Supplementary Material for

Comprehensive Characterization of Recombinant Catalytic Subunit of cAMP-Dependent Protein Kinase by Top-Down Mass Spectrometry

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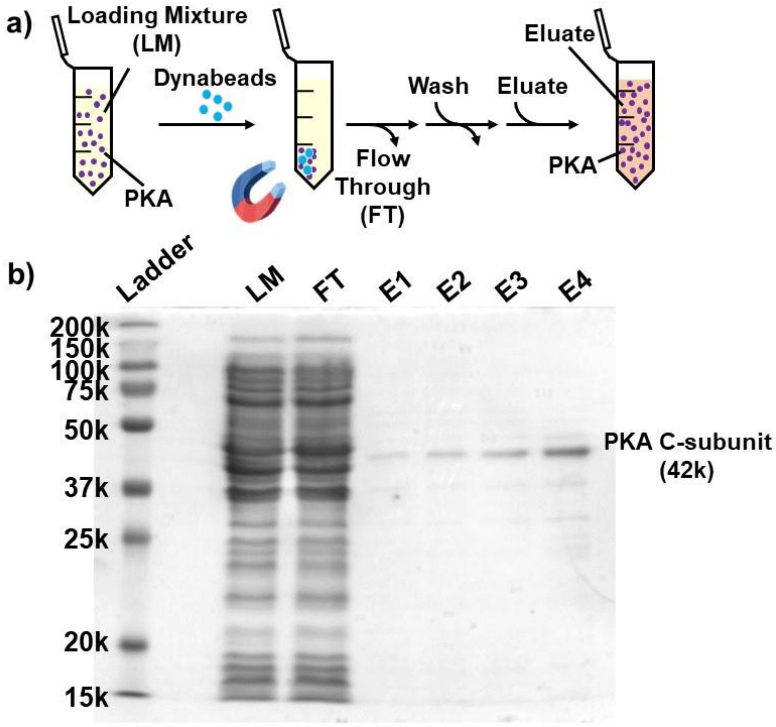

Figure S1. Schematic drawing and SDS-PAGE analysis of affinity purification for PKA C-subunit. a) Schematic of the affinity purification of PKA C-subunit using DynaBead for His-tag purification; b) SDS-PAGE analysis of purified PKA catalytic subunit expressed in *E. coli*.

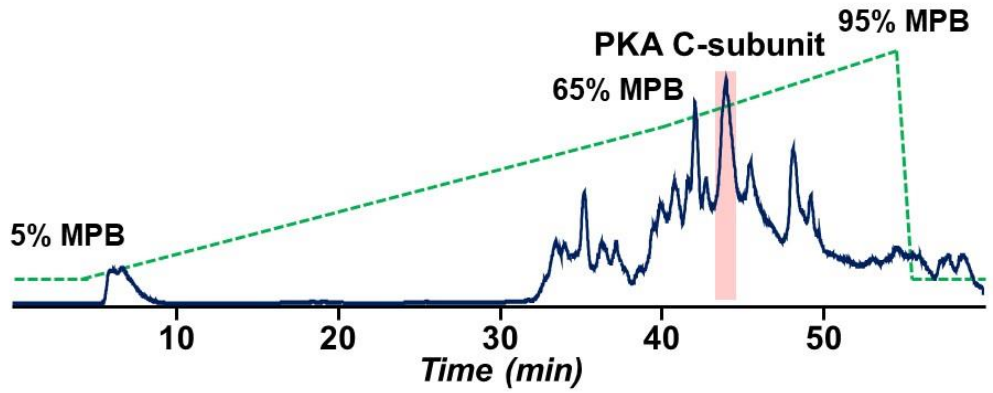

Figure S2. Total ion chromatogram of recombinant PKA C-subunit using liquid chromatography (LC). The LC condition was run with H_2O with 0.1% formic acid (FA) as mobile phase A and 50:50 EtOH:ACN with 0.1% FA as mobile phase B (MPB). The gradient ran at 5% MPB for 5 min, followed by 5% to 65% MPB from 5 to 40 min, 65% to 95% MPB from 40-53 min, and back to 5% MPB. Other peaks in the chromatogram are either low mass proteins or small molecule contamination.

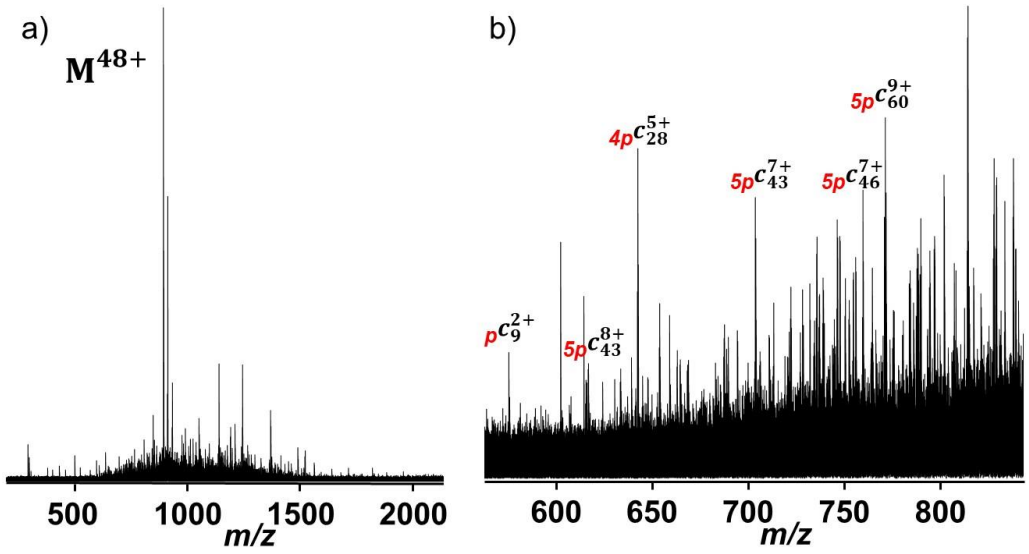

Figure S3. Raw spectra of ECD experiment. a) The precursor ion at charge state 48^+ was subject to ECD fragmentation experiment, and a large number of fragment ions was yielded. b) Zoomed-in spectra from 625 – 850 *m/z* showing several *c* ions with phosphorylations intact.

Figure S4. Fragment ion mapping for CID fragmentation. A series of CID fragment ions was observed at Ser[54-58]Gln, Ser[134-145]Gly, Gly[246-265]Gln, and Tyr[350-357]Arg. *b* ions that contain a large number of phosphorylations were observed at Ser[54-58]Gln, Ser[134-145]Gly, and Gly[246-255]Tyr.

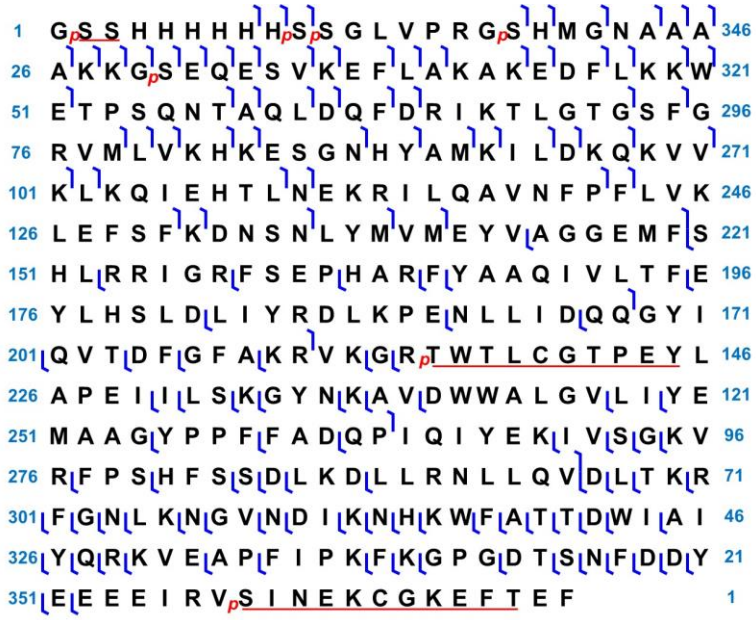

Figure S5. Fragment ion mapping for ECD fragmentation. Most ECD fragment ions were located at both ends of the amino acid sequence.

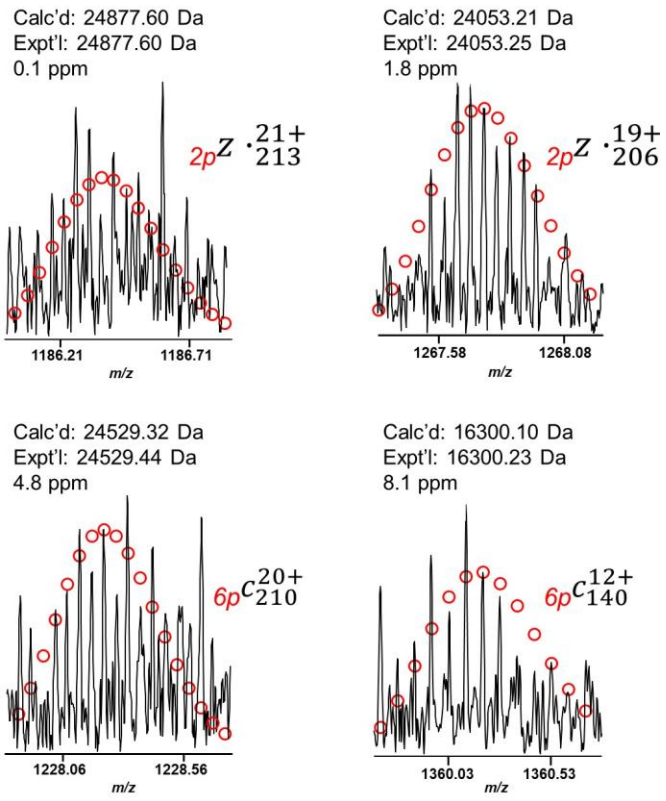

Figure S6. Representative fragment ions from ECD fragmentation. Four ECD fragment ions with large molecular weight were shown.

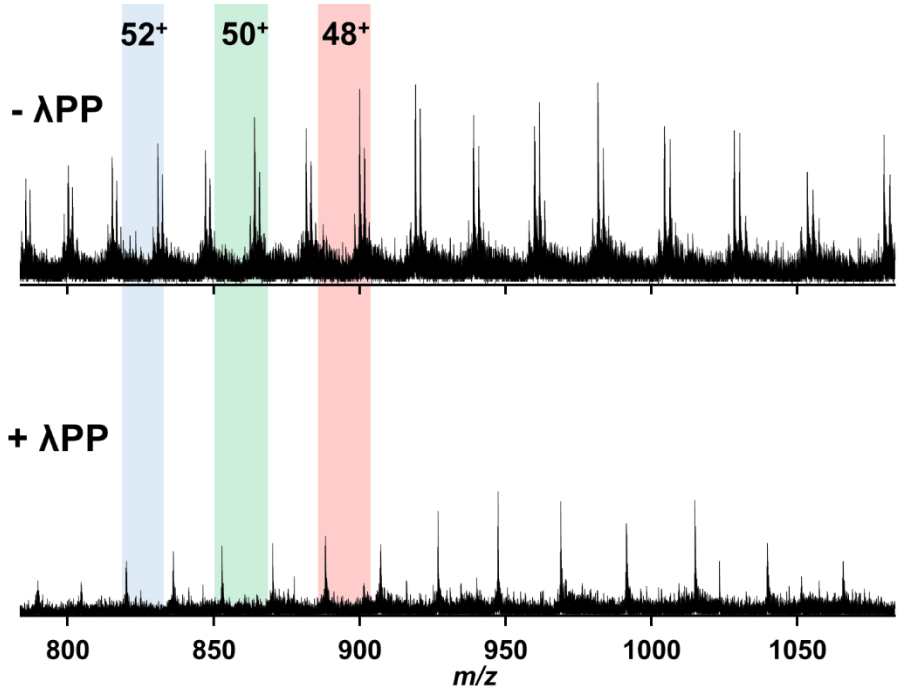

Figure S7. Broadband spectra for the dephosphorylation reaction. A drastic peak shift was observed for charge state 52^+, 50^+, and 48^+ due to the removal of multiple phosphorylations.