Supplementary Materials belonging to
Changes in calmodulin mainchain dynamics upon ligand binding revealed by cross-correlated NMR relaxation measurements.

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Methodology:

The $^{13}CO^{13}C\alpha$-dipole-dipole-CSA cross correlation rates ($\Gamma^{CSA/DD}_{CO/CO-C\alpha}$) were obtained from the "TROSY" effects in the $^{13}CO^{13}C\alpha$ doublets in constant time $^{13}C\alpha$ -coupled 3D HNCO spectra[1]. The data was analyzed using an in-house written simulated annealing routine that fitted the CO time-domain data[1], extracted from the 3D (t1,f2,f3) matrix.

The order parameter of the ^{13}CO-$^{13}C\alpha$ vector was subsequently obtained using the following equation[1]

$$\Gamma^{CSA/DD}_{CO/CO-C\alpha} = \frac{1}{30}\left(\frac{\mu_0}{4\pi}\right)\frac{\hbar\omega_C\gamma_C^2}{r^3_{CO-C\alpha}}\times\left[(\sigma_{11}-\sigma_{33})\times\left(3cos^2\theta_{11,CO-C\alpha}-1\right)+(\sigma_{22}-\sigma_{33})\times\left(3cos^2\theta_{22,CO-C\alpha}-1\right)\right]$$
$$\times\tau_c\times\left\{4S^2_{CO-C\alpha}+\frac{3S^2_{CO-C\alpha}}{1+(\omega_C\tau_c)^2}\right\}$$

where the symbols have their usual meaning; $\theta_{11,CO\text{-}C\alpha}$ and $\theta_{22,CO\text{-}C\alpha}$ describe the angle between the (σ_{11} and σ_{22}) principal axes of the ^{13}CO CSA tensor and the ^{13}CO-$^{13}C\alpha$ vector and σ_{11}-σ_{33} = 156 ppm, σ_{22}-σ_{33} = 80 ppm, $\theta_{11,CO\text{-}C\alpha}=157^0$, $\theta_{22,CO\text{-}C\alpha}=247^0$. The values obtained correspond very closely to solid-state NMR data (σ_{11}- σ_{33} = 154 ppm, σ_{22} - σ_{33} = 81 ppm, $\theta_{11,CO\text{-}C\alpha}=157^0$ and $\theta_{22,CO\text{-}C\alpha}=247^0$).[2-4]

The value for τ_c was obtained from ^{15}N relaxation data.

Note that the spectral density function used

$$J(\omega)=\frac{2}{5}\frac{S^2_{CO-C\alpha}\tau_c}{1+(\omega\tau_c)^2}$$

corresponds to Model 1 of the Model-free program[5]. We use the approximation that the cross-correlation order parameter reports predominantly on the motion of the $CO C\alpha$ vector[1].

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(2) Oas, T. G.; Hartzell, C. J.; McMahon, T. J.; Drobny, G. P.; Dahlquist, F. W. *J Am Chem Soc* **1987**, *109*, 5956-5962.
(3) Teng, Q.; Ph D. thesis, Florida State University: Tallahassee, FL, 1990.
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(5) Mandel, A. M.; Akke, M.; Palmer, A. G. III. *J. Mol. Biol.* **1995**, *246*, 144-163.

Following are three tables with raw relaxation data for CaM-free and CaM-smMLCK-p.

Table 1. ^{15}N relaxation data of smMLCKp-complexed Calmodulin measured at a 500 MHz proton resonance frequency on a Bruker Avance spectrometer and at a temperature of 35 ^{0}C, pH 6.5.

Residue	R_1 (s^{-1})		R_2 (s^{-1})		NOE	
	Rate	Error	Rate	Error	Data	Error
4	1.524	0.053	5.091	0.114	0.169	0.005
5	1.957	0.039	7.464	0.156	0.557	0.023
6	1.994	0.049	8.24	0.161	0.645	0.035
7	2.019	0.033	8.643	0.126	0.638	0.019
9	2.043	0.048	8.786	0.145	0.657	0.038
10	2.061	0.041	9.508	0.203	0.671	0.046
11	2.003	0.045	9.503	0.16	0.618	0.027
12	2.068	0.034	9.554	0.209	0.697	0.025
13	1.996	0.053	9.612	0.236	0.662	0.028
14	1.947	0.039	9.776	0.181	0.689	0.043
15	2.046	0.032	9.468	0.175	0.652	0.048
16	2.137	0.049	9.345	0.129	0.703	0.044
17	1.974	0.038	9.344	0.198	0.658	0.043
18	1.996	0.049	9.938	0.137	0.697	0.042
19	1.961	0.028	9.701	0.14	0.663	0.038
20	2.017	0.046	9.45	0.201	0.683	0.055
21	1.959	0.039	8.138	0.226	0.624	0.014
22	2.078	0.036	8.716	0.127	0.663	0.034
24	2.128	0.036	8.523	0.108	0.698	0.048
25	2.122	0.035	8.242	0.155	0.685	0.026
26	2.028	0.038	9.489	0.158	0.74	0.028
27	2.035	0.027	9.811	0.16	0.657	0.035
28	2.186	0.062	8.617	0.174	0.675	0.032
29	2.144	0.046	8.201	0.145	0.674	0.038
30	2.147	0.053	8.567	0.185	0.686	0.031
31	2.13	0.031	8.572	0.231	0.696	0.044
32	2.17	0.034	8.894	0.23	0.648	0.018
33	2.187	0.043	8.334	0.106	0.652	0.042
34	2.078	0.04	8.565	0.228	0.674	0.031
35	2.225	0.038	8.412	0.152	0.75	0.042
37	2.06	0.058	9.124	0.131	0.645	0.035
39	2.04	0.058	11.173	0.217	6.05	0.014
41	1.988	0.038	8.288	0.103	0.649	0.036
42	1.781	0.085	7.061	0.148	0.558	0.039
44	1.841	0.048	8.766	0.176	0.661	0.024
45	2.117	0.044	8.271	0.17	0.72	0.036

46	2.178	0.047	8.394	0.192	0.636	0.03
48	2.115	0.055	8.907	0.096	0.691	0.021
49	2.119	0.038	8.588	0.219	0.673	0.026
52	2.096	0.029	8.952	0.166	0.652	0.055
54	2.095	0.037	8.454	0.19	0.682	0.046
55	2.105	0.038	9.702	0.172	0.744	0.078
56	2.076	0.046	8.495	0.224	0.67	0.037
57	1.666	0.048	5.897	0.121	0.643	0.028
58	2.1	0.029	8.089	0.084	0.658	0.025
61	2.056	0.055	9.436	0.219	0.68	0.034
63	2.132	0.048	9.337	0.203	0.721	0.035
64	2.183	0.051	8.674	0.186	0.739	0.039
65	1.973	0.043	9.709	0.245	0.729	0.043
67	1.978	0.042	10.079	0.168	0.723	0.024
68	2.083	0.043	9.742	0.191	0.703	0.035
69	2.11	0.055	9.743	0.144	0.709	0.043
70	1.813	0.028	8.404	0.152	0.342	0.022
71	1.995	0.045	10.167	0.173	0.706	0.028
72	2.014	0.064	9.772	0.147	0.704	0.056
73	2.029	0.05	9.55	0.155	0.626	0.039
76	1.961	0.044	7.617	0.173	0.491	0.019
77	1.795	0.047	6.046	0.101	0.31	0.033
78	1.792	0.043	5.529	0.09	0.22	0.006
79	1.66	0.045	4.891	0.165	0.2	0.012
80	1.849	0.033	4.989	0.155	0.228	0.019
81	1.915	0.057	5.961	0.138	0.346	0.014
82	1.957	0.053	7.468	0.215	0.437	0.013
84	2.055	0.035	8.469	0.17	0.635	0.033
85	1.974	0.052	9.949	0.249	0.642	0.021
86	1.961	0.057	9.903	0.213	0.684	0.043
87	2.08	0.04	9.192	0.145	0.682	0.027
88	2.052	0.052	9.094	0.154	0.676	0.023
89	2.047	0.043	10.19	0.178	0.7	0.043
90	1.941	0.035	10.125	0.165	0.713	0.03
91	2.082	0.03	8.556	0.12	0.674	0.031
93	1.928	0.051	9.776	0.181	0.671	0.027
94	2.072	0.047	7.565	0.137	0.638	0.036
95	2.205	0.04	7.788	0.153	0.651	0.031
96	2.2	0.05	7.68	0.149	0.676	0.025
97	2.152	0.027	8.407	0.119	0.706	0.039
98	2.332	0.035	7.776	0.1	0.698	0.025
99	2.236	0.032	8.12	0.094	0.658	0.028
100	2.129	0.041	7.726	0.137	0.692	0.029
101	2.281	0.024	8.368	0.12	0.713	0.036
102	2.228	0.046	7.9	0.117	0.703	0.029

103	2.205	0.042	7.598	0.126	0.693	0.029
105	2.17	0.043	8.397	0.177	0.682	0.042
109	2.173	0.051	9.367	0.198	0.638	0.025
110	2.157	0.037	7.805	0.144	0.631	0.061
111	2.34	0.036	7.869	0.131	0.667	0.026
114	2.016	0.027	6.712	0.092	0.538	0.025
115	1.688	0.037	5.471	0.083	0.219	0.014
116	1.609	0.04	5.149	0.137	0.355	0.009
117	1.959	0.034	7.369	0.14	0.59	0.017
118	2.079	0.036	8.265	0.179	0.626	0.036
119	2.092	0.059	7.906	0.177	0.613	0.029
121	2.041	0.043	8.979	0.112	0.636	0.032
125	2.066	0.037	8.961	0.173	0.648	0.039
126	2.135	0.059	8.524	0.158	0.653	0.025
127	2.167	0.062	9.227	0.173	0.69	0.043
128	2.073	0.043	8.695	0.111	0.739	0.036
129	2.037	0.033	9.451	0.155	0.672	0.026
130	1.571	0.028	6.235	0.168	0.598	0.03
131	2.219	0.033	7.769	0.132	0.67	0.033
133	2.028	0.029	9.382	0.115	0.707	0.041
134	2.104	0.045	8.016	0.124	0.689	0.035
135	2.188	0.028	8.044	0.113	0.69	0.031
136	2.158	0.04	7.988	0.149	0.675	0.032
137	2.187	0.04	8.024	0.186	0.652	0.026
140	2.258	0.038	8.03	0.146	0.682	0.03
141	2.282	0.056	8.358	0.187	0.688	0.034
142	2.227	0.06	8.111	0.146	0.679	0.018
143	2.164	0.033	8.117	0.201	0.653	0.061
145	2.187	0.041	7.98	0.125	0.642	0.035
146	1.937	0.034	8.006	0.17	0.576	0.039
147	1.851	0.039	5.171	0.136	0.354	0.012
148	1.235	0.037	2.655	0.096	-0.365	0.017

Table 2. ^{15}N relaxation data of free Calmodulin measured at a 500 MHz proton resonance frequency on a Bruker Avance spectrometer and at a temperature of 35 ^{0}C, pH 6.5.

Residue	R_1 (s^{-1})		R_2 (s^{-1})		NOE	
	Rate	Error	Rate	Error	Data	Error
3	1.213	0.019	2.79	0.25	-0.589	0.016
4	1.472	0.019	5.455	0.098	0.142	0.006
5	1.751	0.016	8.621	0.119	0.647	0.03
6	1.973	0.026	8.958	0.136	0.719	0.017
7	1.972	0.024	9.144	0.112	0.733	0.03
8	1.903	0.024	9.233	0.148	0.735	0.024
9	1.967	0.015	9.337	0.155	0.681	0.02
10	2.037	0.025	9.715	0.097	0.746	0.025
11	1.986	0.018	9.682	0.103	0.769	0.016
12	2.014	0.026	9.981	0.128	0.746	0.025
13	2.023	0.031	9.916	0.077	0.796	0.03
15	2.008	0.018	9.444	0.131	0.8	0.021
16	2.011	0.013	9.601	0.091	0.774	0.029
17	1.973	0.031	9.871	0.141	0.771	0.024
18	2.032	0.024	9.784	0.1	0.775	0.049
19	1.924	0.032	9.477	0.127	0.771	0.018
20	1.911	0.029	9.447	0.097	0.792	0.022
21	1.907	0.021	8.587	0.079	0.73	0.015
22	1.953	0.026	9.671	0.092	0.76	0.016
23	1.964	0.033	9.174	0.083	0.788	0.025
24	1.965	0.024	9.522	0.079	0.803	0.039
25	1.912	0.023	10.271	0.123	0.813	0.025
26	2.003	0.029	9.71	0.14	0.789	0.025
27	1.899	0.035	9.447	0.155	0.774	0.036
28	1.92	0.044	10.169	0.199	0.787	0.037
29	1.902	0.03	9.995	0.085	0.809	0.027
30	1.934	0.039	10.022	0.126	0.779	0.017
31	1.893	0.03	10.359	0.121	0.781	0.032
32	1.816	0.027	10.395	0.139	0.801	0.036
33	1.962	0.02	9.987	0.138	0.798	0.03
34	1.939	0.021	9.817	0.099	0.77	0.017
36	1.873	0.027	10.605	0.095	0.782	0.029
37	1.911	0.035	9.919	0.106	0.764	0.024
38	1.87	0.022	9.694	0.05	0.787	0.032
39	1.828	0.026	9.892	0.113	0.772	0.032
40	1.833	0.026	8.16	0.191	0.735	0.034
42	1.581	0.045	7.353	0.359	0.664	0.034
44	1.868	0.032	8.917	0.096	0.736	0.027
45	1.981	0.032	9.272	0.113	0.753	0.033
46	2.012	0.023	9.54	0.094	0.769	0.02
47	1.973	0.02	9.712	0.146	0.777	0.023
48	1.985	0.027	9.704	0.135	0.777	0.027
49	1.966	0.029	9.518	0.093	0.77	0.03

51	1.976	0.024	9.646	0.066	0.789	0.028
52	1.956	0.032	9.597	0.112	0.766	0.036
53	2.007	0.014	9.895	0.076	0.77	0.027
54	1.878	0.022	9.428	0.07	0.619	0.022
55	1.96	0.028	9.119	0.098	0.74	0.034
57	1.382	0.025	6.877	0.122	0.698	0.023
58	1.82	0.021	9.911	0.148	0.794	0.024
59	1.783	0.035	9.073	0.091	0.786	0.025
60	1.922	0.018	9.634	0.078	0.801	0.03
61	1.951	0.025	9.477	0.173	0.784	0.029
62	1.949	0.028	9.818	0.1	0.808	0.026
63	1.988	0.036	9.713	0.125	0.728	0.027
64	1.979	0.034	9.553	0.18	0.831	0.038
65	1.982	0.023	10.54	0.112	0.795	0.026
67	1.897	0.042	10.336	0.124	0.806	0.02
69	2.046	0.036	10.229	0.077	0.775	0.037
70	1.912	0.036	9.849	0.113	0.774	0.037
71	2.015	0.017	10.104	0.109	0.785	0.02
74	1.814	0.041	9.246	0.204	0.682	0.036
75	1.911	0.037	11.472	0.23	0.522	0.02
77	1.884	0.034	8.529	0.227	0.537	0.028
80	1.813	0.019	10.169	0.396	0.556	0.028
81	1.986	0.034	8.949	0.166	0.65	0.068
82	2.026	0.02	8.992	0.123	0.671	0.026
84	1.968	0.02	9.528	0.118	0.759	0.035
85	2.041	0.029	9.739	0.13	0.798	0.014
86	2.022	0.023	9.856	0.098	0.765	0.028
87	2.029	0.023	10.337	0.182	0.758	0.044
89	2.056	0.028	9.867	0.118	0.806	0.026
90	1.972	0.023	9.807	0.089	0.788	0.035
91	1.974	0.035	9.802	0.12	0.791	0.018
92	1.944	0.037	9.6	0.104	0.751	0.032
93	1.863	0.036	9.631	0.068	0.782	0.029
94	1.925	0.03	8.81	0.066	0.77	0.014
95	2.003	0.035	9.313	0.067	0.777	0.033
96	2.023	0.03	9.018	0.086	0.776	0.02
97	1.962	0.035	9.468	0.092	0.81	0.039
98	1.929	0.025	9.99	0.121	0.765	0.031
100	1.904	0.027	9.195	0.119	0.768	0.031
101	1.983	0.036	9.936	0.13	0.812	0.032
102	1.872	0.026	10.247	0.099	0.825	0.02
103	1.961	0.017	10.047	0.117	0.784	0.013
104	1.846	0.029	10.17	0.116	0.799	0.033
105	1.895	0.03	10.658	0.102	0.781	0.038
106	1.955	0.026	10.4	0.081	0.788	0.033
107	1.972	0.024	10.017	0.099	0.773	0.019
108	1.913	0.022	10.189	0.082	0.797	0.031
109	1.933	0.018	10.165	0.087	0.771	0.031

110	1.9	0.033	10.256	0.116	0.793	0.039
111	1.929	0.035	10.023	0.111	0.782	0.029
112	1.781	0.024	9.878	0.115	0.776	0.028
113	1.854	0.029	8.761	0.151	0.75	0.029
115	1.628	0.022	5.961	0.108	0.405	0.019
116	1.49	0.02	5.668	0.05	0.314	0.01
117	1.815	0.036	8.23	0.127	0.683	0.036
118	2.021	0.025	9.64	0.166	0.763	0.023
119	2.025	0.023	8.968	0.093	0.73	0.021
120	1.972	0.025	9.574	0.075	0.758	0.023
123	2	0.029	9.664	0.106	0.762	0.02
125	2.044	0.032	10.213	0.093	0.762	0.02
126	2.06	0.023	9.542	0.086	0.773	0.034
127	2	0.012	9.612	0.102	0.785	0.045
128	1.971	0.025	9.558	0.113	0.76	0.026
129	1.907	0.022	9.141	0.102	0.793	0.025
130	1.399	0.017	7.194	0.096	0.756	0.046
131	1.884	0.021	9.909	0.098	0.799	0.033
132	1.761	0.023	9.092	0.102	0.79	0.021
133	1.892	0.031	9.862	0.093	0.829	0.033
134	1.973	0.034	9.539	0.136	0.813	0.039
135	1.974	0.032	9.637	0.094	0.791	0.018
136	1.968	0.031	9.423	0.052	0.804	0.034
137	1.964	0.025	9.3	0.162	0.797	0.029
138	1.971	0.025	9.616	0.073	0.777	0.021
139	1.917	0.019	9.573	0.088	0.807	0.029
140	2.007	0.023	9.702	0.083	0.787	0.038
141	2.014	0.028	9.898	0.126	0.76	0.022
142	2.026	0.021	9.901	0.084	0.779	0.05
143	1.964	0.02	9.947	0.088	0.792	0.026
144	2.007	0.019	9.513	0.112	0.783	0.033
145	1.968	0.027	9.401	0.108	0.747	0.033
146	1.806	0.024	8.556	0.129	0.698	0.023
147	1.915	0.025	6.118	0.123	0.502	0.021

Table 3. The cross correlation rates between the CSA of ^{13}CO and the dipolar ^{13}CO-$^{13}C\alpha$ of smMLCKp-complexed and free Calmodulin measured at a 500 MHz proton resonance frequency on a Bruker Avance spectrometer and at a temperature of 35 ^{0}C, pH 6.5.

Residue	Free		Complex	
	Rate	Error	Rate	Error
3	-1.034	0.037	-1.270	0.045
5	-1.543	0.055	-1.490	0.026
6	-1.085	0.068	-1.371	0.036
8	-0.858	0.100	-1.701	0.020
9	-1.314	0.087	-1.527	0.070
11	-1.210	0.070	-1.653	0.047
12	-1.670	0.097	-1.623	0.013
13	-1.240	0.109	-1.648	0.033
15	-1.585	0.117	-1.732	0.059
17	-1.524	0.108	-1.906	0.043
18	-1.391	0.081	-1.582	0.062
19	-1.501	0.101	-1.839	0.083
20	-1.504	0.103	-1.910	0.060
22	-1.229	0.069	-1.800	0.086
24	-1.841	0.077	-1.806	0.113
25	-1.445	0.114	-2.177	0.072
26	-1.433	0.092	-1.266	0.023
27	-1.418	0.072	-1.874	0.068
28	-1.680	0.082	-1.466	0.035
29	-1.228	0.072	-1.659	0.095
30	-1.409	0.065	-1.600	0.009
31	-1.899	0.077	-1.464	0.094
32	-1.485	0.063	-1.484	0.046
33	-1.469	0.056	-1.832	0.092
35	-1.824	0.089	-1.796	0.056
36	-1.349	0.105	-1.560	0.070
37	-1.458	0.067	-1.385	0.054
38	-1.729	0.095	-1.559	0.113
40	-1.365	0.077	-1.818	0.069
41	-1.357	0.093	-1.514	0.176
44	-1.299	0.085	-1.631	0.047
45	-1.529	0.091	-1.649	0.025
46	-1.699	0.100	-1.580	0.073
47	-1.320	0.066	-1.713	0.044
49	-1.740	0.065	-1.496	0.054
50	-0.918	0.083	-1.119	0.047
51	-1.115	0.116	-1.971	0.045
54	-1.374	0.068	-1.663	0.009
55	-1.588	0.046	-1.772	0.040
56	-1.696	0.156	-1.610	0.019
59	-1.871	0.106	-1.837	0.034
60	-1.351	0.123	-1.897	0.102

61	-1.656	0.105	-1.692	0.120
62	-1.606	0.092	-2.160	0.139
63	-1.491	0.099	-1.735	0.025
64	-1.482	0.069	-1.800	0.202
66	-1.219	0.040	-1.734	0.266
67	-1.338	0.087	-1.892	0.091
71	-1.539	0.066	-1.378	0.152
72	-1.695	0.074	-1.464	0.065
73	-1.399	0.076	-1.558	0.090
77	-0.867	0.061	-1.201	0.209
78	-1.040	0.062	-1.309	0.154
79	-1.087	0.039	-1.247	0.057
81	-1.260	0.100	-1.775	0.027
85	-0.996	0.075	-1.736	0.035
86	-1.197	0.062	-1.620	0.031
87	-1.652	0.067	-1.598	0.112
89	-1.159	0.070	-1.707	0.090
90	-1.392	0.081	-1.756	0.066
92	-1.041	0.114	-1.677	0.081
93	-1.277	0.083	-1.631	0.050
95	-1.597	0.070	-1.781	0.026
97	-1.648	0.082	-1.734	0.086
98	-1.427	0.070	-1.873	0.008
99	-1.610	0.073	-1.850	0.126
100	-1.753	0.094	-1.702	0.136
101	-1.195	0.060	-1.489	0.087
102	-1.447	0.040	-1.644	0.020
104	-1.346	0.034	-1.388	0.067
105	-1.245	0.054	-1.543	0.079
107	-1.423	0.089	-1.698	0.058
109	-1.387	0.072	-1.670	0.038
110	-1.453	0.025	-1.710	0.029
111	-0.703	0.092	-2.044	0.103
113	-1.186	0.026	-1.577	0.044
114	-1.455	0.069	-1.669	0.058
115	-1.351	0.071	-1.721	0.005
116	-1.596	0.074	-1.698	0.035
117	-0.955	0.085	-1.651	0.068
118	-1.145	0.091	-1.859	0.091
120	-1.179	0.074	-1.513	0.026
121	-0.914	0.052	-1.787	0.060
122	-1.659	0.097	-1.190	0.087
124	-1.034	0.036	-1.829	0.056
125	-1.138	0.087	-1.693	0.046
126	-1.351	0.049	-1.511	0.086
127	-1.374	0.060	-1.662	0.034
128	-1.097	0.053	-1.808	0.044
129	-1.257	0.048	-1.942	0.056

130	-1.545	0.085	-1.639	0.098
133	-1.736	0.100	-1.888	0.079
134	-1.541	0.101	-1.704	0.032
135	-1.569	0.108	-1.639	0.099
136	-1.635	0.106	-1.999	0.086
137	-1.574	0.090	-1.724	0.036
138	-1.758	0.067	-1.702	0.047
139	-1.218	0.073	-1.666	0.086
140	-1.428	0.086	-1.582	0.035
141	-1.697	0.066	-1.886	0.118
142	-1.389	0.031	-1.401	0.027
143	-1.378	0.056	-1.639	0.046
144	-1.501	0.067	-1.786	0.055
145	-1.571	0.053	-1.341	0.006
146	-0.826	0.042	-1.348	0.051
147	-0.519	0.025	-0.912	0.038