Supporting Materials for "Retention of Conformational Entropy upon Calmodulin Binding to Target Peptides is Driven by Transient Salt Bridges" by Dayle M.A. Smith, T.P. Straatsma and Thomas C. Squier

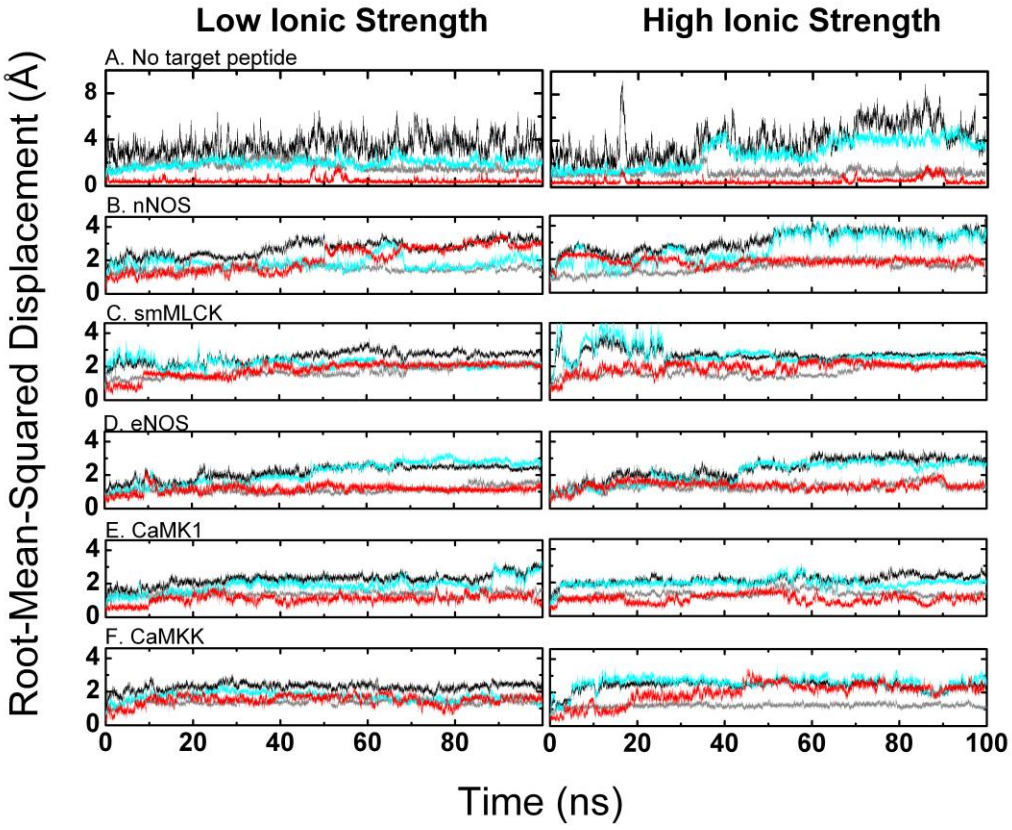


Figure S1. Time-dependent changes in root-mean squared displacement (RMSD) at low- (left panels) and high- (right panels) ionic strength of CaM C_α atoms in full length CaM (black lines), C-domain (residues 83-148) (grey lines), N-domain (residues 1-72)(cyan lines), and central linker (residues 73-82) (red lines) in comparison to initial equilibrated calcium-activated CaM (A) or complexes between CaM and target peptides derived from neuronal nitric oxide synthase (nNOS; 2o60.pdb) (B), epithelial nitric oxide synthease (eNOS; 1niw.pdb) (C), smooth myosin light chain kinase (smMLCK, 1qtx.pdb) (D), CaM-dependent protein kinase I (CaMK1; 1mxe.pdb)(E), and CaM-dependent protein kinase kinase (CaMKK; 1ckk.pdb)(F). .

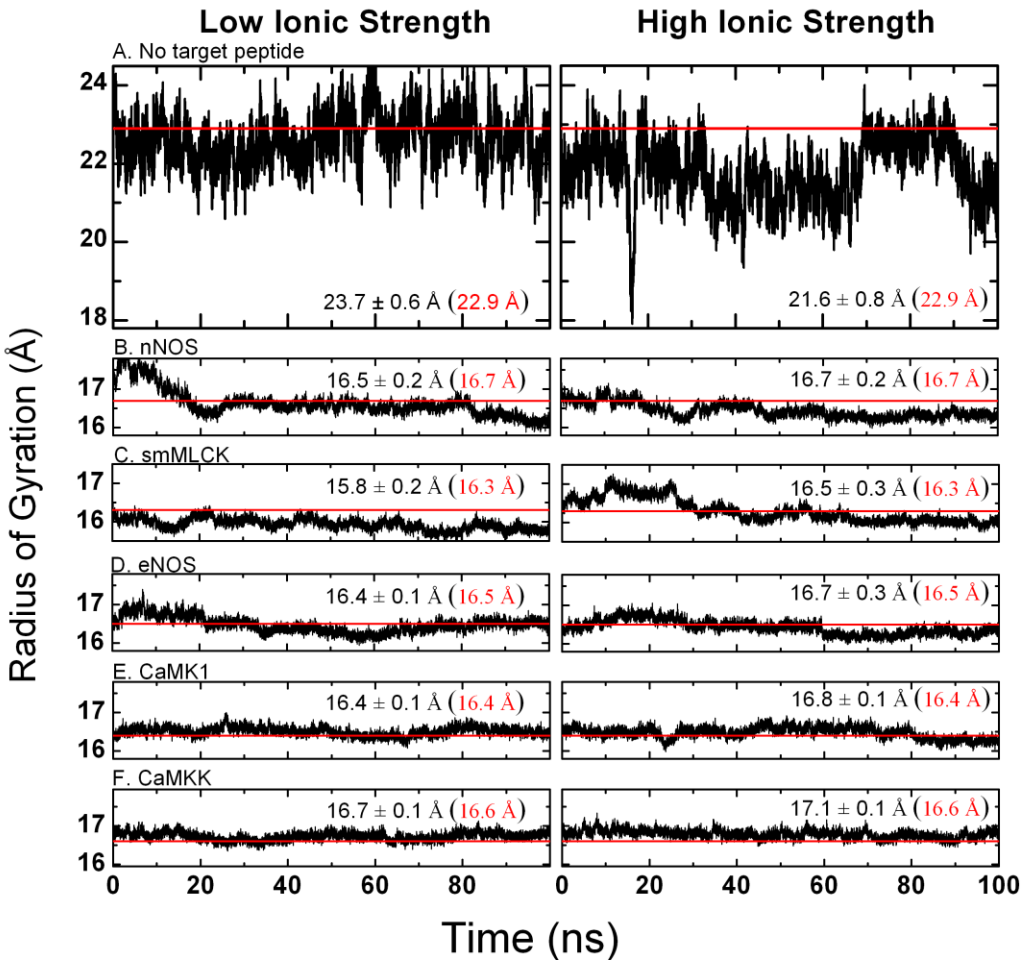


Figure S2. Time-dependent changes in radius of gyration (Rg) for CaM backbone atoms at low- (left panels) and high- (right panels) ionic strength for full length CaM calcium-activated CaM (1x02.pdb) (A) and following association with target peptides derived from neuronal nitric oxide synthase (nNOS; 2o60.pdb) (B), epithelial nitric oxide synthease (eNOS; 1niw.pdb) (C), smooth myosin light chain kinase (smMLCK, 1qtx.pdb) (D), CaM-dependent protein kinase I (CaMK1; 1mxe.pdb)(E), and CaM-dependent protein kinase kinase (CaMKK; 1ckk.pdb)(F). Average radius of gyration from simulations (black numbers); Rg calculated from high-resolution structures (red numbers).

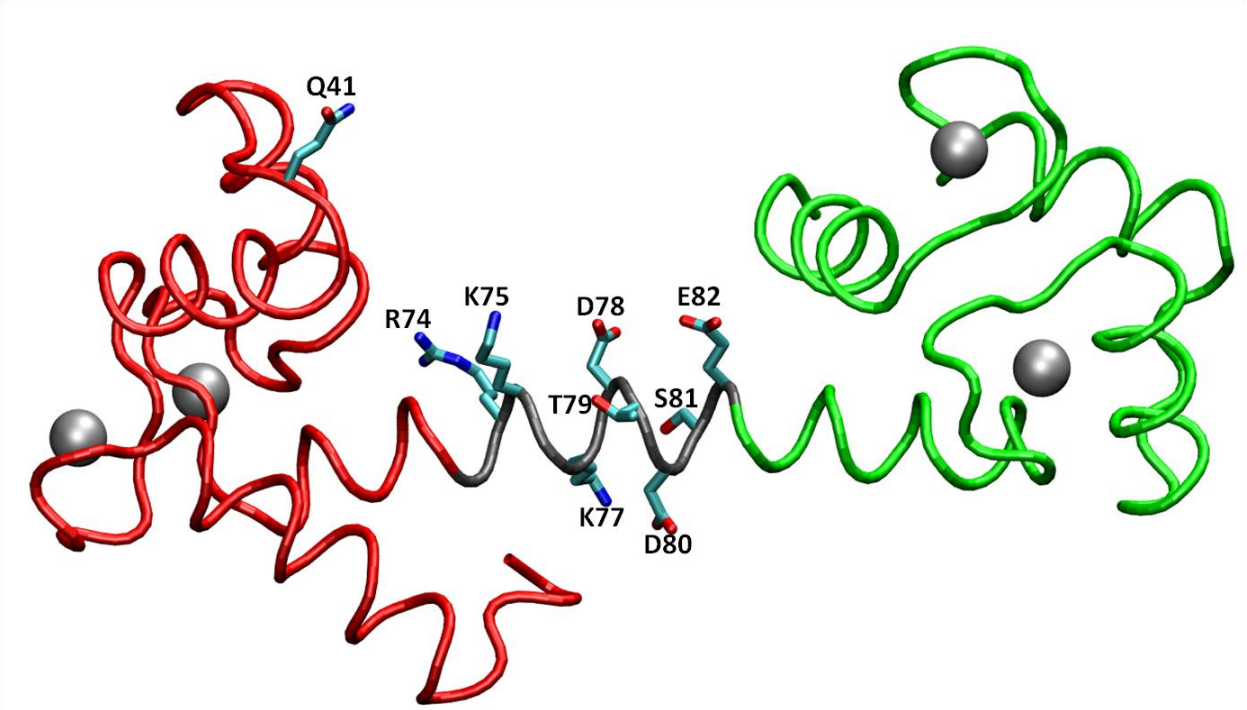


Figure S3. Conformationally-sensitive CaM sidechains referred to in Figure 1 (shown as sticks). Red: CaM N-domain; Green: CaM C-domain; Grey: CaM central linker.

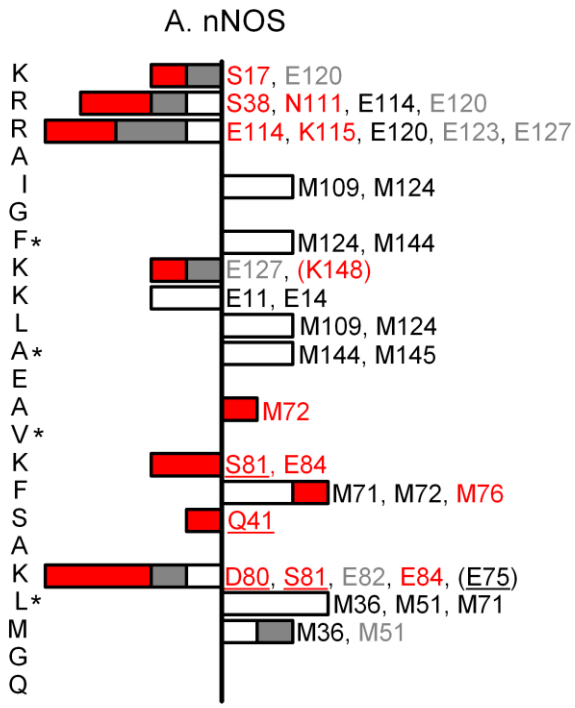


Figure S4: *Ionic Strength Dependence of Contact Side-Chain Interactions*. Specific contact interactions within 4 Å between side-chains in target peptides (sequence is indicated on left) and CaM. Polar (left direction) or hydrophobic Met (right direction) contact interactions between side-chains in CaM and indicated target peptide that are independent of ionic strength (open bars) or present only at low- (gray bars) or high- (red bars) ionic strengths (more red or grey bars in the left direction correspond to a greater number of ionic strength dependent polar CaM-target sidechain interactions). Data plotted from Table S2. Residues indicated with parentheses are electrostatically repulsive CaM-target sidechain interactions. Underlined residues are those described in the article text. Asterisks refer to each target's binding motifs from the literature (eNOS and nNOS have a 1-5-8-14 CaM binding motif, smMLCK has a 1-8-14 motif, CaMKK has a 1-16 motif, and CaMK1 is 1-5-10; see article text for references).

Figure S4, cont'd.

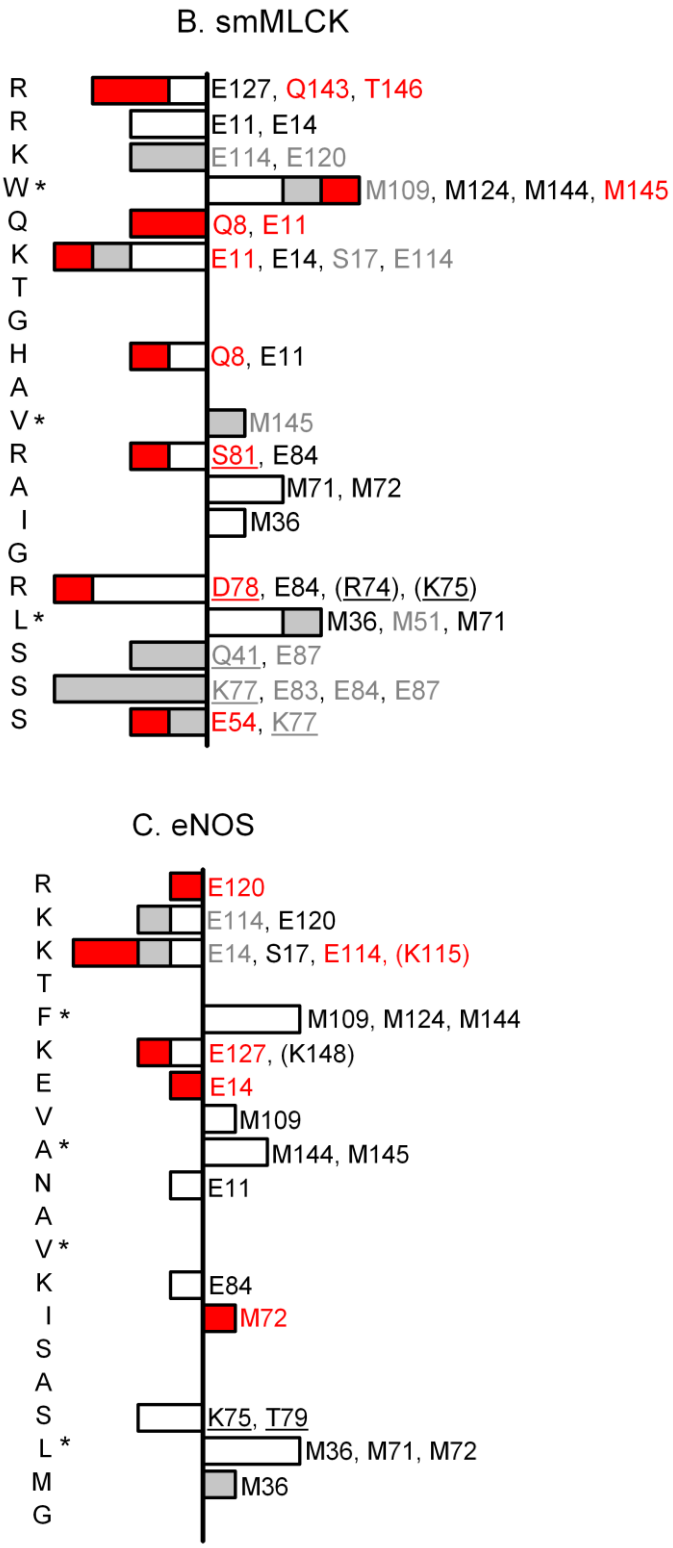
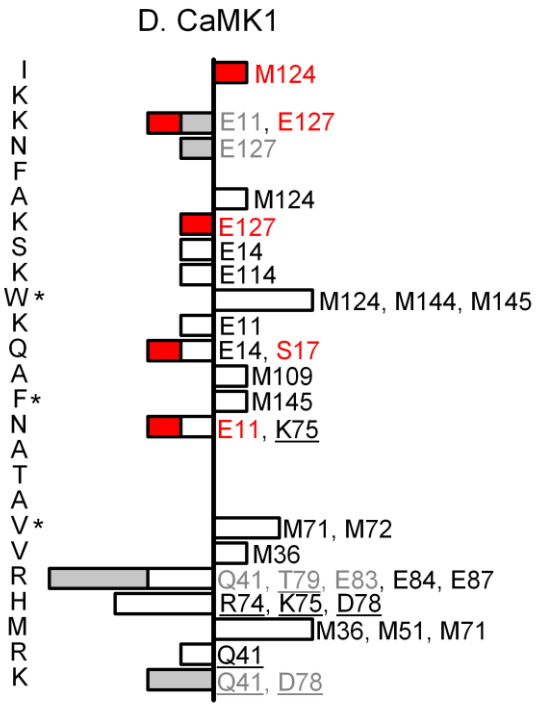
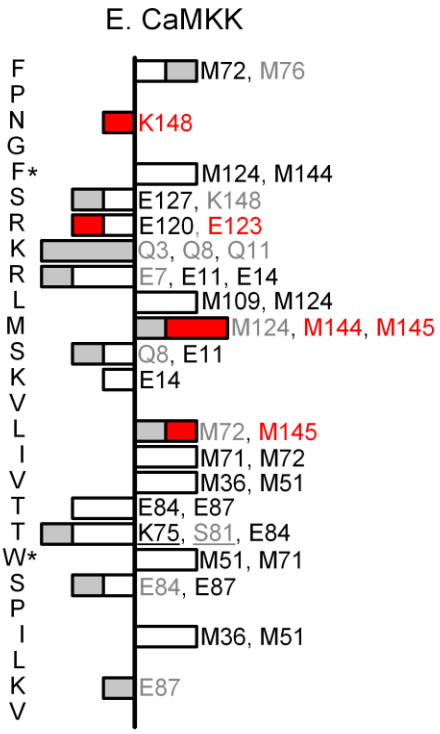


Figure S4, cont'd.





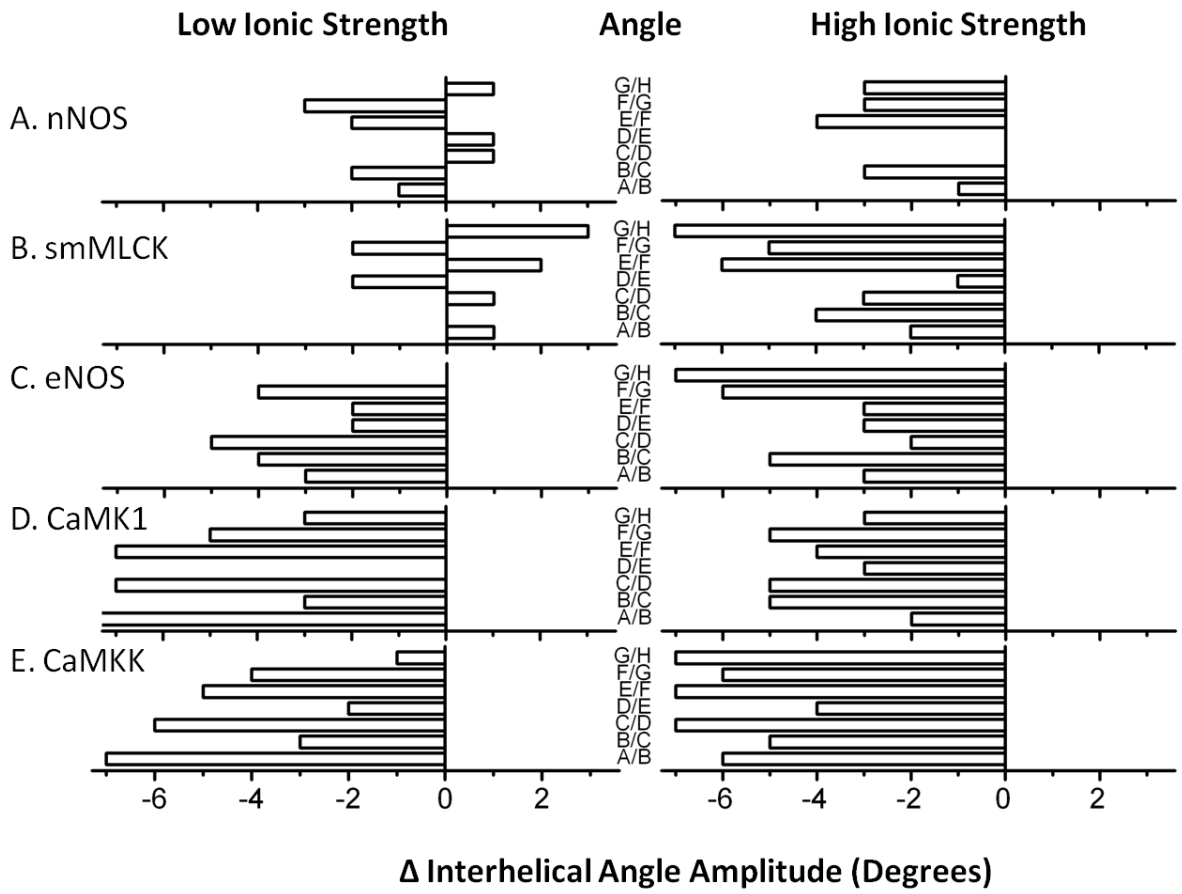


Figure S5: Amplitude changes upon target binding in the inter-helical bending for CaM in complex with target peptides for indicated helices. Data taken from Table S5.

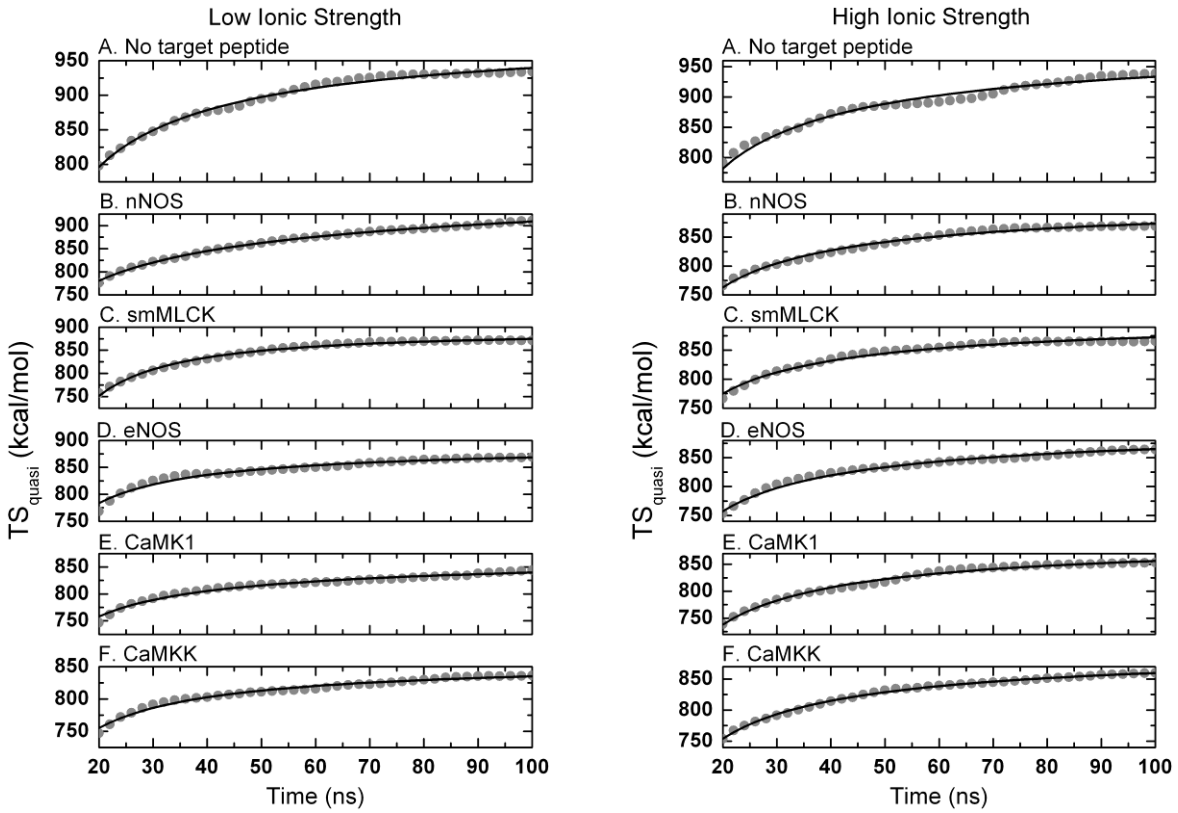


Figure S6: Extrapolation of absolute quasiharmonic entropies calculated from CaM backbone atom fluctuations (kcal/mol) at infinite simulation time (ns) from fitting to $TS(t) = TS_{\infty} - At^{-n}$. Grey circles are quasiharmonic entropies at 300 K calculated for CaM backbone atoms from 10 to *t* ns in 2 ns increments. Black lines are the fitted curves. Panel A: unbound CaM; Panels B-F: CaM bound to target peptides. See Table S7 for results from the least-squares fit.

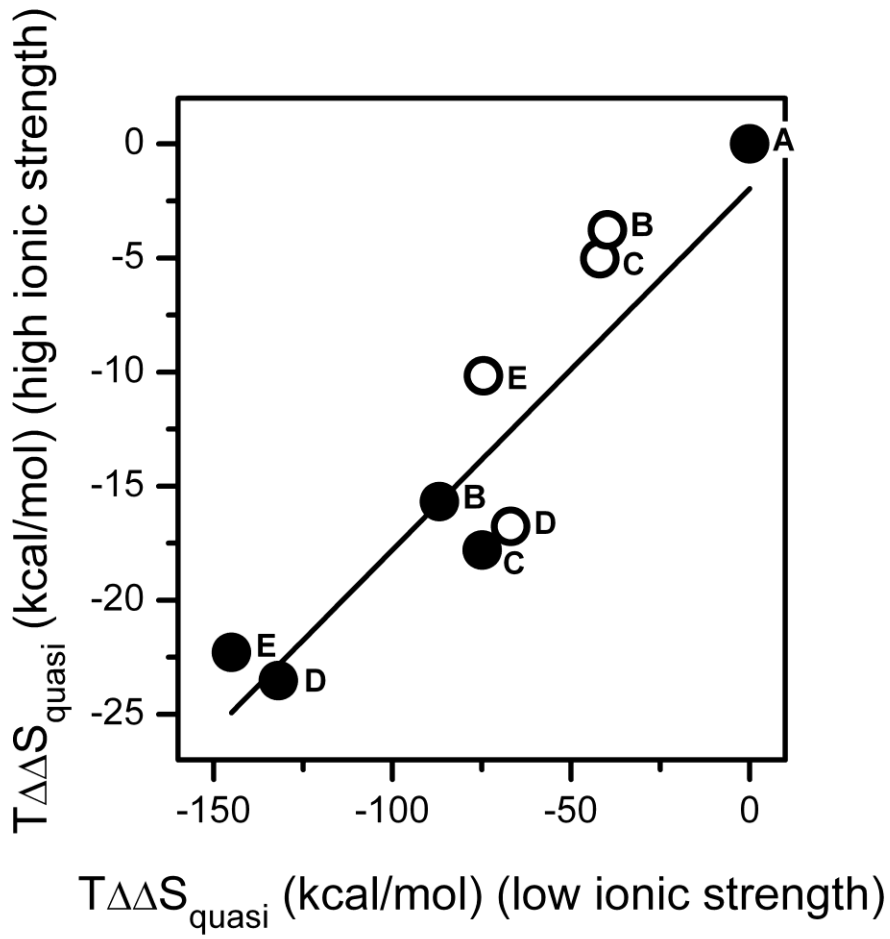


Figure S7: *Ionic Strength Dependent Changes in Calculated Quasiharmonic Conformational Entropy are Proportional for Backbone and Sidechain Motions*. Target-dependent differences in quasiharmonic conformational entropy ($T\Delta\Delta S_{conf}$, relative to nNOS-CaM) calculated for backbone atoms (open circles) or all CaM heavy atoms (filled circles) between complexes of calcium-activated CaM bound to different target peptides at low and high ionic strengths. Points are labeled according to peptide targets: (A) neuronal nitric oxide synthase (nNOS; 2o60.pdb); (B), smooth myosin light chain kinase (smMLCK, 1qtx.pdb); (C), epithelial nitric oxide synthease (eNOS; 1niw.pdb); (D), CaM-dependent protein kinase I (CaMK1; 1mxe.pdb); (E), and CaM-dependent protein kinase kinase (CaMKK; 1ckk.pdb)(F). Line represents nonlinear least squares fit to the data, where $R^2 = 0.87$ and slope = 0.17.

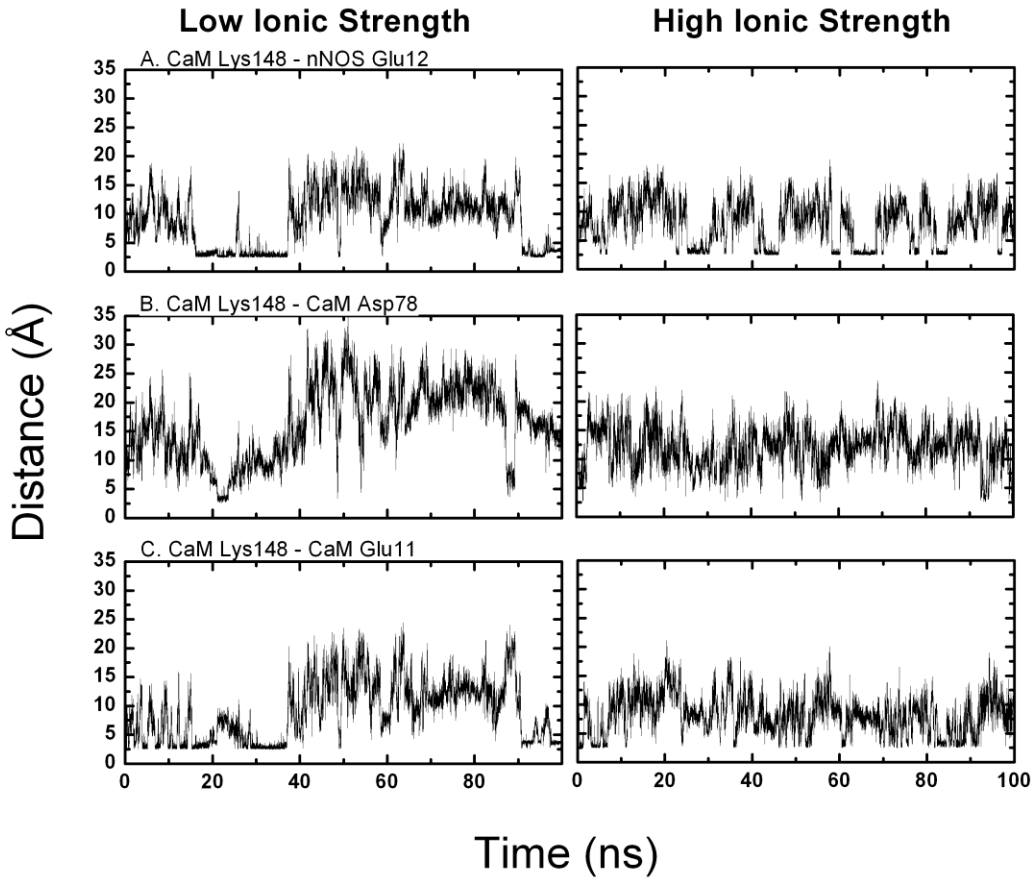


Figure S8. Distances between Lys148 at the C-terminus of CaM with Glu12 in the target peptide (A), Asp78 in the central linker of CaM (B), or Glu11 in the N-domain of CaM (C) at low (left panels) and high (right panels) ionic strength.

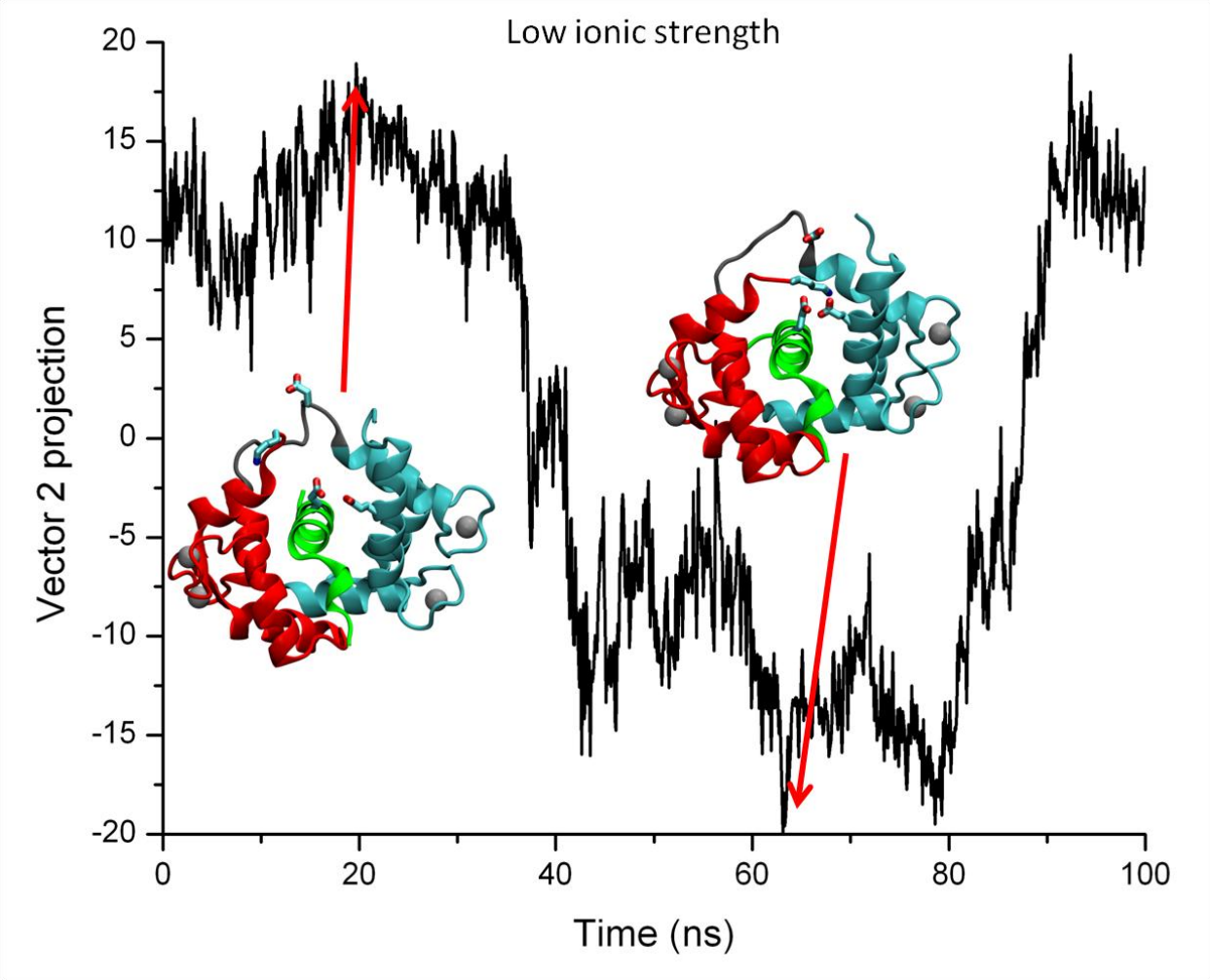


Figure S9: Eigenvector projections for first nNOS-CaM eigenvector that includes transient salt bridge movements. Covariance analysis was done on full CaM and bound nNOS heavy atoms after superimposing CaM backbone atoms. nNOS-CaM structures at the eigenvector extremes are shown in ribbons format. Red: CaM N-domain; cyan: CaM C-domain; grey: CaM linker; Green: nNOS peptide. Calcium ions are shown as silver Van der Waals spheres.

Figure S9, cont'd.

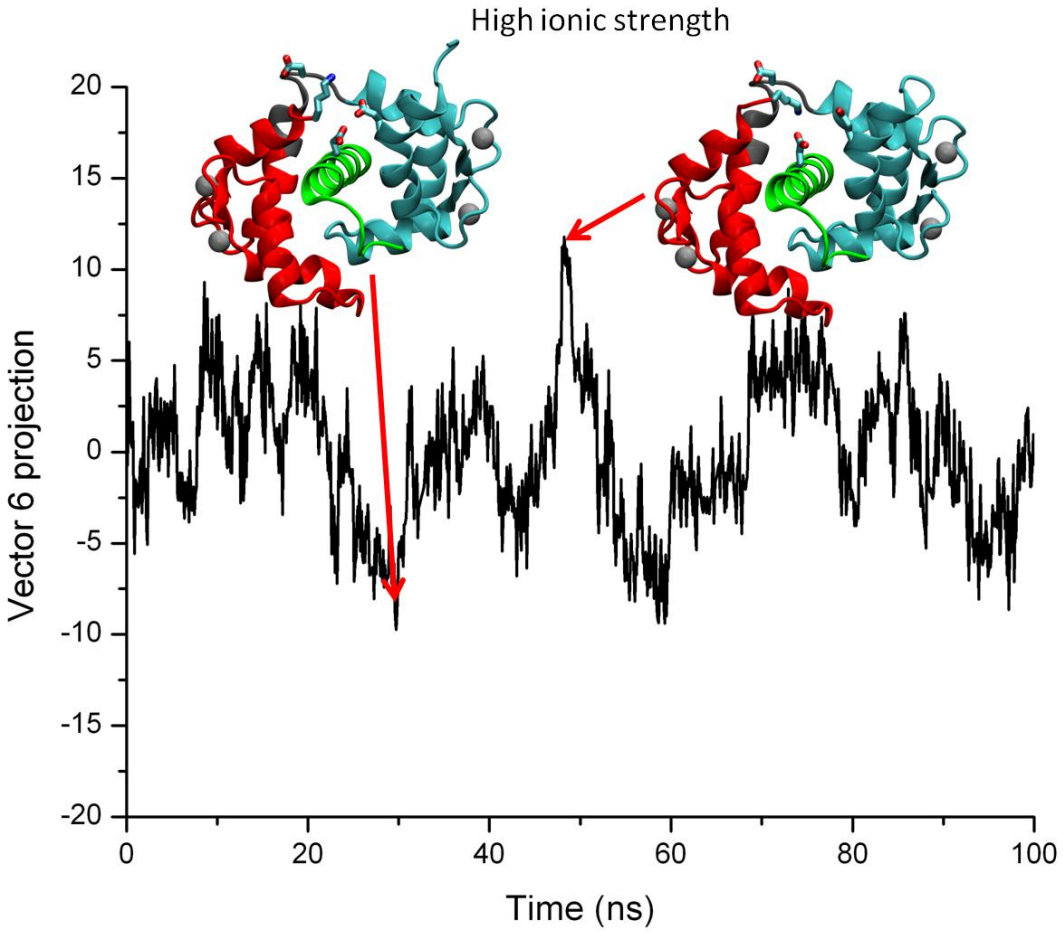


Table S1: Solvated protein system specifications.

				low ionic strength set		high ionic strength set		
System	**PDB ID**	**solute atoms**	**initial cubic box side (Å)**	**water molecules**	**Na+ ions**	**water molecules**	**K+ ions**	**Cl- ions**
CaMKK-CaM	1CKK	2721	81.54	16,890	11	16,810	51	40
CaMK1-CaM	1MXE	2712	82.08	17,346	8	17,262	50	42
eNOS-CaM	1NIW	2594	83.27	18,112	12	18,030	53	41
smMLCK-CaM	1QTX	2606	81.19	16,688	10	16,610	49	39
nNOS-CaM	2O60	2658	81.44	16,908	10	16,830	49	39
CaM	1X02	2266	97.90	35,192	16	35,046	89	73

Table S2: Selected CaM-target contacts less than 4 Å calculated from mean closest distance analysis. The majority of complexes involve the same antiparallel binding mechanism in which the N-domain of CaM binds toward the C-terminus of the peptide; in the case of CaMKK binding involves a parallel binding mechanism where the N-domain of CaM binds toward the N-terminus of the peptide. Residues indicated with parentheses are electrostatically repulsive CaM-target sidechain interactions. Highlighted residues refer to each target's binding motifs from the literature. eNOS and nNOS have a 1-5-8-14 CaM binding motif, smMLCK has a 1-8-14 motif, CaMKK has a 1-16 motif, and CaMK1 is 1-5-10. See text for references.

Table S2, cont'd.

Low ionic strength											
CaMKK	CaM		CaMK1	CaM		eNOS	CaM		smMLCK	CaM		nNOS	CaM
			I1										
			K2										
			K3	E11									
			N4	E127								K1	E120
			F5									R2	E114 E120
F26	M72 M76		A6	M124		R1						R3	E120 E123 E127
P25			K7			K2	E114 E120		R1	E127		A4	
N24			S8	E14		K3	E14 S17		R2	E11 E14		I5	M109 M124
G23			K9	E114		T4			K3	E114 E120		G6	
F22	M124 M144		W10	M124 M144 M145		F5	M109 M124 M144		W4	M109 M124 M144		F7	M124 M144
S21	E127 K148		K11	E11		K6	(K148)		Q5			K8	E127
R20	E120		Q12	E14		E7			K6	E14 S17 E114		K9	E11 E14
K19	Q3 Q8 E11		A13	M109		V8	M109		T7			L10	M109 M124
R18	E7 E11 E14		F14	M145		A9	M144 M145		G8			A11	M144 M145
L17	M109 M124		N15	K75		N10	E11		H9	E11		E12	
M16	M124		A16			A11			A10			A13	
S15	Q8 E11		T17			V12			V11	M145		V14	
K14	E14		A18			K13	E84		R12	E84		K15	
V13			V19	M71 M72		I14			A13	M71 M72		F16	M71 M72
L12	M72		V20	M36		S15			I14	M36		S17	
I11	M71 M72		R21	Q41 T79 E83 E84 E87		A16			G15			A18	
V10	M36 M51		H22	R74 K75 D78		S17	K75 T79		R16	E84 (R74) (K77)		K19	E82 (K75)
T9	E84 E87		M23	M36 M51 M71		L18	M36 M71 M72		L17	M36 M51 M71		L20	M36 M51 M71
T8	K75 S81 E84		R24	Q41		M19	M36		S18	Q41 E87		M21	M36 M51
W7	M51 M71		K25	Q41 D78		G20			S19	K77 E83 E84 E87		G22	
S6	E84 E87								S20	K77		Q23	
P5													
I4	M36 M51												
L3													
K2	E87												
V1													

Table S2, cont'd.

High ionic strength											
CaMKK	CaM		CaMK1	CaM		eNOS	CaM		smMLCK	CaM		nNOS	CaM
			I1	M124									
			K2										
			K3	E127									
			N4									K1	S17
			F5									R2	S38 N111 E114
F26	M72		A6	M124		R1	E120					R3	E114 K115 E120
P25			K7	E127		K2	E120		R1	E127 Q143 T146		A4	
N24	K148		S8	E14		K3	S17 E114		R2	E11 E14		I5	M109 M124
G23			K9	E114		T4			K3			G6	
F22	M124 M144		W10	M124 M144 M145		F5	M109 M124 M144		W4	M124 M144 M145		F7	M124 M144
S21	E127		K11	E11		K6	E127 (K148)		Q5	Q8 E11		K8	(K148)
R20	E120 E123		Q12	E14 S17		E7	(E14)		K6	E11 E14 E114		K9	E11 E14
K19			A13	M109		V8	M109		T7			L10	M109 M124
R18	E11 E14		F14	M145		A9	M144 M145		G8			A11	M144 M145
L17	M109 M124		N15	E11 K75		N10	E11		H9	Q8 E11		E12	
M16	M144 M145		A16			A11			A10			A13	M72
S15	E11		T17			V12			V11			V14	
K14	E14		A18			K13	E84		R12	S81 E84		K15	S81 E84
V13			V19	M71 M72		I14	M72		A13	M71 M72		F16	M71 M72 M76
L12	M145		V20	M36		S15			I14	M36		S17	Q41
I11	M71 M72		R21	E84 E87		A16			G15			A18	
V10	M36 M51		H22	R74 K75 D78		S17	K75 T79		R16	(R74) (K75) D78 E84		K19	D80 S81 E84 (K75)
T9	E84 E87		M23	M36 M51 M71		L18	M36 M71 M72		L17	M36 M71		L20	M36 M51 M71
T8	K75 E84		R24	Q41		M19			S18			M21	M36
W7	M51 M71		K25			G20			S19			G22	
S6	E87								S20	E54		Q23	
P5													
I4	M36 M51												
L3													
K2													
V1													

Table S3: Interactions between target sidechains and conformationally-sensitive CaM sidechains calculated from < 4.0 Å minimum distance analysis described in Figure 1.

High ionic strength simulation set							
target		**CaM res**			**target res**		**Average minimum distance (Å)**
CaMKK		LYS	75		THR	8	2.70±0.90
CaMK1		GLN	41		ARG	24	3.00±0.60
CaMK1		ARG	74		HIS	22	2.20±0.50
CaMK1		LYS	75		HIS	22	2.50±0.20
CaMK1		ASP	78		HIS	22	2.30±0.60
eNOS		LYS	75		SER	17	2.70±0.50
eNOS		THR	79		SER	17	2.80±0.80
smMLCK		ARG	74		ARG	16	4.00±0.50
smMLCK		LYS	75		ARG	16	3.60±0.50
smMLCK		ASP	78		ARG	16	3.00±0.60
smMLCK		SER	81		ARG	12	2.90±1.00
nNOS		GLN	41		SER	17	2.60±0.30
nNOS		LYS	75		LYS	19	2.90±0.70
nNOS		ASP	80		LYS	19	3.80±1.20
nNOS		SER	81		LYS	15	2.50±0.40

Table S3, cont'd.

Low ionic strength simulation set						
target	**CaM res**			**target res**		**Average minimum distance (Å)**
CaMKK	LYS	75		THR	8	2.30±0.20
CaMK1	GLN	41		ARG	24	2.50±0.30
CaMK1	ARG	74		HIS	22	2.10±0.30
CaMK1	LYS	75		HIS	22	2.50±0.20
CaMK1	ASP	78		HIS	22	2.10±0.40
CaMK1	ASP	78		LYS	25	2.80±1.60
CaMK1	THR	79		ARG	21	2.90±0.70
eNOS	LYS	75		SER	17	2.40±0.30
eNOS	THR	79		SER	17	2.80±1.00
smMLCK	GLN	41		SER	18	2.10±0.40
smMLCK	ARG	74		ARG	16	2.80±0.50
smMLCK	LYS	77		ARG	16	2.90±1.20
smMLCK	LYS	77		SER	19	2.70±0.90
smMLCK	LYS	77		SER	20	3.00±1.00
nNOS	LYS	75		LYS	19	2.80±0.60
nNOS	ASP	80		LYS	19	1.90±0.20
nNOS	GLU	82		LYS	19	2.70±1.40

Table S4: Coulomb interaction energy between CaM and target sidechains in smMLCK and nNOS.

High ionic strength simulation set							
target		**CaM res**			**Target res**		**Coulomb interaction (kcal/mol)**
smMLCK		ARG	74		ARG	16	0.56±0.28
smMLCK		LYS	75		ARG	16	1.47±0.98
nNOS		LYS	75		LYS	19	2.20±1.00
Low ionic strength simulation set							
target		**CaM res**			**Target res**		**Coulomb interaction (kcal/mol)**
smMLCK		ARG	74		ARG	16	2.41±1.36
smMLCK		LYS	77		ARG	16	1.67±0.94
nNOS		LYS	75		LYS	19	2.03±2.07

Table S5: Inter-helical angles for unbound, Ca^{2+}-saturated CaM and CaM bound to targets indicated (averages, standard deviations and amplitudes) from simulations using low and high ionic strength conditions.

			Low Ionic Strength				High Ionic Strength		
	angle		avg	stdev	amplitude		avg	stdev	amplitude
CaMKK	AB		98	3	8		97	3	9
CaMK1			79	2	7		90	7	13
smMLCK			96	6	16		87	6	13
eNOS			90	5	12		89	8	12
nNOS			86	8	14		86	7	14
no target			85	7	15		89	7	15
CaMKK	BC		92	4	11		87	7	12
CaMK1			86	4	11		94	7	12
smMLCK			87	8	14		97	6	13
eNOS			92	5	10		94	5	12
nNOS			85	6	12		91	7	14
no target			92	7	14		92	8	17
CaMKK	CD		83	4	9		85	5	9
CaMK1			100	2	8		88	6	11
smMLCK			85	8	16		88	7	13
eNOS			88	4	10		88	9	14
nNOS			95	8	16		92	6	16
no target			93	8	15		90	7	16
CaMKK	DE		84	4	10		84	5	11
CaMK1			92	5	12		90	7	12
smMLCK			83	5	11		94	6	14
eNOS			87	5	10		92	6	12
nNOS			83	6	13		93	8	15
no target			93	4	12		91	9	15
CaMKK	EF		100	4	9		95	5	10
CaMK1			80	3	7		94	8	13
smMLCK			97	8	16		94	5	11
eNOS			95	5	12		92	10	14
nNOS			91	7	14		87	6	13
no target			97	5	14		93	9	17
CaMKK	FG		89	5	11		92	6	11
CaMK1			90	3	10		84	6	12
smMLCK			95	7	13		81	4	12
eNOS			86	6	11		85	6	11
nNOS			91	6	12		87	8	14
no target			94	8	15		91	9	17
CaMKK	GH		80	5	12		84	6	11
CaMK1			99	4	10		89	9	15
smMLCK			82	7	16		92	4	11
eNOS			87	6	13		92	6	11
nNOS			88	8	14		94	7	15
no target			83	7	13		86	9	18

Table S6: Experimental conformational entropies (Frederick, K. K., Marlow, M. S., Valentine, K. G., and Wand, A. J. (2007), *Nature 448*, 325-329) and calculated quasiharmonic entropies. TΔS is relative to unbound CaM; TΔΔS is relative to nNOS-CaM.

	Experimental conformational entropies (kcal/mol)			
	target peptide	**TΔS**	**TΔΔS**	
	nNOS	-3.80		
	smMLCK	-13.12	-9.32	
	eNOS	-11.02	-7.22	
	CaMK1	-15.77	-11.97	
	CaMKK	-15.18	-11.38	
	Quasiharmonic entropies (kcal/mol) for CaM backbone atoms			
MD conditions	**target peptide**	**TS**	**TΔS**	**TΔΔS**
low ionic strength	nNOS	910.86	-23.34	
	smMLCK	871.04	-63.15	-39.82
	eNOS	868.96	-65.23	-41.90
	CaMK1	843.95	-90.24	-66.90
	CaMKK	836.36	-97.84	-74.50
	no target peptide	934.19		
high ionic strength	nNOS	869.41	-68.93	
	smMLCK	865.60	-72.75	-3.81
	eNOS	864.36	-73.98	-5.05
	CaMK1	852.89	-85.45	-16.52
	CaMKK	859.65	-78.69	-9.76
	no target peptide	938.35		
	Quasiharmonic entropies (kcal/mol) for all CaM heavy atoms			
MD conditions	**target peptide**	**TS**	**TΔS**	**TΔΔS**
low ionic strength	nNOS	2274.08	-72.07	
	smMLCK	2187.25	-158.91	-86.84
	eNOS	2199.21	-146.95	-74.88
	CaMK1	2142.21	-203.95	-131.87
	CaMKK	2129.06	-217.10	-145.02
	no target peptide	2346.16		
high ionic strength	nNOS	2206.48	-139.17	
	smMLCK	2190.80	-154.85	-15.68
	eNOS	2188.68	-156.97	-17.80
	CaMK1	2182.96	-162.69	-23.53
	CaMKK	2184.19	-161.46	-22.29
	no target peptide	2345.65		

Table S7: Extrapolation of absolute quasiharmonic entropies calculated from CaM backbone atom fluctuations (kcal/mol) at infinite simulation time (ns) from fitting to $TS(t) = TS_\infty - At^{-n}$. Quasiharmonic entropies were calculated at 300 K from 10 to *t* ns in 2 ns increments. TS_∞, A and n are fitting parameters; R^2 is the goodness-of-fit; TS_{100} is the quasiharmonic entropy calculated from 10-100 ns (Table S6). Fitted curves are plotted in Figure S6.

High Ionic Strength					
CaM target	**TS_∞**	**A**	**n**	**R^2**	**TS_{100}/TS_∞**
no target peptide	1001.89	1934.15	0.73	0.98	0.94
nNOS	919.60	1522.17	0.76	0.99	0.95
smMLCK	913.41	1344.98	0.76	0.98	0.95
eNOS	913.10	1389.74	0.73	0.99	0.95
CaMK1	904.12	1680.24	0.77	0.99	0.94
CaMKK	905.82	1398.63	0.74	1.00	0.95
Low Ionic Strength					
CaM target	**TS_∞**	**A**	**n**	**R^2**	**TS_{100}/TS_∞**
no target peptide	1004.07	1823.27	0.73	0.99	0.93
nNOS	1035.51	928.99	0.43	1.00	0.88
smMLCK	893.32	6276.52	1.27	0.99	0.98
eNOS	891.51	1901.56	0.96	0.97	0.97
CaMK1	874.61	1085.41	0.75	0.98	0.96
CaMKK	862.93	1341.83	0.84	0.98	0.97