

Supporting Information for

Metal Affinity Capture Tandem Mass Spectrometry for the Selective Detection of Phosphopeptides

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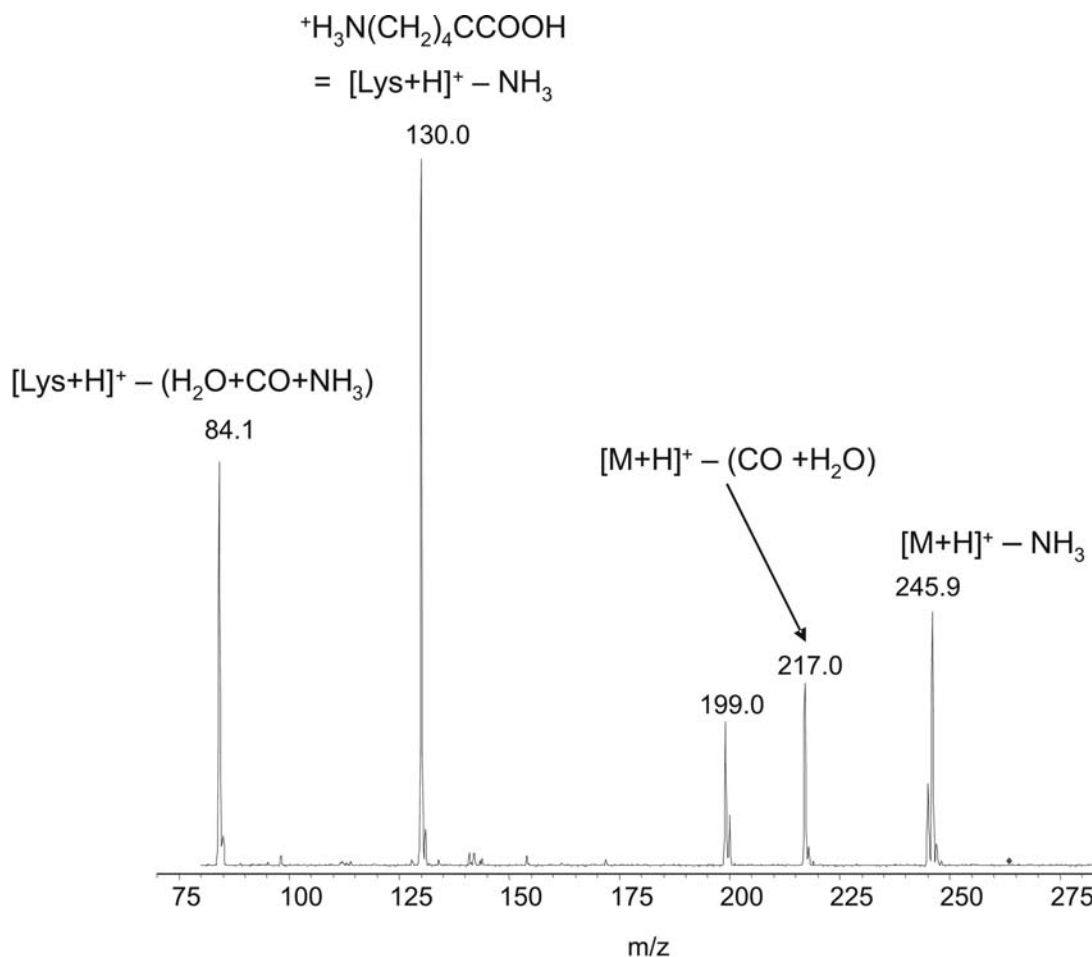


Figure S1. Collision-induced dissociation mass spectrum of protonated N_α, N_α -bis-(carboxymethyl)lysine ($LysNTA + H$)⁺ at m/z 263. CID spectrum obtained on a Bruker Esquire quadrupole ion trap.

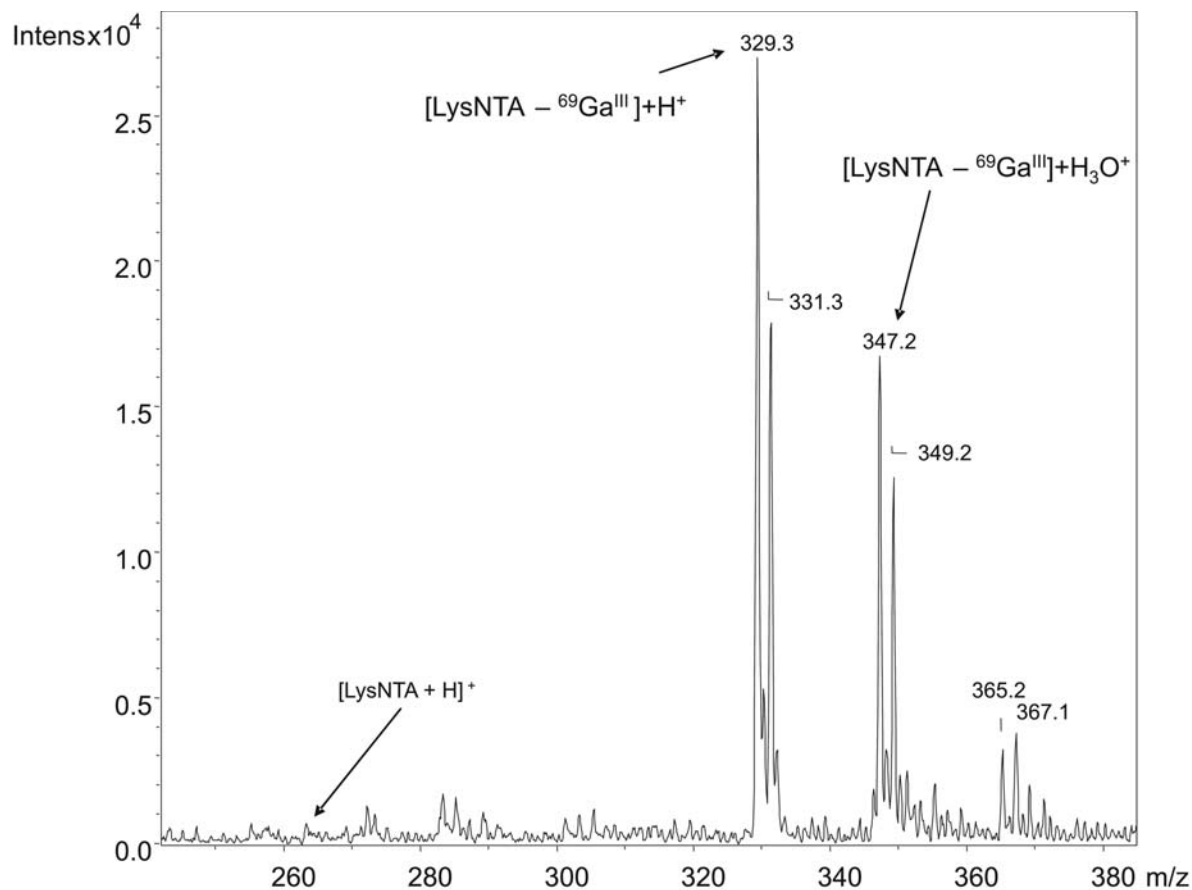


Figure S2. Electrospray mass spectrum (Bruker Esquire ion trap) of a complex formed from 25 μM LysNTA and 50 μM GaCl_3 in 1:1 $\text{CH}_3\text{OH}/\text{H}_2\text{O}$ buffered with 1% acetic acid. The position of the peak of $(\text{LysNTA} + \text{H})^+$ is denoted by an arrow. The Ga-containing ions show splitting due to the presence of ⁶⁹Ga and ⁷¹Ga isotopes.

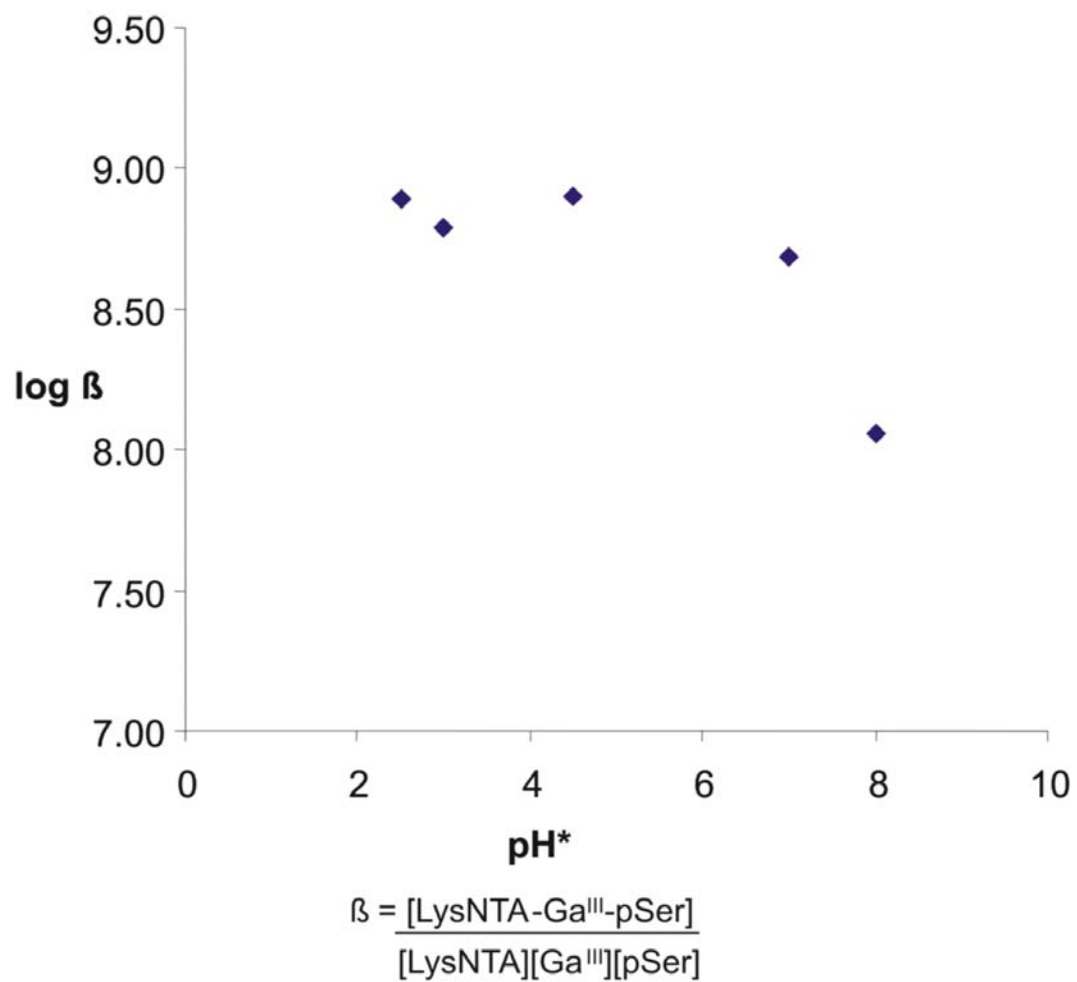


Figure S3. Formation constants for ternary complexes of LysNTA, Ga^{III} and phosphoserine.

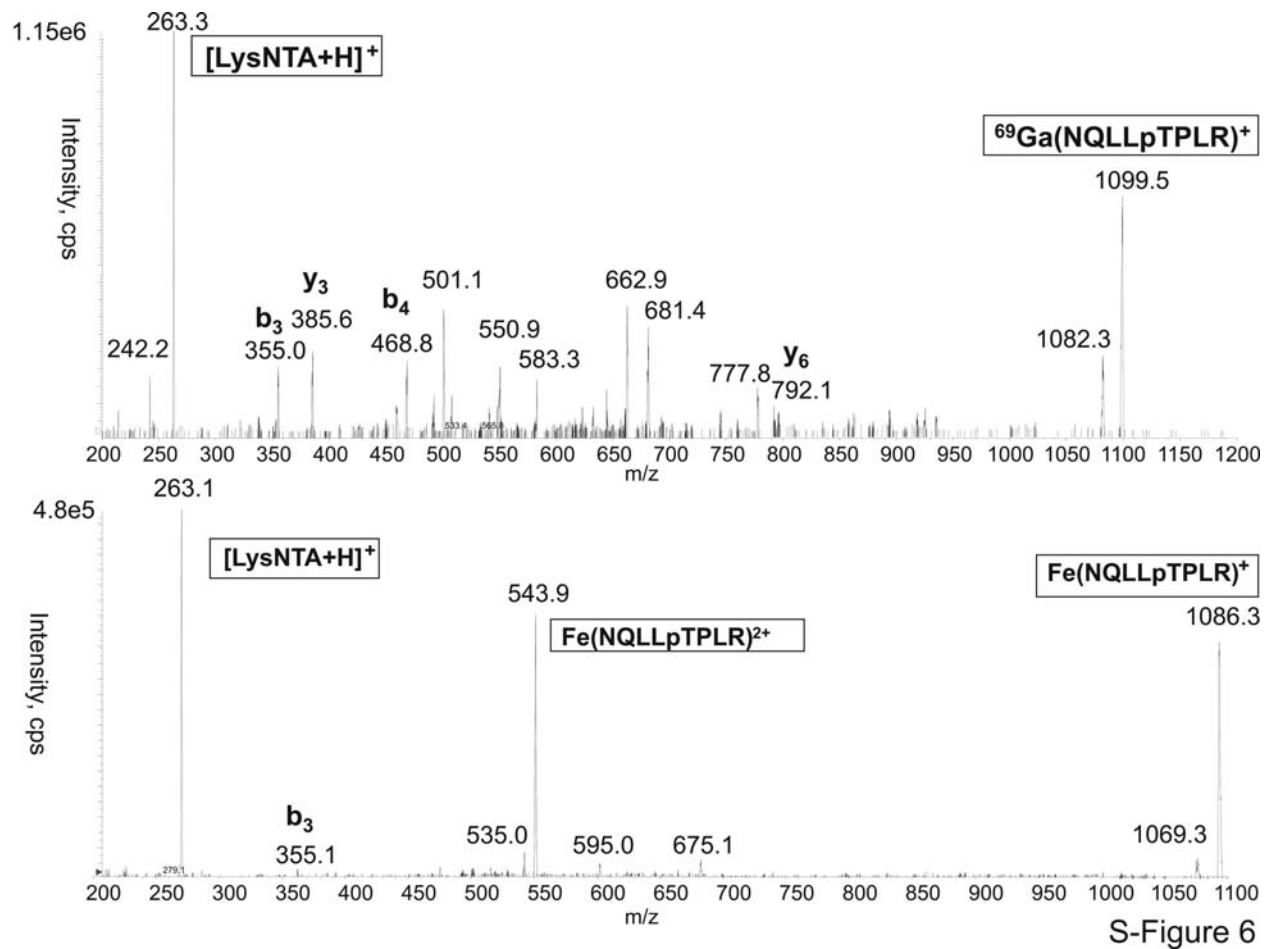


Figure S4. CID mass spectra of doubly charged LysNTA complexes with NQLLpTPLR and Ga^{III} (top) and Fe^{III} (bottom).

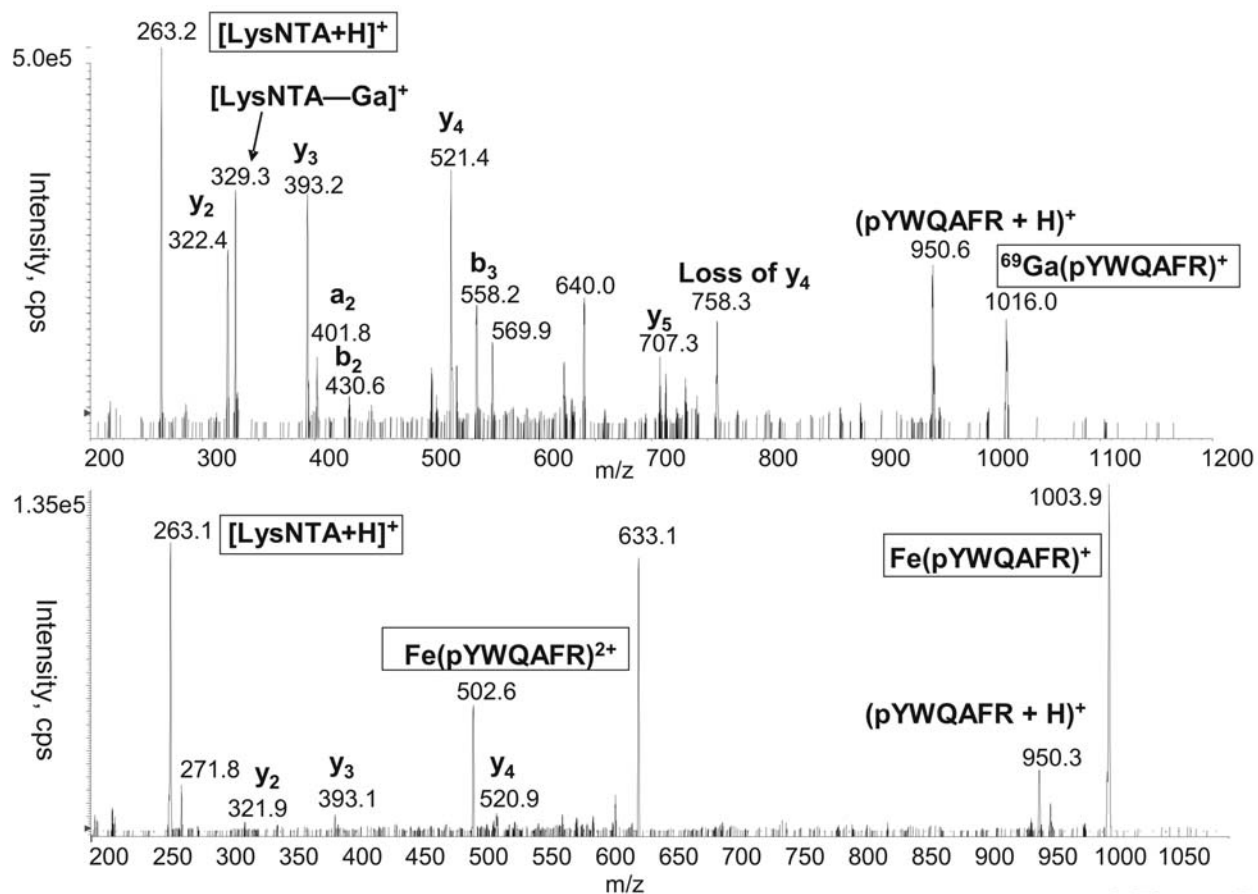


Figure S5. CID mass spectra of doubly charged LysNTA complexes with pYWQAFR and Ga^{III} (top) and Fe^{III} (bottom).

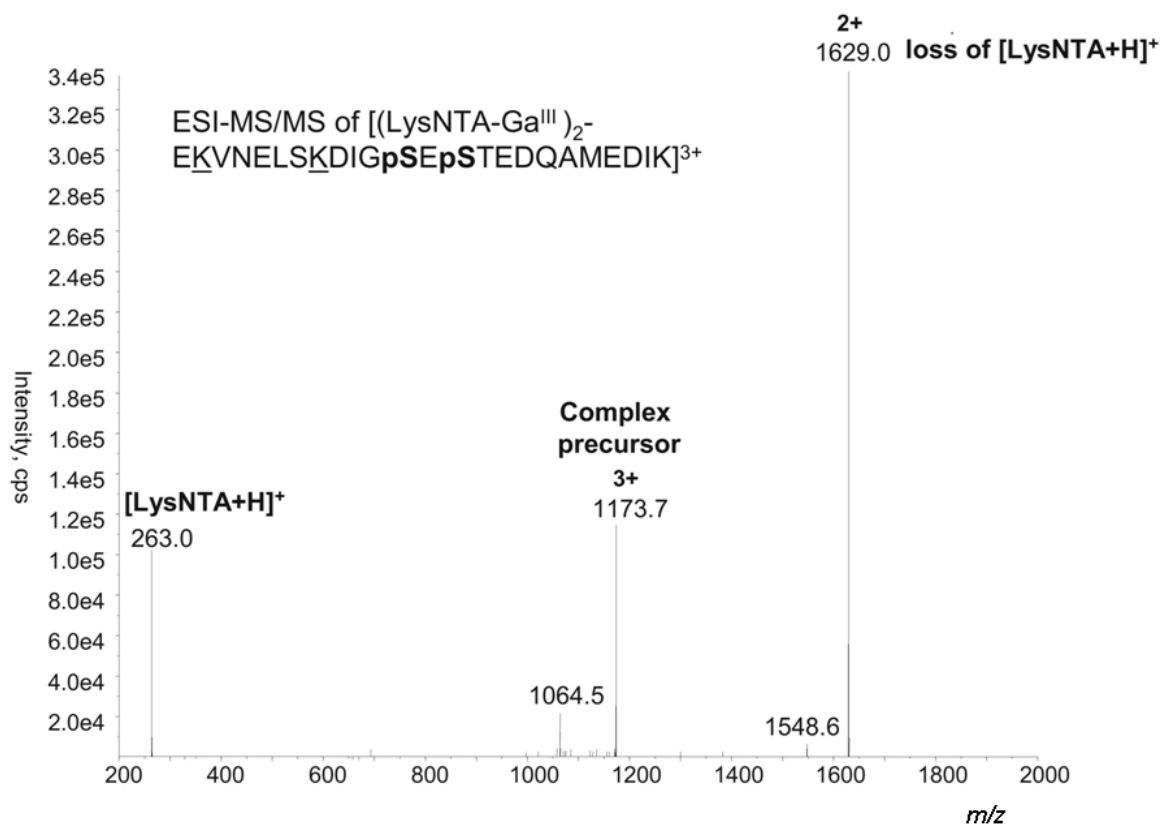


Figure S6. CID mass spectrum of a triply charged LysNTA- Ga^{III} complex with the α -casein tryptic peptide EKVNELSKDIGpSEpSTEDQAMEDIK.

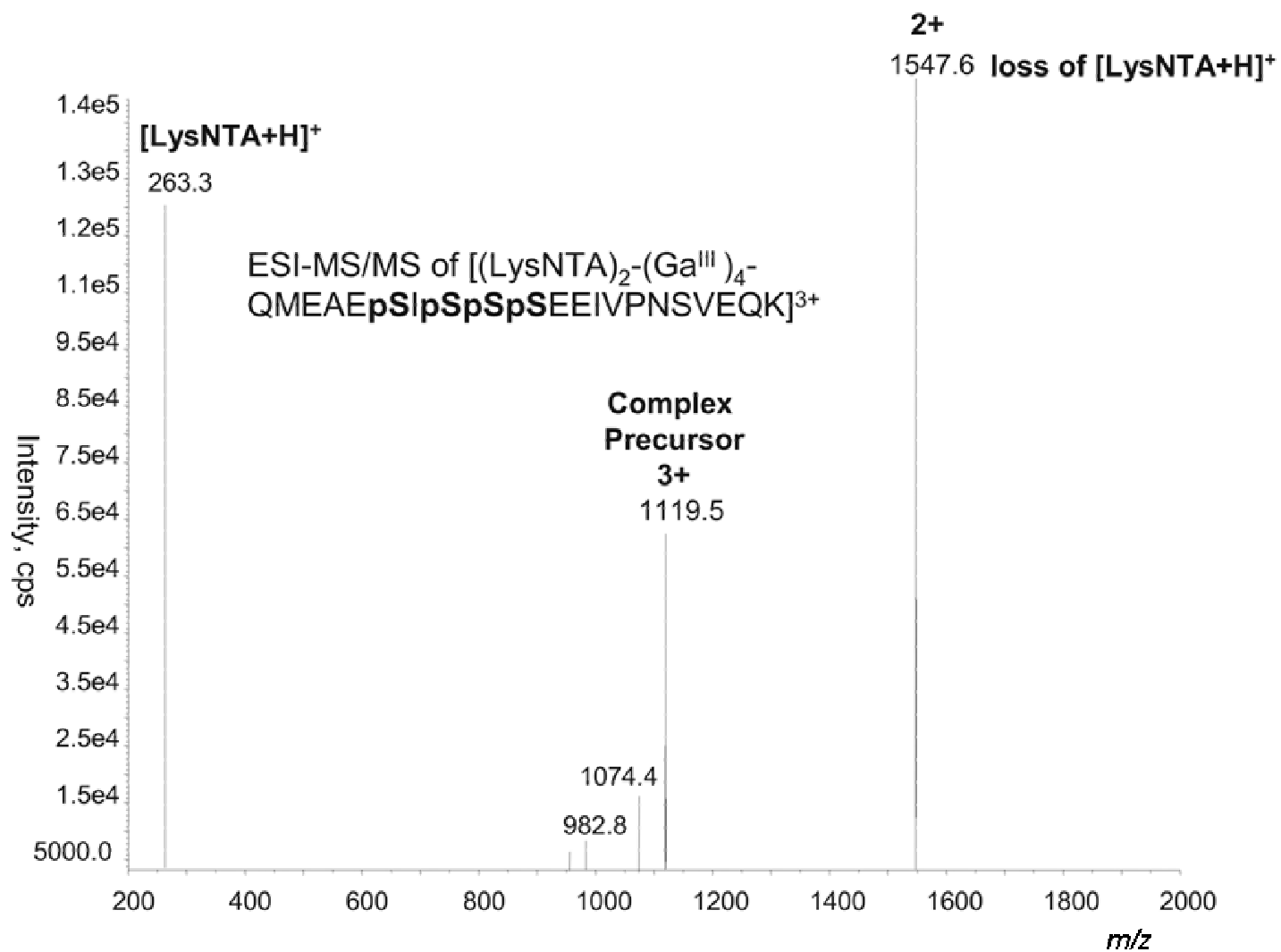


Figure S7. CID mass spectrum of a triply charged LysNTA- Ga^{III} complex with the α -casein tryptic peptide QMEAEpSIpSpSpSEEIVPNSVEQK.

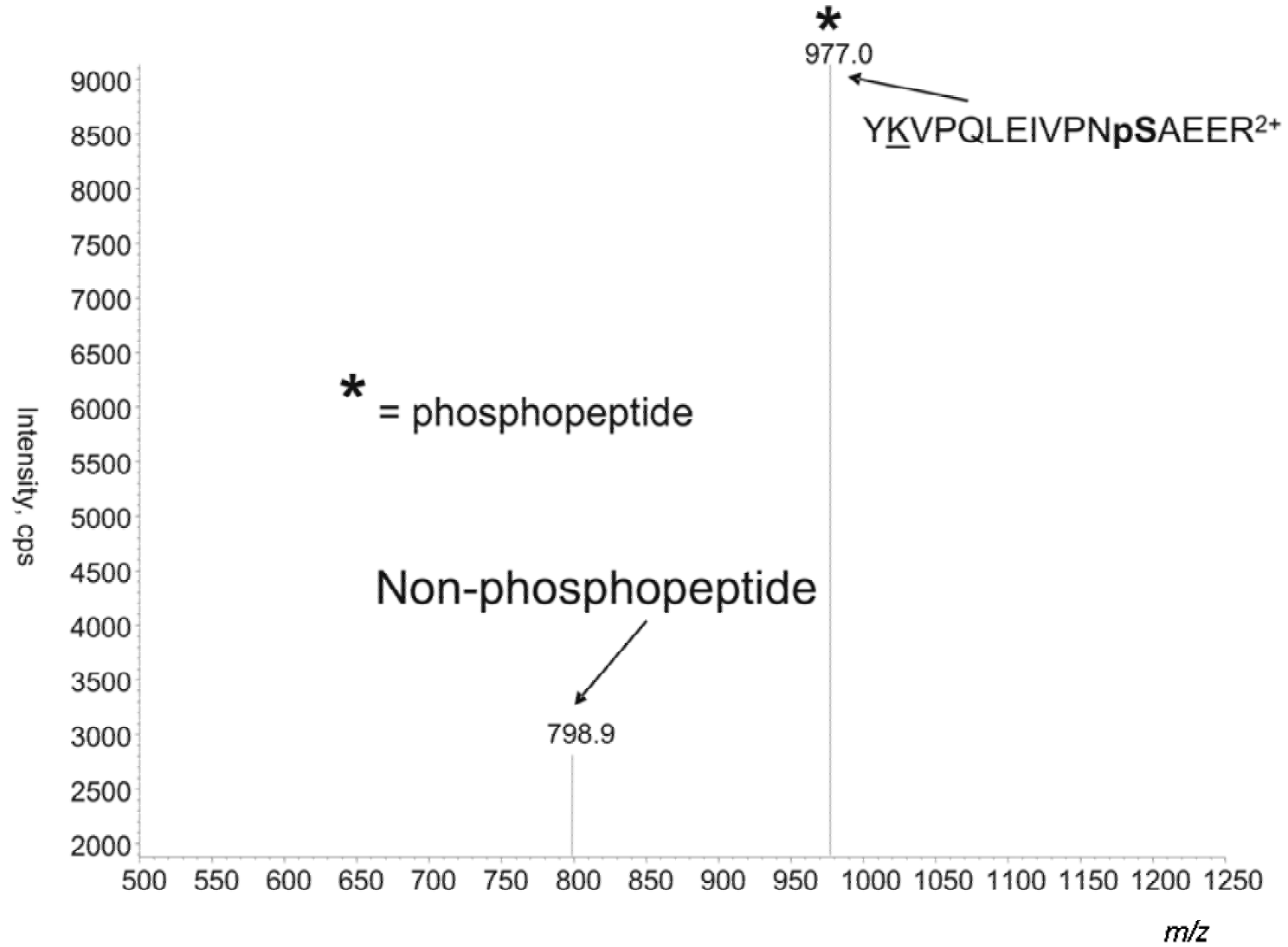


Figure S8. Neutral loss scan (49 Da difference) of phosphopeptides from trypsinolysis of 40 nM α -casein.

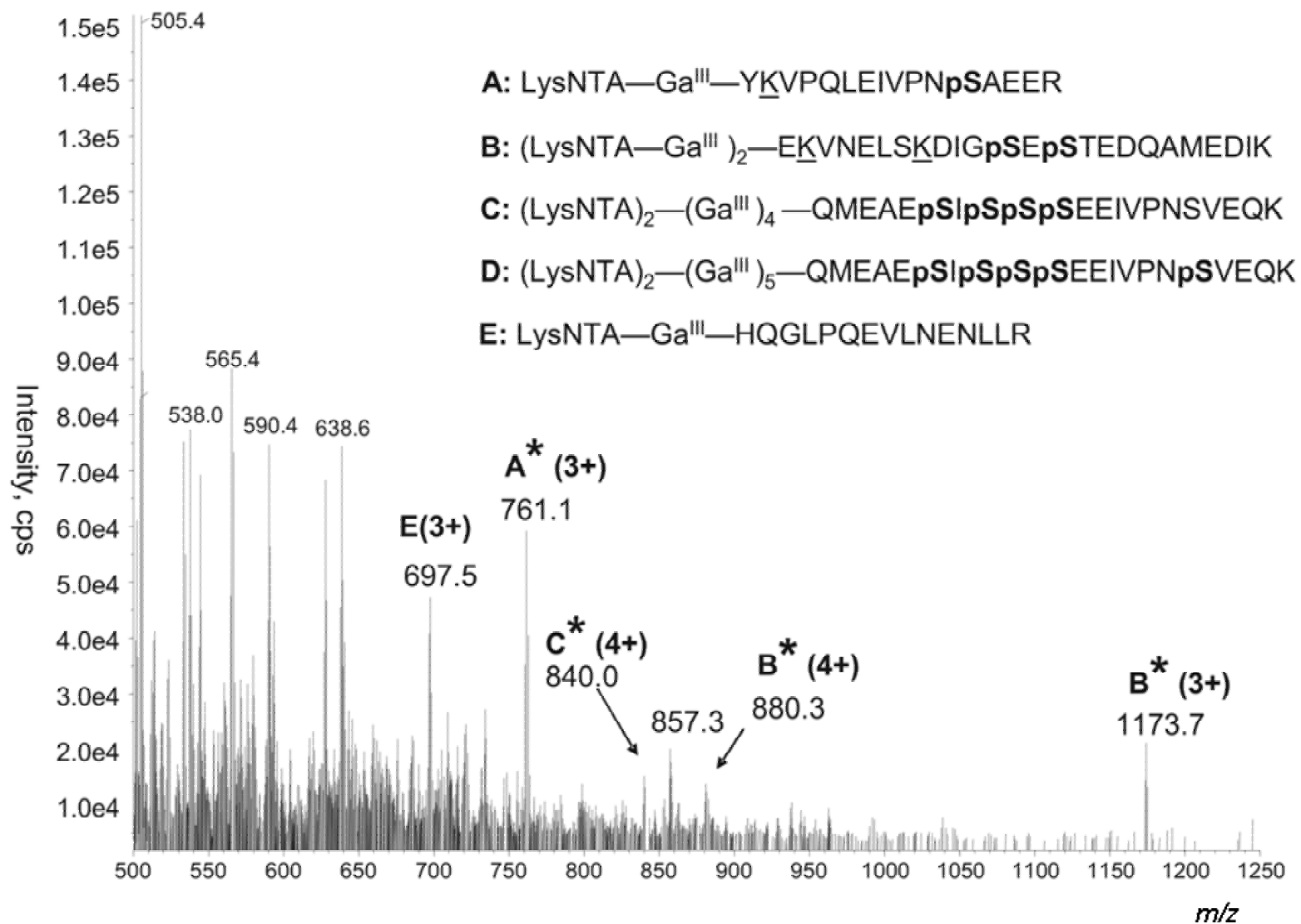


Figure S9. Parent scan (m/z 263) detection of LysNTA-Ga^{III} phosphopeptide complexes from trypsinolysis of 40 nM α -casein.