

**HD Exchange and PLIMSTEX Determine the Affinities and Order of Binding of Ca²⁺
with Troponin C**

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Supporting information

SUPPLEMENTAL FIGURES

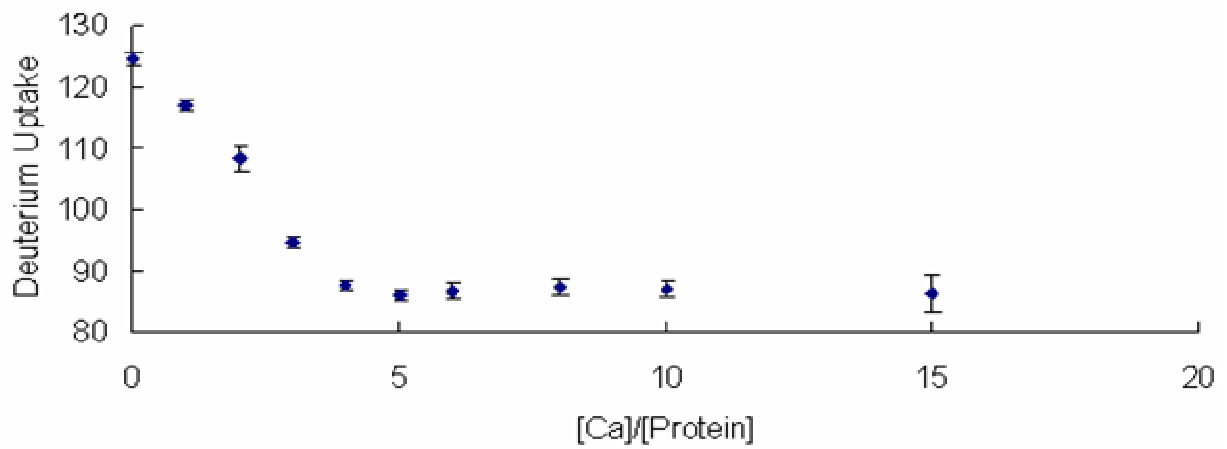


Figure S1. “Sharp-break” PLIMSTEX titration curve; protein concentration = 15 μM .

The junction of the two arms of the curves indicates the stoichiometry of Ca^{2+} binding is

4:1.

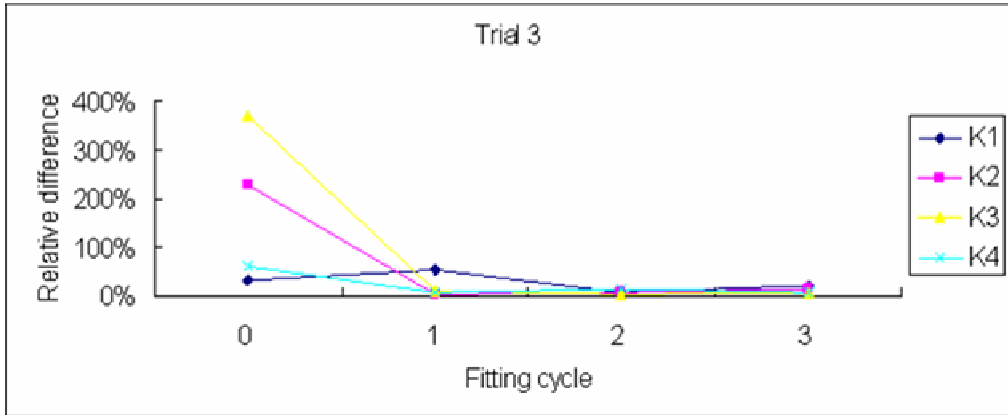
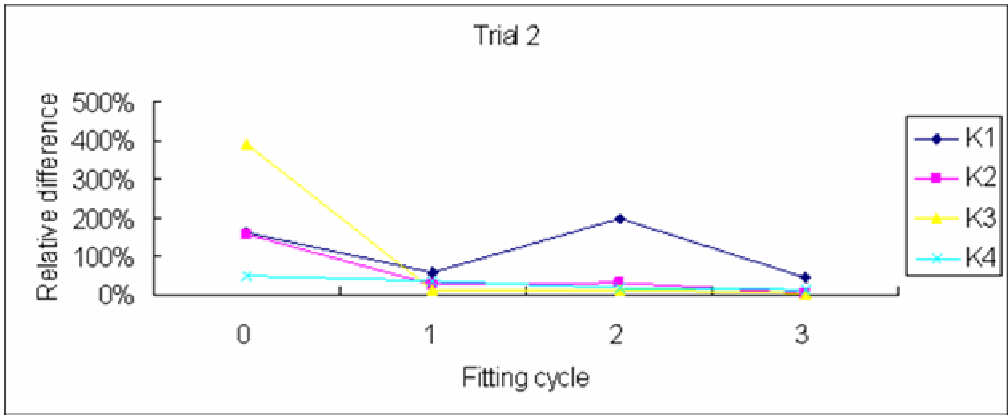
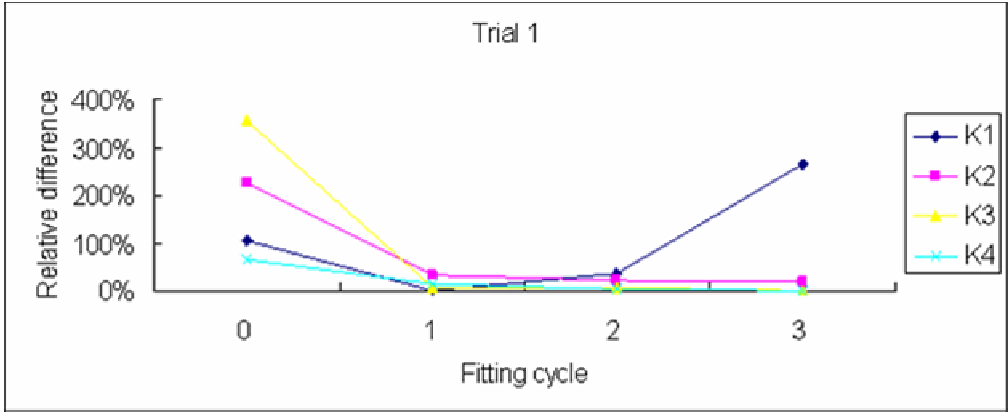


Figure S2. Relative differences between K's values before and after each cycle of fitting as a function of the number of fitting cycles.

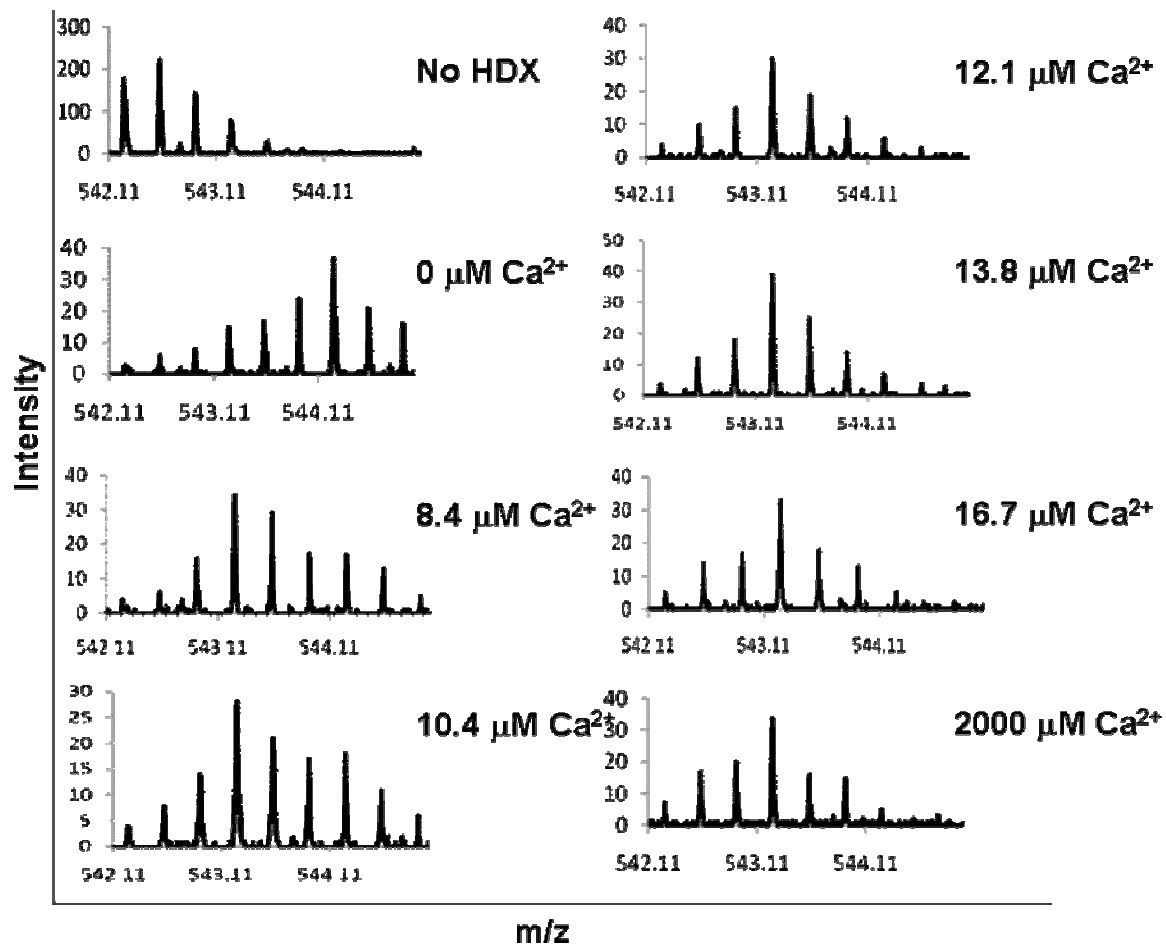


Figure S3. ESI mass spectra of the deuterated triply charged peptic peptide 136-149 (EF-IV) with respect to Ca^{2+} concentration.

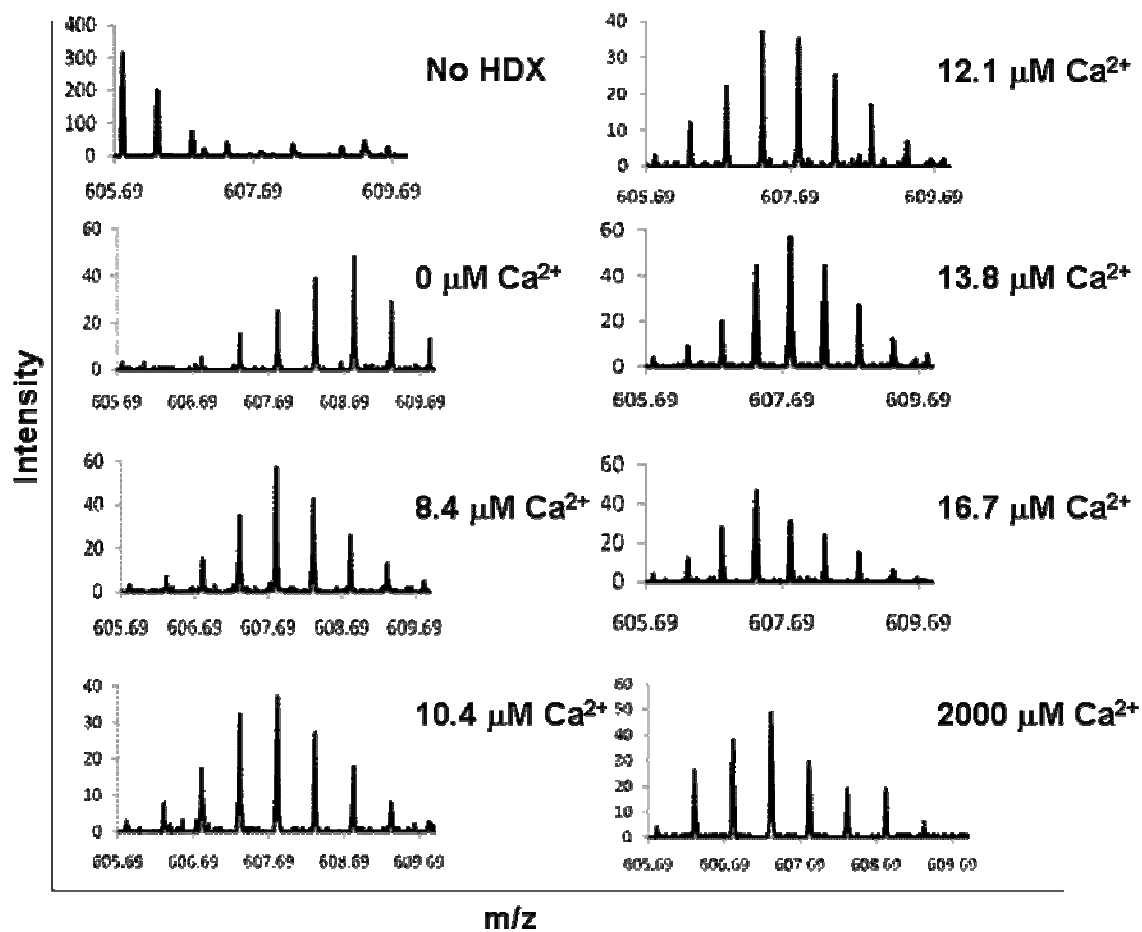


Figure S4. ESI mass spectra of deuterated doubly charged peptic peptide 100-109 (EF-III) with respect to Ca^{2+} concentration.

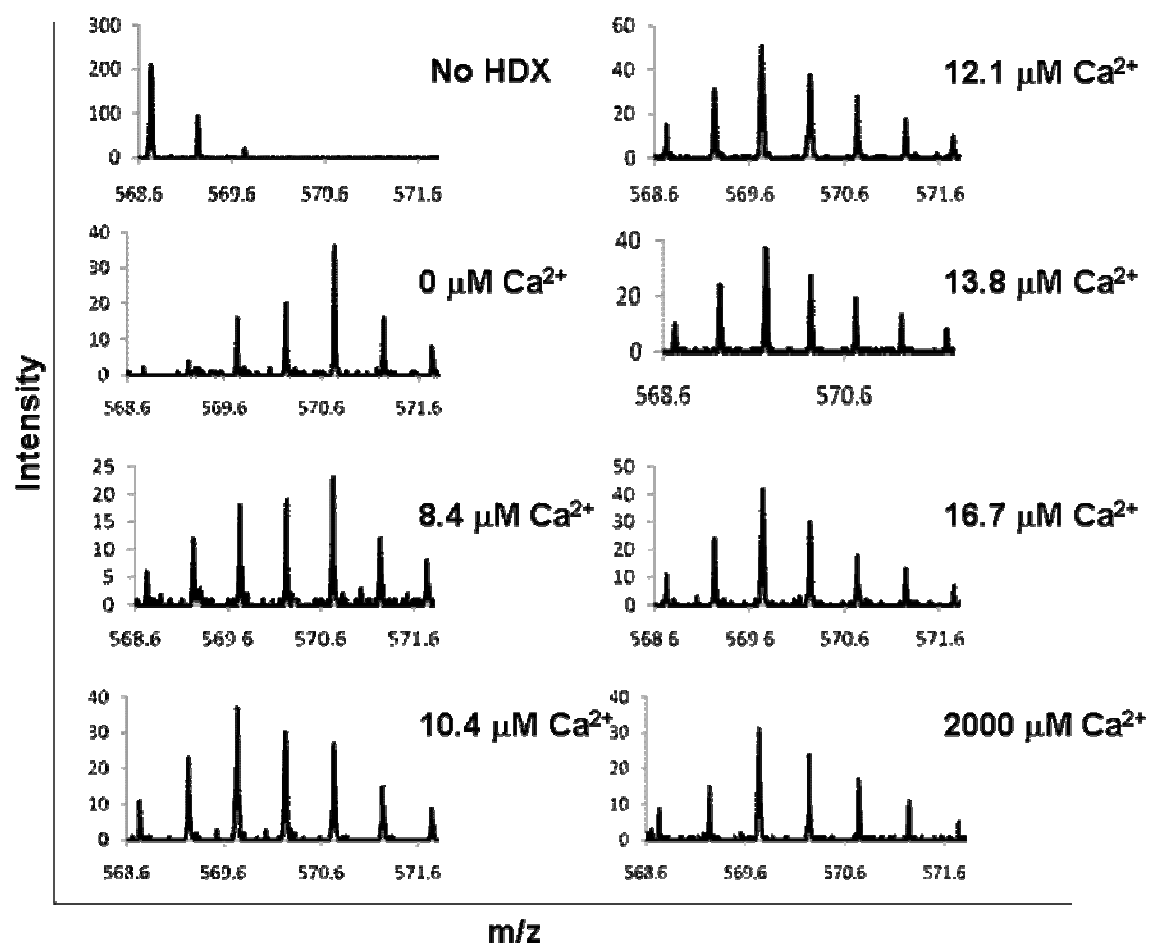


Figure S5. ESI mass spectra of the deuterated doubly charged peptic peptide 63-73 (EF-II) with respect to Ca^{2+} concentration.

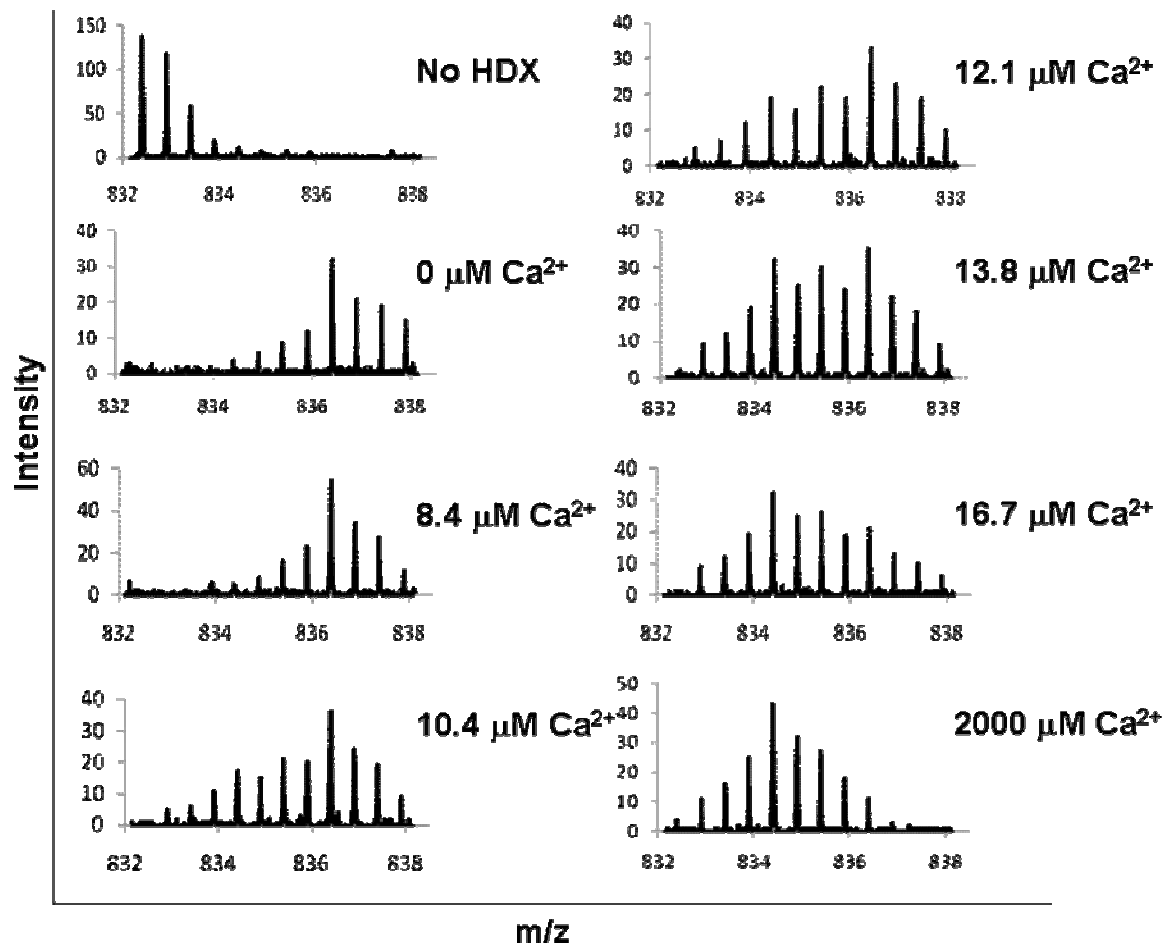


Figure S6. Spectra of the deuterated doubly charged peptic peptide 28-44 (EF-I) with respect to Ca^{2+} concentration.

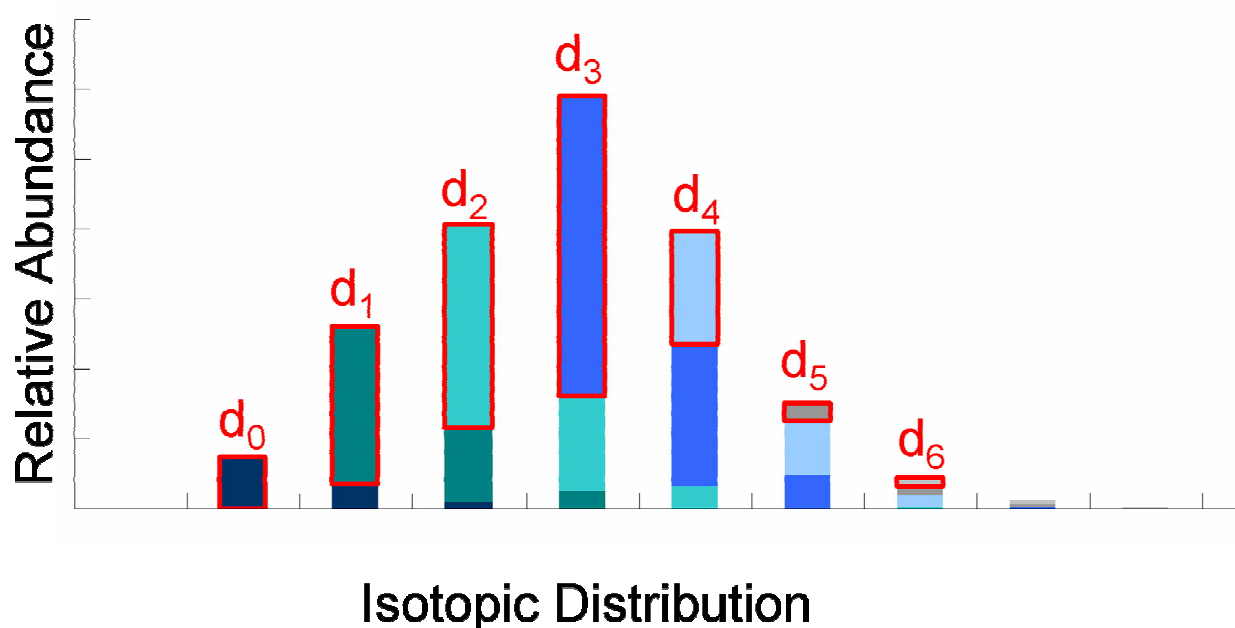


Figure S7. Deuterium distribution convolved with the native isotopic distribution of doubly charged peptide 63-73. Bars represent the different numbers of deuteriums (d_0 , d_1 , d_2 ...) comprising each peak, and their heights include other isotopic contributions (C-13, N-15, O-18) (dark blue to light blue). Removing the native isotopic distribution leaves the deuterium distribution (bars outlined with red frames).