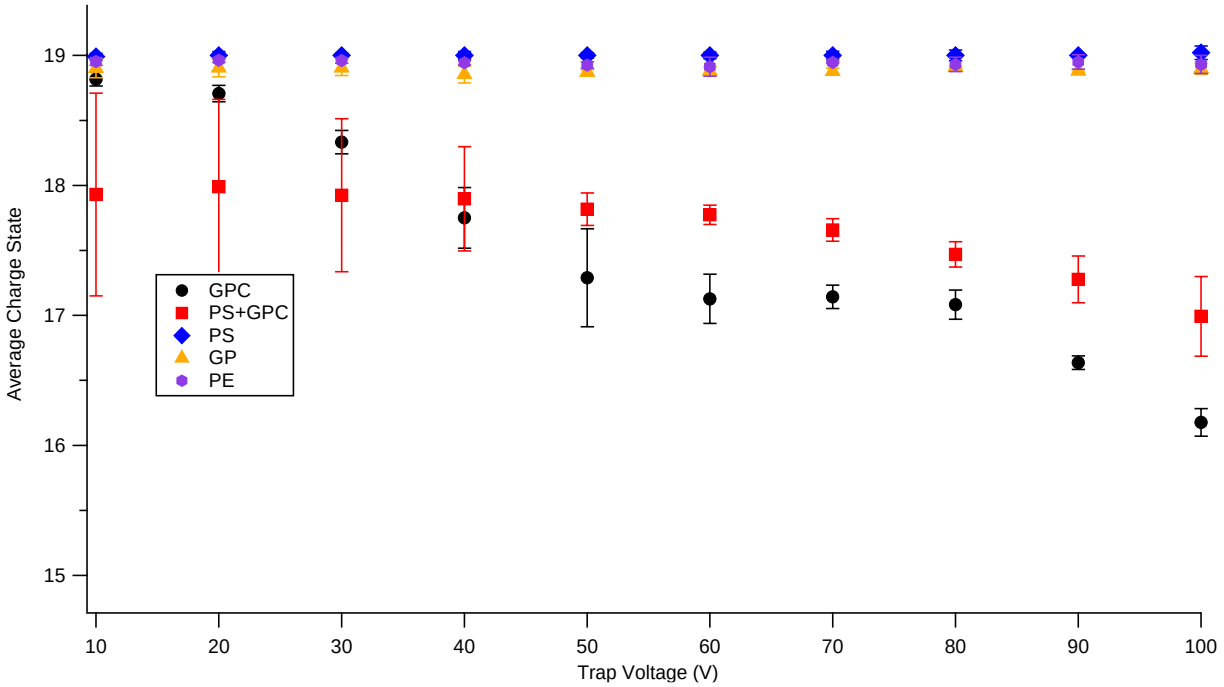

Figure S15. Change in weighted-average charge state of isolated TF^{19+} with increasing collisional activation. Charge state distributions were determined using Unidec, and error bars correspond to the weighted standard deviation of the charge state distribution. Significant loss of charge is observed only when GPC is present. The average charge state was also determined in these CID experiments to investigate charge stripping in mixed-binding environments (Figure 5). When PS, PE, and PG dissociate, no reduction in charge is observed. However, if GPC is present, either alone or in combination with another head group, charge stripping upon dissociation is observed, with an average of 2-3 charges lost at 100 V Trap CE. The rate of charge loss is similar across all the experiments with GPC present, suggesting that GPC dissociates more readily than the other head groups. This agrees with predicted behavior based on GB and the above results for Ubq and LZ and confirms that GPC dissociates readily and retains its charge stripping effect even in the presence of other head groups. This particular figure shows quantitation for only the initial trial in which unfolding of transferrin was not observed.

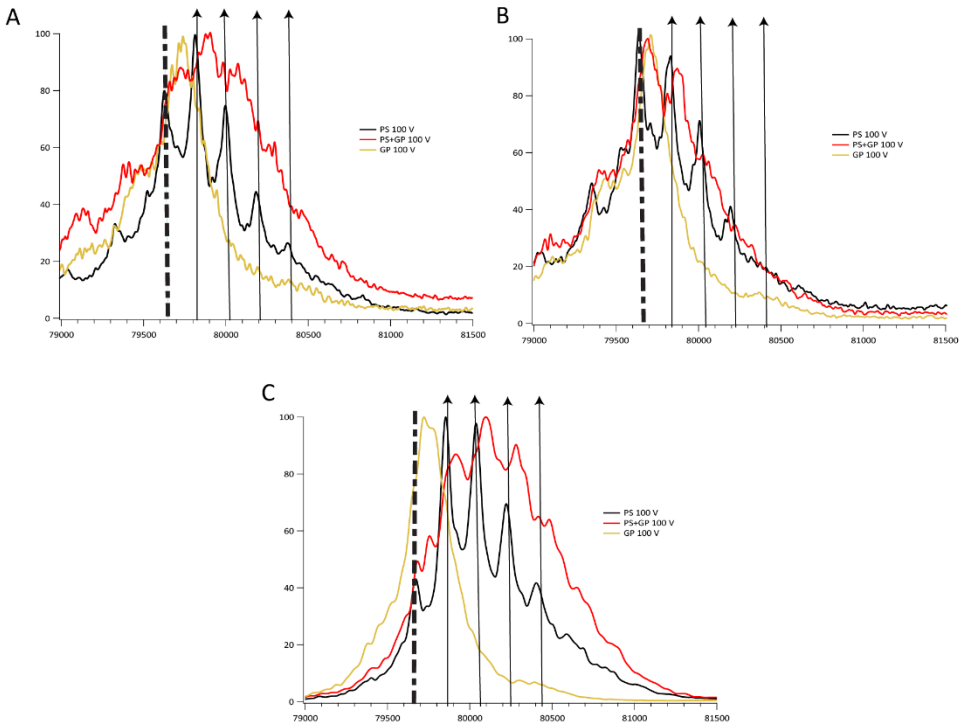

Figure S16. Overlaid deconvolved mass spectra for TF^{19+} with PS bound (black), PG bound (gold), and PS + PG bound (red) at a Trap CE of 100 V. The dashed line corresponds to the base mass of TF with no adducts. For TF with PG, no adducts are resolved, while for TF with PS several PS adducts are clearly resolved (stars). For TF with both PS and PG, partially-resolved adduct peaks are observed at *m*/*z* slightly higher than that of the PS adducts, likely corresponding to PS adducts with some additional salt adduction.