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; 2060.pdb) (B), epithelial nitric oxide synthese (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 (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.

B. smMLCK



C. eNOS



Figure S4, cont'd.



E. CaMKK





Δ Interhelical Angle Amplitude (Degrees)

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.



				low ionic s	trength 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	2060	2658	81.44	16,908	10	16,830	49	39			
CaM	1X02	2266	97.90	35,192	16	35,046	89	73			

 Table S1: Solvated protein system specifications.

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.

٧1	K2	5	14	P5	95	W۷	T8	T9	V10	111	L12	V13	K14	S15	M16	L17	R18	K19	R20	S21	F22	G23	N24	P25	F26						СаМКК	
	E87		M36 M51		E84 E87	M51 M71	<u>K75</u> S81 E84	E84 E87	M36 M51	M71 M72	M72		E14	Q8 E11	M124	M109 M124	E7 E11 E14	Q3 Q8 E11	E120	E127 K148	M124 M144				M72 M76						CaM	
						K25	R24	M23	H22	R21	V20	V19	A18	T17	A16	N15	F14	A13	Q12	K11	W10	К9	8S	K7	A6	F5	N4	K3	K2	11	CaMK	
						<u>Q41 D78</u>	<u>041</u>	M36 M51 M71	<u>R74 K75 D78</u>	<u>Q41 T79</u> E83 E84 E87	M36	M71 M72				<u>K75</u>	M145	M109	E14	E11	M124 M144 M145	E114	E14		M124		E127	E11			1 CaM	
						G20	M19	L18	S17	A16	S15	114	K13	V12	A11	N10	A9	8/	E7	K6	F5	Т4	K3	K2	R1						eNOS	Lov
							M36	M36 M71 M72	<u>K75 T79</u>				E84			E11	M144 M145	M109		(K148)	M109 M124 M144		E14 S17	E114 E120							CaM	v ionic strength
					S20	S19	S18	L17	R16	G15	114	A13	R12	V11	A10	6H	G8	77	K6	Q5	W4	K3	R2	R1							SmMLCK	
					<u>K77</u>	<u>K77</u> E83 E84 E87	<u>041</u> E87	M36 M51 M71	E84 (R74) (K77)		M36	M71 M72	E84	M145		E11			E14 S17 E114		M109 M124 M144	E114 E120	E11 E14	E127							CaM	
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					123	322	121	.20	(19	18	\$17	-16	(15	/14	13	12	11	.10	K9	K8	F7	G6	5	A4	R3	R2	K1				SON	
							M36 M51	M36 M51 M71	<u>E82 (K75)</u>			M71 M72					M144 M145	M109 M124	E11 E14	E127	M124 M144		M109 M124		E120 E123 E127	E114 E120	E120				CaM	

Table S2, cont'd.

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V1	K2	ω	14	P5	<u> 56</u>	W7	T8	T9	V10	111	L12	V13	K14	S15	M16	L17	R18	K19	R20	S21	F22	G23	N24	P25	F26						aMKK	
			M36 M51		E87	M51 M71	<u>K75</u> E84	E84 E87	M36 M51	M71 M72	M145		E14	E11	M144 M145	M109 M124	E11 E14		E120 E123	E127	M124 M144		K148		M72						CaM	
	-																														0	
						K25	R24	M23	H22	R21	V20	V19	A18	T17	A16	N15	F14	A13	Q12	K11	W10	K9	8S	K7	A6	F5	N4	K3	K2	11	aMK1	
							<u>Q41</u>	M36 M51 M71	<u>R74 K75 D78</u>	E84 E87	M36	M71 M72				E11 <u>K75</u>	M145	M109	E14 S17	E11	M124 M144 M145	E114	E14	E127	M124			E127		M124	CaM	
						G20	M19	L18	S17	A16	S15	114	K13	V12	A11	N10	A9	8٨	E7	K6	F5	T4	K3	K2	R1						eNOS	Hig
								M36 M71 M72	<u>K75 T79</u>			M72	E84			E11	M144 M145	M109	(E14)	E127 (K148)	M109 M124 M144		S17 E114	E120	E120						CaM	sh ionic strength
																															s	
					S20	S19	S18	L17	R16	G15	114	A13	R12	V11	A10	H9	G8	77	K6	Q5	W4	κ	R2	R1							mMLCK	
					E54			M36 M71	(R74) (K75) D78 E84		M36	M71 M72	<u>S81</u> E84			Q8 E11			E11 E14 E114	Q8 E11	M124 M144 M145		E11 E14	E127 Q143 T146							CaM	
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					Q23	G22	M21	L20	K19	A18	S17	F16	K15	V14	A13	E12	A11	L10	К9	K8	F7	G6	15	A4	R3	R2	K1				nNOS	
							M36	M36 M51 M71	D80 <u>S81</u> E84 (K75)		<u>041</u>	M71 M72 M76	<u>S81</u> E84		M72		M144 M145	M109 M124	E11 E14	(K148)	M124 M144		M109 M124		E114 K115 E120	S38 N111 E114	S17				CaM	

Table S2, cont'd.

High ionic strength simulation set												
target		CaM	res		target	res	Average minimum distance (Å)					
СаМКК		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: Interactions between target sidechains and conformationally-sensitive CaM sidechainscalculated from < 4.0 Å minimum distance analysis described in Figure 1.

Table S3, cont'd.

Low ionic strength simulation set												
target		CaM	res		target	res	Average minimum distance (Å)					
СаМКК		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 io	nic st	re	ngth sin	nulati	on set						
target	target CaM res				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						

angleangleanglestdevamplitudeangleanglestdevamplitudeCaMKI smMLCK a NOS98389739smMLCK a NOS9951289812no target9051289812no target8571589714no target8571589712CaMKK c GMKI smMLCK8681494712SmMLCK c GMKI smMLCK8641194712CaMKI c GMKI smMLCK9251094512no target9251094512no target9271492814smMLCK c GMKI smMLCK9271492814smMLCK c GMKI smMLCK9381688713smMLCK c GMKI smMLCK c GMKI smMLCK9381590716smMLCK c GMKI c GMKI smMLCK smMLCK9381590712smMLCK c GMKI c GMKI smMLCK9381590712smMLCK c GMKI smMLCK9451194611smMLCK c GMKI smMLCK9451194612smMLCK c GMKI smMLCK9781590 <th></th> <th></th> <th>Low</th> <th>v Ionic Str</th> <th>ength</th> <th>Hig</th> <th>h Ionic Str</th> <th>ength</th>			Low	v Ionic Str	ength	Hig	h Ionic Str	ength
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eNOS nNOS AB 90 5 12 89 8 12 no target 86 8 14 86 7 14 no target 85 7 15 89 7 15 CaMKL CaMKL eNOS nNOS 86 4 11 87 7 12 66 4 11 97 6 13 90 55 10 94 5 12 notarget 87 8 14 97 6 13 notarget 92 7 14 92 8 17 caMKI 85 6 12 91 7 14 sommLock 92 7 14 92 8 17 caMKI 84 9 88 16 88 7 13 sommLock 83 5 11 94 6 14 sommLock 83 5 12<	smMLCK		96	6	16	87	6	13
nNOS no target 86 8 14 86 7 14 no target 85 7 15 89 7 15 CaMKK CaMKK SmNOS nNOS nNOS 92 4 11 87 7 12 SmMLK CaMK CAMK CAMK CAMK CAMK CAMK CAMK CAMK CA	eNOS	AB	90	5	12	89	8	12
no target 85 7 15 89 7 15 CAMKK CAMKI smMLCK eNOS nNOs nNos nNos nNOs nNOS nNOS BC 92 4 11 87 7 12 6 86 4 11 94 7 12 caMKI eNOS 85 6 12 91 7 14 no target 92 7 14 92 8 107 14 caMKK 83 4 9 85 5 9 100 2 8 88 6 11 caMKK 83 4 9 85 5 9 14 92 3 14 100 2 8 88 6 11 14 10 14 10 14 10 14 10 14 10 14 10 14 10 14 10 14 10 14 11 11 11 11 11 11 11	nNOS		86	8	14	86	7	14
CaMKK CaMK1 CaMK1 CaMK1 eNOS nN03 BC Image: second	no target		85	7	15	89	7	15
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SmMLCK eNOS nNOS CD 85 8 16 88 7 13 nNOS no target 95 8 16 92 6 16 no target 93 8 15 90 7 16 CaMKK CaMK1 SmMLCK eNOS nNOS 92 5 12 90 7 12 SmMLCK eNOS nNOS 92 5 12 90 7 12 SmMLCK eNOS nNOS 83 6 13 93 8 15 nNOS 83 6 13 93 8 15 nNOS 83 6 13 93 8 15 no target 97 8 16 94 5 11 SmMLCK eNOS nNOS 97 8 16 94 5 11 GAMKK caMKI smMLCK eNOS nNOS 91 7 14 87 6 13 91 7 13 81 4 12 14	CaMK1		100	2	8	88	6	11
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CaMKK CaMKK R I	no target		93	8	15	90	7	16
CaMKK CaMK1 DE 84 4 10 84 5 11 smMLCK eNOS nNOS DE B3 5 12 90 7 12 smMLCK eNOS B7 5 10 92 6 12 nNOS 83 6 13 93 8 15 no target 93 4 12 91 9 15 CaMKK smMLCK station 93 4 12 91 9 15 CaMKK smMLCK station 93 4 12 91 9 15 CaMKK station 93 4 12 91 9 15 CaMKK station 97 5 12 92 10 14 nNOS 91 7 14 87 6 13 no target FG 95 7 13 81 4 12 smMLCK g								
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SmMLCK eNOS nNOS no target DE 83 5 11 94 6 14 eNOS no target 87 5 10 92 6 12 no target 93 4 12 91 9 15 CaMKK CaMK1 SmMLCK eNOS no target FF 100 4 9 95 5 10 CaMKK CaMK1 smMLCK eNOS no target FF 100 4 9 95 5 10 CaMKK cAMK1 smMLCK eNOS no target FG 100 4 9 95 5 10 FG 97 8 16 94 5 11 97 7 14 87 6 13 91 7 14 87 6 11 99 3 10 84 6 12 SmMLCK eNOS no target FG 95 7 13 81 4 12 99 4 10 85 6 11<	CaMK1		92	5	12	90	7	12
eNOS nNOS DE 83 6 10 92 6 12 nNOS 83 6 13 93 8 15 no target 93 4 12 91 9 15 CaMKK 93 4 12 91 9 15 CaMKI 80 3 7 94 8 13 smMLCK 97 8 16 94 5 11 eNOS 91 7 14 87 6 13 no target 97 5 14 93 9 17 caMKK 89 5 11 92 6 11 caMKK 90 3 10 84 6 12 smMLCK 95 7 13 81 4 12 eNOS FG 95 7 13 81 4 12 eNOS 91 6 12	smMLCK		83	5	11	94	6	14
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CaMKK CaMKI SmMLCK eNOS nNOS EF 100 4 9 95 5 10 eNOS nNOS 80 3 7 94 8 13 smMLCK eNOS nNOS 97 8 16 94 5 11 97 95 5 12 92 10 14 97 5 14 93 9 17 GaMKK CaMK1 smMLCK eNOS no target 97 5 14 93 9 17 GaMKK CaMK1 smMLCK eNOS no target FG 89 5 11 92 6 11 GMKK CaMK1 smMLCK eNOS FG 95 7 13 81 4 12 GMKK CaMK1 smMLCK eNOS FG 91 6 12 87 8 14 GH 80 5 12 84 6 11 SmMLCK eNOS GH 87 6 13 92 6 11 SMMLCK eNOS 88 8	no target		93	4	12	91	9	15
CaMKK CaMK1 smMLCK eNOS FF 100 4 9 95 5 10 eNOS 97 8 16 94 8 13 eNOS 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 97 5 11 92 6 11 CaMKK 99 7 13 81 4 12 SmMLCK 95 7 13 81 4 12 eNOS 91 6 12 87 8 14 no target 94 8 15 91 9 17 caMKK GH 6H 12 87 8 14 no target 94 8								
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CaMKK CaMK1 89 5 11 92 6 11 SmMLCK eNOS 90 3 10 84 6 12 eNOS 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 994 8 15 91 9 17 CaMKK 99 4 10 89 9 15 SmMLCK 99 4 10 89 9 15 smMLCK 6H 87 6 13 92 6 11 eNOS 88 8 14 94 7 15 no target 83 7 13 86 9 18								
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smMLCK eNOS FG 95 7 13 81 4 12 nNOS 86 6 11 85 6 11 nNOS 91 6 12 87 8 14 no target 94 8 15 91 9 17 CaMKK 6 7 16 99 9 15 SmMLCK 84 6 11 10 89 9 15 SmMLCK 99 4 10 89 9 15 11 NOS 84 6 11 11 11 11 11 11 NOS 84 10 89 9 15 11 11 11 11 NOS 88 8 14 94 7 15 no target 83 7 13 86 9 18	CaMK1		90	3	10	84	6	12
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nNOS no target 91 6 12 87 8 14 no target 94 8 15 91 9 17 CaMKK	eNOS	FG	86	6	11	85	6	11
no target 94 8 15 91 9 17 CaMKK Image:	nNOS		91	6	12	87	8	14
CaMKK 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	no target		94	8	15	91	9	17
CaMKK 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	_							
CaMK1 smMLCK eNOS 99 4 10 89 9 15 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	CaMKK		80	5	12	84	6	11
SmMLCK eNOS GH 82 7 16 92 4 11 nNOS 87 6 13 92 6 11 nNOS 88 8 14 94 7 15 no target 83 7 13 86 9 18	CaMK1		99	4	10	89	9	15
eNOS 6H 87 6 13 92 6 11 nNOS 88 8 14 94 7 15 no target 83 7 13 86 9 18	smMLCK		82	7	16	92	4	11
nNOS 88 8 14 94 7 15 no target 83 7 13 86 9 18	eNOS	GH	87	6	13	92	6	11
no target 83 7 13 86 9 18	nNOS		88	8	14	94	7	15
	no target		83	7	13	86	9	18

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.

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. T Δ S is relative to unbound CaM; T Δ \DeltaS is relative to nNOS-CaM.

	Experiment			
	target peptide	TΔS	τδας	
	nNOS	-3.80		
	smMLCK	-13.12	-9.32	
	eNOS	-11.02	-7.22	
	CaMK1	-15.77	-11.97	
	CaMKK	-15.18	-11.38	
	Quasiha	armonic entropies (kcal/mol) for CaM ba	ckbone atoms	
MD conditions	target peptide	TS	TΔS	τδας
	nNOS	910.86	-23.34	
	smMLCK	871.04	-63.15	-39.82
low ionic strongth	eNOS	868.96	-65.23	-41.90
low forme strength	CaMK1	843.95	-90.24	-66.90
	CaMKK	836.36	-97.84	-74.50
	no target peptide	934.19		
	nNOS	869.41	-68.93	
	smMLCK	865.60	-72.75	-3.81
high ionic strongth	eNOS	864.36	-73.98	-5.05
nigh tonic strength	CaMK1	852.89	-85.45	-16.52
	CaMKK	859.65	-78.69	-9.76
	no target peptide	938.35		
	Quasih	armonic entropies (kcal/mol) for all CaN	A heavy atoms	
MD conditions	target peptide	TS	TΔS	τδας
	nNOS	2274.08	-72.07	
	smMLCK	2187.25	-158.91	-86.84
low ionic strength	eNOS	2199.21	-146.95	-74.88
iow ionic sciengen	CaMK1	2142.21	-203.95	-131.87
	CaMKK	2129.06	-217.10	-145.02
	no target peptide	2346.16		
	nNOS	2206.48	-139.17	
	smMLCK	2190.80	-154.85	-15.68
high ionic strongth	eNOS	2188.68	-156.97	-17.80
mgn tonic strength	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∞	Α	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							
СаМКК	905.82	1398.63	0.74	1.00	0.95							
	Low lo	onic Streng	th									
CaM target	TS∞	Α	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							
СаМКК	862.93	1341.83	0.84	0.98	0.97							