Supporting Figures/Tables

Relative Cosolute Size Influences the Kinetics of Protein-Protein Interactions

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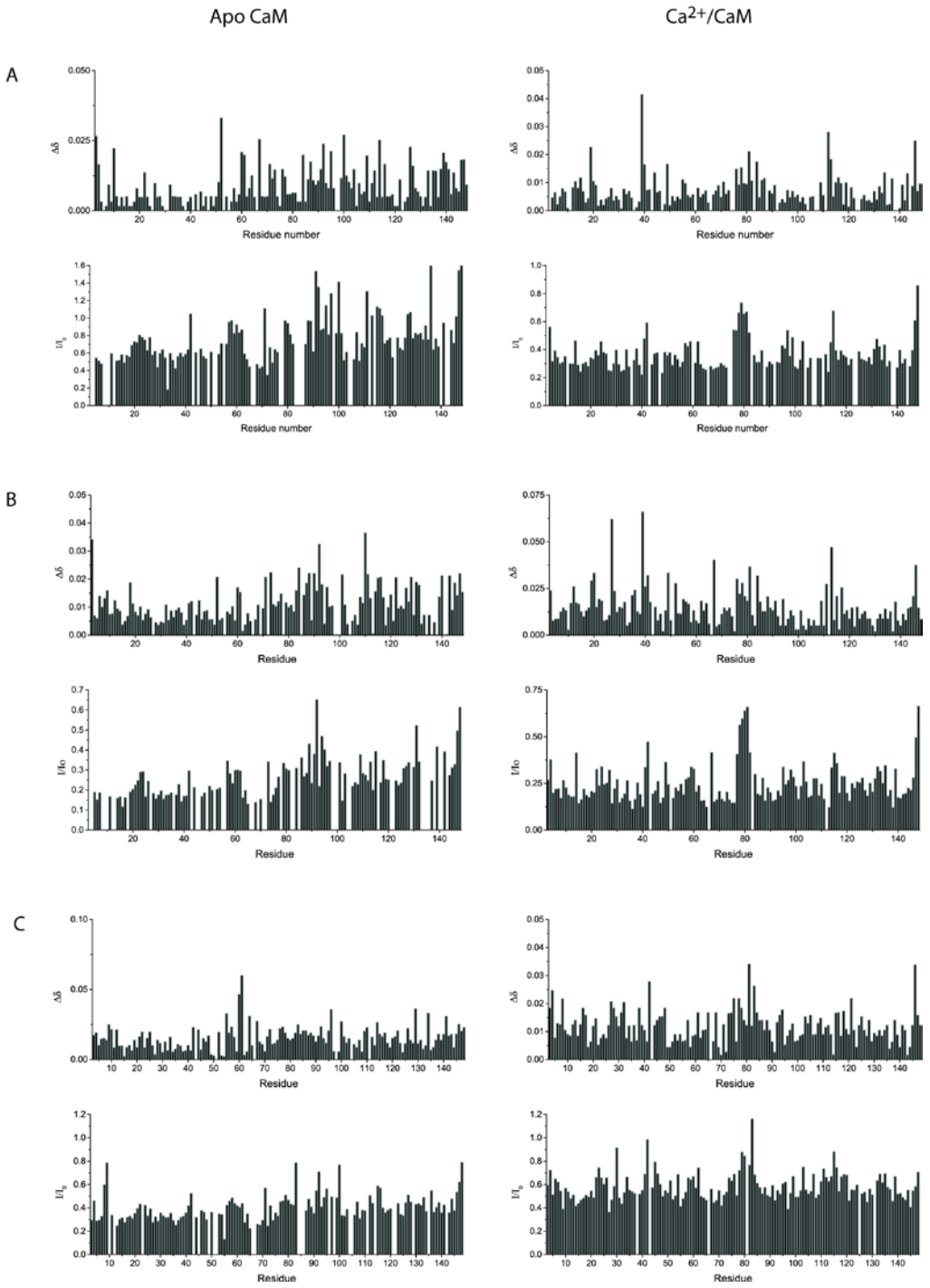

Figure S1: NMR analysis of CaM under conditions of volume exclusion. ^{1}H-heteronuclear single quantum coherence (HSQC) experiments were carried out for ^{15}N-labeled apo CaM (left column) and Ca^{2+}/CaM (right column) in the presence and absence of 20% ficoll-70 (A), dextran-10 (B), and sucrose (C). Values for chemical shifts and peak intensities were determined and compared with the condition of no added polymer or sucrose to determine changes in chemical shifts and changes in peak intensities.

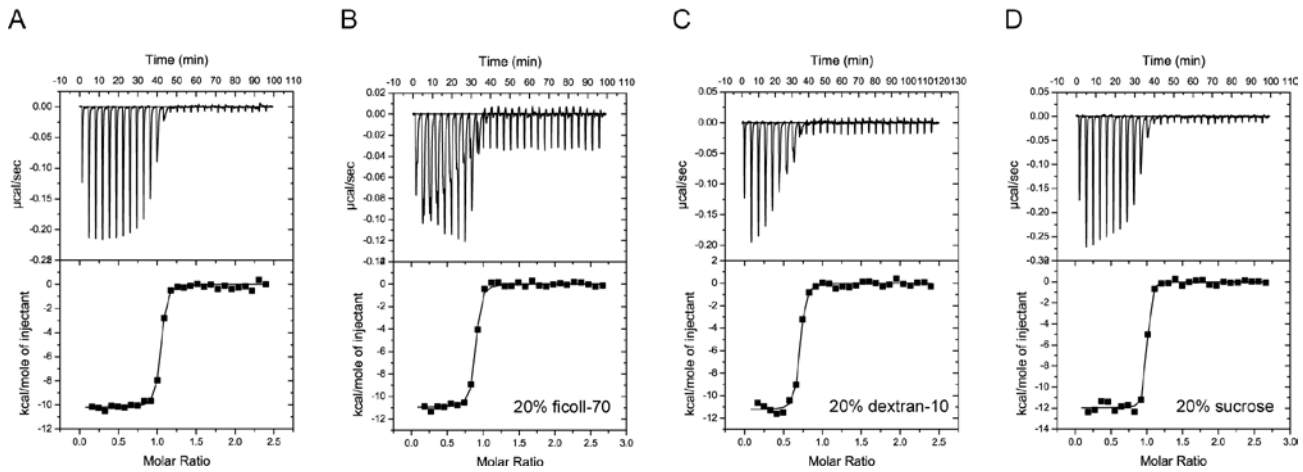

Figure S2: CaM-CaMKI peptide binding affinities. Representative ITC experiments of CaMKI peptide titrated into CaM in the absence (A) or presence of 20% ficoll-70 (B), dextran-10 (C), or sucrose (D). Upper panels show raw thermogram data plotted as a function of experimental time and lower panels show integrated peak intensities as a function of [CaMKI peptide]/[CaM] molar ratio. Data have been fit to single site binding models to determine K_d values (fitting parameters delineated in Table S2).

Experimental Condition	K_A	*n*	$K_d = K_A^n$ (μM)
None	1. 56 ±0.04	1.59 ±0.06	2.2
20% ficoll-70	1.83 ±0.08	1.60 ±0.02	2.6
20% dextran-10	1.85 ±0.06	1.61 ±0.09	2.6
20% sucrose	2.19 ±0.06	1.69 ±0.003	3.8

Table S1. Fitting parameters and dissociation constants for Ca^{2+} affinity for CaM (n=2). The macroscopic association constant, K_A, and the Hill coefficient, *n*, were determined by monitoring Tyr fluorescence during titration with Ca^{2+} and fitting the data to the Hill equation as described in the Materials and methods. Average values from two independent experiments are reported and errors were determined for each condition. A macroscopic dissociation constant for the CaM C-lobe, K_d was calculated from the relationship $K_d = K_A^n$.

Experimental Condition	N	K_a (M^{-1})	ΔH (cal/mol)	ΔS (cal/mol/deg)
None	0.99 ±0.02	1.61×10^{8} ±4.8×10^{7}	-9812 ±368	4.54 ±0.63
20% ficoll-70	1.01 ±0.15	1.40×10^{8} ±1.8×10^{7} *	-10745 ±205	1.22 ±0.44
20% dextran-10	0.81 ±0.11	9.75×10^{7} ±1.7×10^{7} *	-10375 ±866	8.00 ±8.30
20% sucrose	1.05 ±0.09	3.24×10^{8} ±1.4×10^{8} *	-12805 ±845	4.21 ±3.73

Table S2. Summary of ITC data for CaM +CaMKI peptide affinity measurements. For ITC measurements values of N (stoichiometry of binding), K_a, ΔH, and ΔS were obtained by fitting the heat signature of a series of injections of CaMKI peptide into solutions of CaM with a single site binding model in the Microcal software. All parameters are reported as averages (n=2) with standard deviations. Asterisk (*) denotes that the standard deviation for each volume exclusion experiment overlaps with the range determined in the absence of reagent; signifying affinities are not statistically different in the absence of polymer or sucrose.