



**Supplementary Figure 2:** (A) Change in fluorescence anisotropy of FI-CaNp upon titration with  $\text{CaM}_{1-148}$  in the presence of saturating  $\text{Ca}^{2+}$ . The normalized anisotropy is plotted against the ratio of  $\text{CaM}_{1-148}$  to CaNp. Curves through the data were simulated to a one-site binding isotherm for several  $K_d$  values: 1 pM (solid line), 1 nM (dotted line), 10 nM (large dashed line), and 100 nM (dotted/dashed line). Inset shows a detail view of the data near the break point. (B) To illustrate the separation in  $K_d$  values, simulated curves were plotted as a function of  $[\text{CaM}]_{\text{Free}}$ .