

Supporting Information

Conformational Selection and Functional Dynamics of Calmodulin: A ^{19}F Nuclear Magnetic Resonance Study

Joshua Hoang[†] and R. Scott Prosser^{*,†,‡}

[†]Department of Chemistry, University of Toronto, UTM, 3359 Mississauga Road North, Mississauga, ON L5L 1C6, Canada.

[‡]Department of Biochemistry, University of Toronto, 1 King's College Circle, Toronto, ON, M5S 1A8, Canada.

Supporting Figures

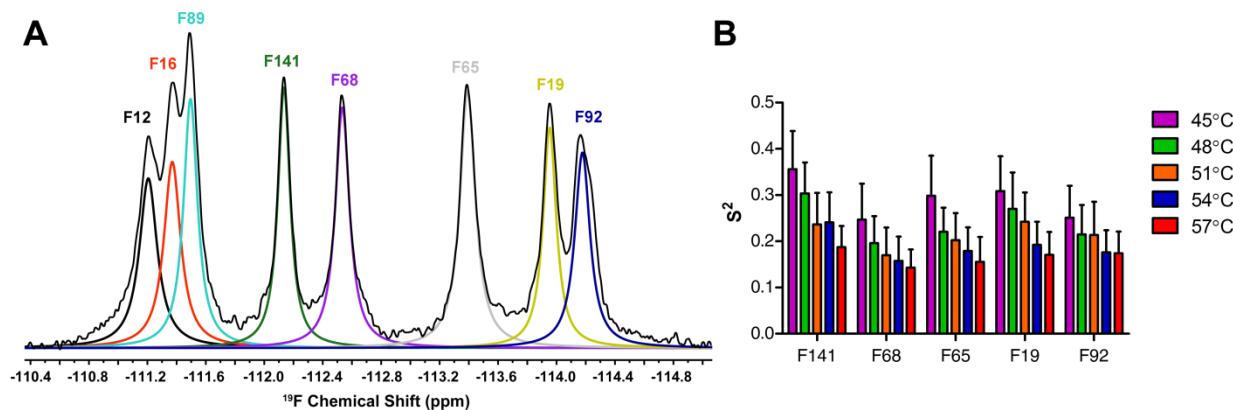


Figure S1. (A) ^{19}F NMR spectrum of 3-FPhe fractionally labeled CaM-4Ca²⁺ at 51°C with the corresponding phenylalanine assignments. A spectral deconvolution is shown to highlight each of the eight resonances. (B) Order parameter values from a Lipari Szabo analysis based on ^{19}F T₁ and T₂ data from the 3-FPhe reporters. Residues F12, F16, and F89 are excluded from the analysis due to significant spectral overlap.

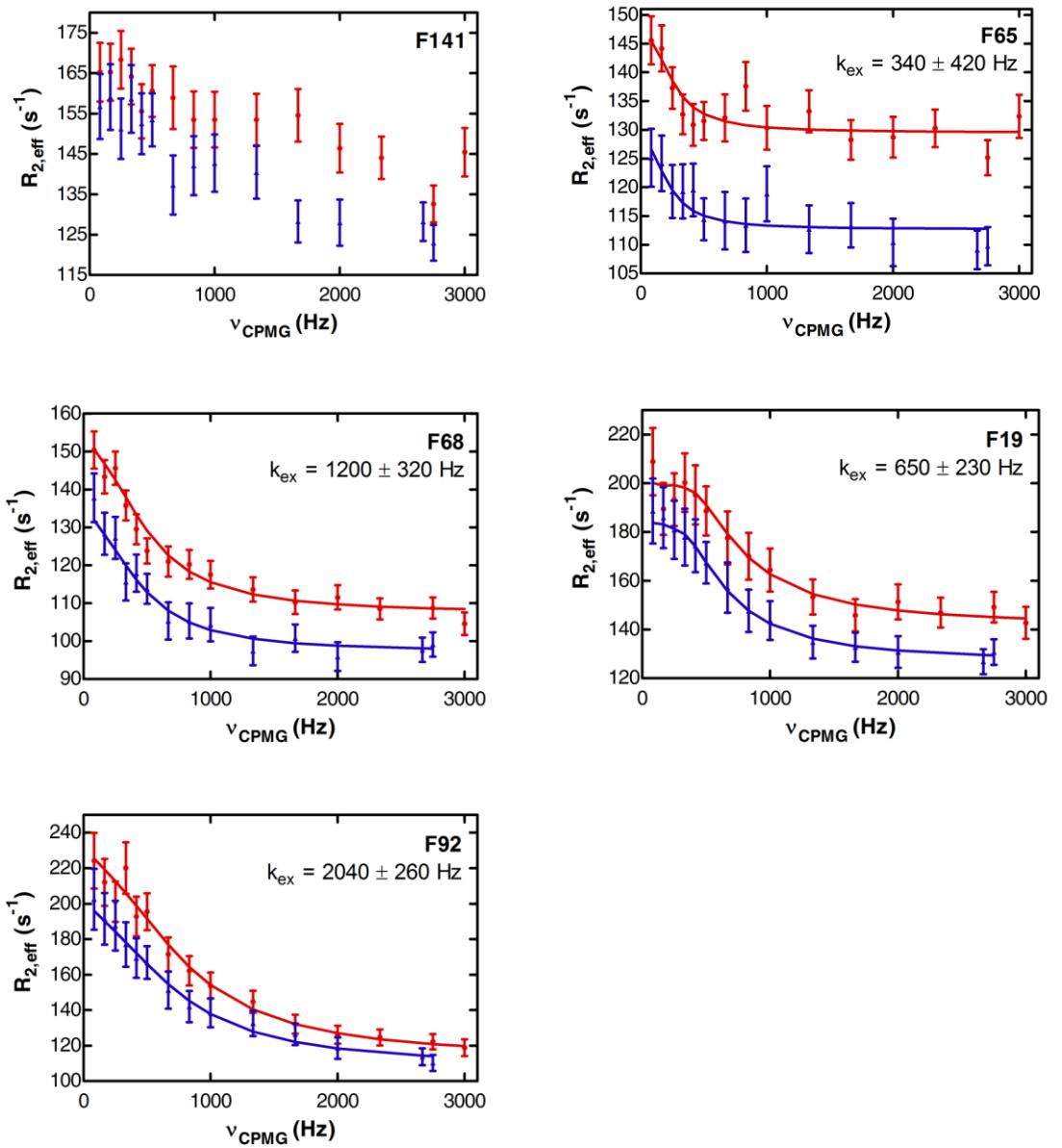


Figure S2. ^{19}F CPMG relaxation dispersion profiles collected at 37°C. F12, F16, and F89 here are excluded due to poor peak separation in the spectrum. Using two field strengths, 11.7 T (blue) and 14.1 T (red), a global fit to a two-site exchange model of residues F68, F65, and F19 give an estimate of the exchange rate and relative population of the *I* state at 37°C. The exchange rate from F92 is near the fast-exchange regime and thus was omitted from the global fit. At 37°C, the CPMG dispersion from F141 did not produce a reliable fit to extract an exchange rate.

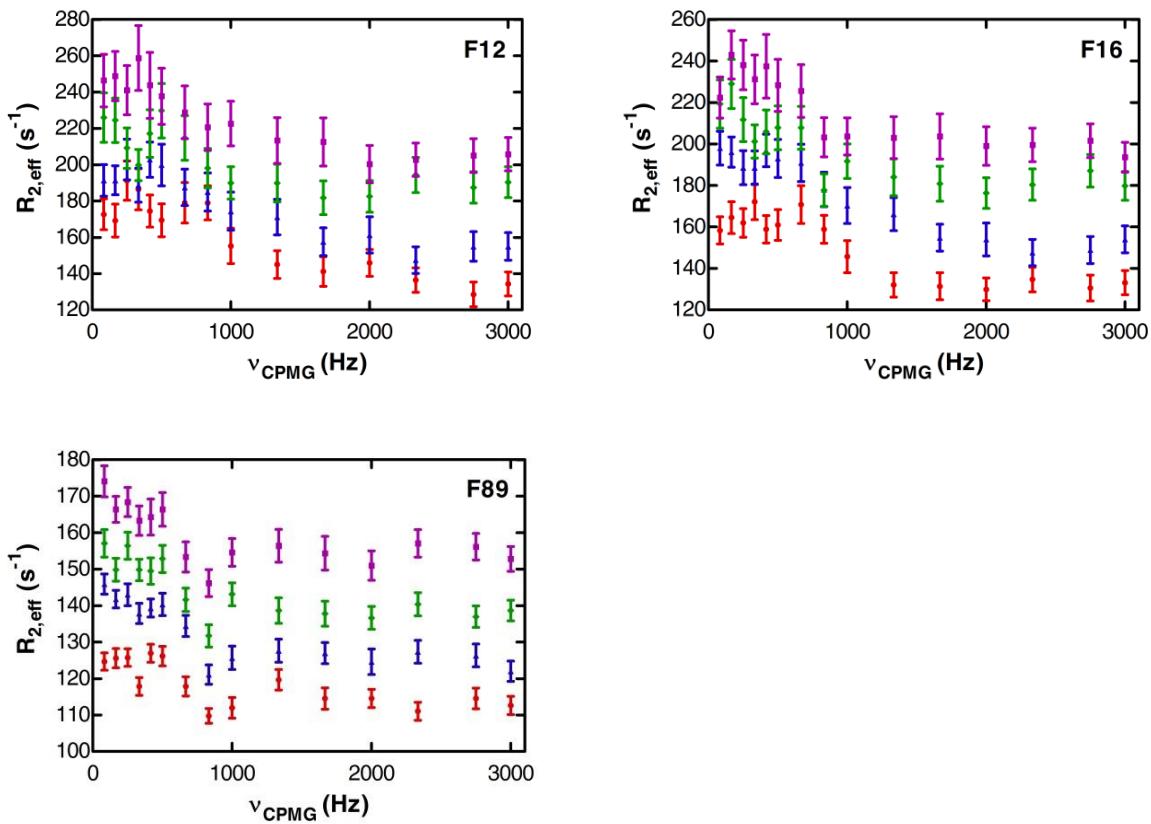


Figure S3. ^{19}F CPMG relaxation dispersion profiles for three of the eight phenylalanine reporters (F12, F16, F89) collected at 41°C (purple), 45°C (green), 51°C (blue), and 57°C (red). Though these residues exhibit a dispersion, the curves were not fit due to the additional error added as a result of spectral overlap.

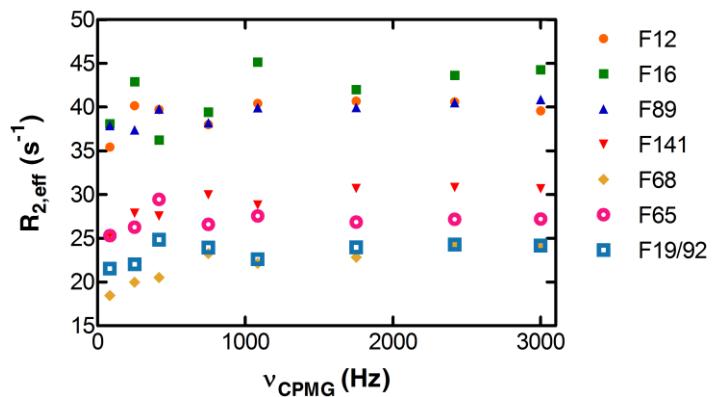


Figure S4. ^{19}F CPMG relaxation dispersion profiles for full length CaM-4Ca $^{2+}$ in the presence of 5% trifluoroethanol (TFE) at 57°C. The lack of dispersion indicates TFE stabilizes the native state and prevents excursions to the near-native intermediate.

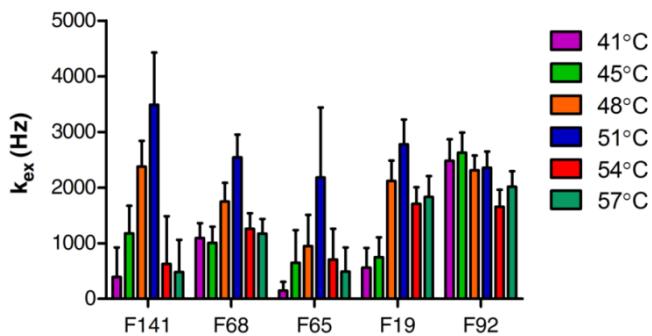


Figure S5. Exchange rates (k_{ex}), extracted from the CPMG experiments, as a function of temperature.

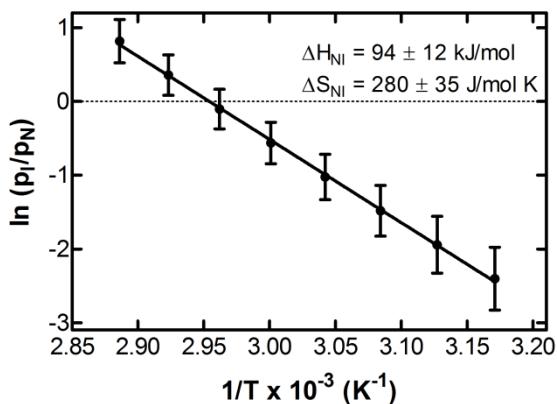


Figure S6. A van't Hoff analysis associated with the formation of the water-depleted intermediate state. In this analysis, it is assumed the equilibrium constant, K_{NI} , is equal to the ratio of the populations of the two states (p_l/p_N). The populations of the native, p_l , and near-native, p_N , states were obtained from previous ^{19}F chemical shift analysis.¹

Supporting Tables

Table S1. Exchange (k_{ex}), folding (k_{IN}), and unfolding (k_{NI}) rates extracted from CPMG dispersion experiments for the phenylalanine residues.

Temperature (°C)	Exchange Rate (Hz)								
	Residue								
	F141			F68			F65		
Temperature (°C)	k_{ex}	k_{fold}	k_{unfold}	k_{ex}	k_{fold}	k_{unfold}	k_{ex}	k_{fold}	k_{unfold}
57	480 ± 500	340 ± 370	140 ± 150	1180 ± 270	830 ± 300	340 ± 120	490 ± 430	350 ± 320	140 ± 130
54	630 ± 470	490 ± 390	140 ± 110	1260 ± 280	970 ± 380	280 ± 110	710 ± 550	550 ± 460	160 ± 140
51	3490 ± 940	2840 ± 1220	640 ± 280	2550 ± 410	2070 ± 770	480 ± 180	2200 ± 1260	1780 ± 1180	400 ± 270
48	2380 ± 860	2040 ± 1050	330 ± 170	750 ± 340	1500 ± 630	240 ± 100	950 ± 560	820 ± 570	130 ± 90
45	1120 ± 580	1050 ± 670	120 ± 80	1000 ± 290	900 ± 450	110 ± 50	650 ± 590	580 ± 580	70 ± 70
41	400 ± 530	360 ± 520	30 ± 40	1100 ± 270	1010 ± 500	80 ± 40	150 ± 170	140 ± 160	10 ± 10
37	N.A	N.A	N.A	1240 ± 320	1180 ± 630	60 ± 30	350 ± 420	330 ± 430	20 ± 20

Temperature (°C)	Exchange Rate (Hz)					
	Residue					
	F19			F92		
Temperature (°C)	k_{ex}	k_{fold}	k_{unfold}	k_{ex}	k_{fold}	k_{unfold}
57	1830 ± 380	1300 ± 450	530 ± 180	2010 ± 280	1420 ± 440	290 ± 180
54	1710 ± 300	1310 ± 480	390 ± 140	1660 ± 310	1280 ± 460	380 ± 140
51	2780 ± 440	2270 ± 830	510 ± 190	2360 ± 290	1920 ± 680	440 ± 150
48	2120 ± 370	1820 ± 740	300 ± 120	2310 ± 270	1990 ± 770	320 ± 130
45	750 ± 360	670 ± 420	80 ± 50	2630 ± 370	3250 ± 1000	280 ± 120
41	690 ± 300	640 ± 400	50 ± 30	2480 ± 390	2300 ± 1070	180 ± 90
37	650 ± 230	620 ± 360	30 ± 20	2040 ± 260	1940 ± 940	100 ± 50

Table S2. CPMG relaxation dispersion data ($R_{2,\text{eff}}$ vs. ν_{CPMG}) for full length CaM-4Ca²⁺, TR1C, TR2C, and full length CaM-4Ca²⁺ in the presence of 5% TFE at 45°C.

ν_{CPMG} (Hz)	Full-length CaM-4Ca ²⁺					
	$R_{2,\text{eff}}$ (s ⁻¹)					
	Residue					
ν_{CPMG} (Hz)	F19	F65	F68	F89	F92	F141
83	159.6	120.3	126.9	157.1	164.5	154.4
167	170.9	123.7	129.2	149.8	168.9	146.7
250	163.9	117.0	121.3	156.4	162.2	155.8
333	173.7	119.0	128.7	149.8	166.3	146.6
417	164.0	115.7	121.2	149.5	154.4	142.6
500	161.2	113.7	112.2	152.8	147.3	142.6
667	145.3	117.7	113.9	141.6	140.1	139.9
833	147.5	112.6	107.5	131.7	136.3	133.6
1000	140.4	110.7	104.2	143.1	132.7	133.9
1333	135.0	112.6	100.0	138.6	121.6	135.0
1667	135.0	112.6	99.2	137.8	118.5	133.6
2000	128.5	109.9	98.5	136.6	114.0	127.8
2333	126.4	108.5	96.5	140.4	108.1	129.4
2750	127.6	107.3	95.7	136.9	107.5	124.3
3000	127.4	112.6	95.8	138.6	105.1	130.7

ν_{CPMG} (Hz)	Individual Domains of CaM-4Ca ²⁺					
	$R_{2,\text{eff}}$ (s ⁻¹)					
	Residue					
	TR1C			TR2C		
ν_{CPMG} (Hz)	F19	F65	F68	F89	F92	F141
50	90.5	39.3	56.5	29.6	33.0	31.1
100	62.1	32.9	35.0	31.9	32.2	34.3
150	88.0	37.9	55.6	28.5	28.6	33.5
200	88.8	36.9	54.5	31.1	32.7	34.1
300	89.2	36.7	57.0	31.1	31.2	31.8
500	86.1	37.0	55.2	30.3	29.9	33.6
700	90.7	35.6	54.8	33.5	27.9	33.5
900	87.1	35.4	52.2	32.7	28.3	33.6
1100	77.2	32.4	46.6	32.4	27.7	33.8
1500	81.8	33.7	42.7	31.9	24.4	32.9
1900	68.1	32.0	40.2	32.2	20.1	32.4
2300	64.8	31.8	36.8	31.2	19.9	32.3
2700	64.0	30.8	33.2	32.1	24.1	29.6

v_{CPMG} (Hz)	Full-length CaM-4Ca²⁺ with 5% TFE					
	R_{2,eff} (s⁻¹)					
	Residue					
F19	F65	F68	F89	F92	F141	
83	66.6	74.5	56.3	96.1	26.8	73.6
250	62.1	70.6	56.4	100.1	30.5	77.7
417	63.7	68.3	51.5	97.5	26.8	69.6
750	60.5	73.2	56.3	96.9	26.6	73.9
1083	63.7	68.3	57.4	96.5	28.7	73.3
1750	63.7	71.9	56.4	97.6	25.9	77.8
2417	66.1	70.1	57.4	97.0	22.7	73.0
3000	66.1	74.5	57.1	97.0	22.9	77.6
3333	67.2	71.9	61.1	96.2	22.5	75.7

Supplemental References

- (1) Kitevski-LeBlanc, J. L., Hoang, J., Thach, W., Larda, S. T., and Prosser, R. S. (2013) F-19 NMR studies of a desolvated near-native protein folding intermediate, *Biochemistry* 52, 5780-5789.