Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Paramagnetic relaxation enhancements for the characterization of the conformational heterogeneity in two-domain proteins

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PRE~~re~~-derived MO in the slow exchange limit

In the unrealistic assumption that the exchange rate among the different conformations of the protein is slower than its tumbling rate,

$$R_{2j}^{PRE} = pR_{2j}^{sel} + \sum_i p_i R_{2ij} \qquad \text{(S1)}$$

where R_{2j}^{PRE}, R_{2j}^{sel} and R_{2ij} are the measured ~~pre-~~PRE of the j residue, the ~~pre-~~PRE calculated for a selected conformation and the ~~pre-~~PRE calculated for the other i conformations sampled by the protein, respectively, and p and p_i indicate the corresponding weights.

If $R_{2j}^{sel} > R_{2j}^{PRE}$ it can be immediately stated that the selected conformation cannot be the only one belonging to the structure ensemble (and thus $p<1$). Since the largest admissible value for p is in this case equal to $R_{2j}^{PRE} / R_{2j}^{sel}$, obtained when the R_{2ij} contributions to R_{2j}^{PRE} are negligible,[1] it is possible to define

$$\mathrm{MO}_j = R_{2j}^{PRE} / R_{2j}^{sel} \text{ for } R_{2j}^{sel} > R_{2j}^{PRE} \qquad \text{(S2)}$$

If $R_{2j}^{sel} < R_{2j}^{PRE}$ the largest weight p of the selected conformation is achieved when the other conformations of the ensemble provide the largest possible ~~pre~~PRE, i.e. $R_{2ij} = R_{2j}^{\max}$, so that $\sum_i p_i = 1 - p$ can have the smallest value. In this case,

$$\mathrm{MO}_j = \frac{R_{2j}^{PRE} - R_{2j}^{\max}}{R_{2j}^{sel} - R_{2j}^{\max}} \text{ for } R_{2j}^{sel} < R_{2j}^{PRE} \qquad \text{(S3)}$$

$R_{2j}^{\max}$ is the largest ~~pre-~~PRE calculated for each residue taking into account all the conformations within the pool of 33,000 conformations covering the whole conformational space possibly sampled by the protein.[2] The MO of the conformation is then defined as the smallest MO_j, where j varies on all residues for which experimental PRE data are available.

It can be actually expected that in reality some conformations are sampled within the protein reorientation time and others in longer times. Therefore, some conformations can be representative for

sub-ensembles of conformations experienced in time smaller than the reorientation time of the protein; their relaxation rate must thus be calculated using an average correlation time which takes into account local mobility.

Reference List

(1) A. N. Volkov, M. Ubbink and N. A. J. Van Nuland, *J.Biomol.NMR*, 2010, ***48***, 225-236.

(2) I. Bertini, A. Giachetti, C. Luchinat, G. Parigi, M. V. Petoukhov, R. Pierattelli, E. Ravera and D. I. Svergun, *J.Am.Chem.Soc.*, 2010, ***132***, 13553-13558.

Table S1. Axial and rhombic anisotropies and Euler angles (in ZYZ convention) of the ~~pcs~~PCS-derived magnetic susceptibility tensors and of the ~~rdc~~RDC-derived average tensors for the different lanthanides coordinated in the second binding site of the N-terminal domain of CaM

Magnetic susceptibility anisotropy tensors calculated from the PCSs of the N-terminal domain

	$\Delta\chi_{ax}$ (10^{-32} m^3)	$\Delta\chi_{rh}$ (10^{-32} m^3)	Euler angles (radians)[a]			Q-factor
298 K						
Tb	36 ± 1	-16.5 ± 0.9	1.77 ± 0.04	-0.88 ± 0.05	0.71 ± 0.04	0.20
Tm	31 ± 3	-9 ± 1	0.48 ± 0.04	-0.51 ± 0.11	1.81 ± 0.04	0.17
Dy	36 ± 1	-13 ± 2	1.32 ± 0.07	-0.72 ± 0.11	0.31 ± 0.02	0.35
278 K						
Tb	47 ± 1	-17 ± 3	-1.29 ± 0.03	-1.48 ± 0.05	2.59 ± 0.03	0.12
Tm	31 ± 2	-10 ± 1	-2.64 ± 0.03	-2.51 ± 0.07	1.30 ± 0.02	0.11
Dy	54 ± 2	-16 ± 2	2.02 ± 0.02	1.60 ± 0.04	0.43 ± 0.02	0.13

Average tensors calculated from the RDCs of the C-terminal domain

	$\Delta\overline{\chi}_{ax}$ (10^{-32} m^3)	$\Delta\overline{\chi}_{rh}$ (10^{-32} m^3)	Euler angles (radians)[b]		
298 K					
Tb	-2.7 ± 0.1	1.3 ± 0.1	2.81 ± 0.01	-2.11 ± 0.02	1.60 ± 0.03
Tm	1.7 ± 0.1	-0.8 ± 0.1	2.85 ± 0.05	-2.50 ± 0.08	1.33 ± 0.04
Dy	2.2 ± 0.1	-1.4 ± 0.1	1.10 ± 0.03	-1.64 ± 0.05	0.92 ± 0.06
278 K					
Tb	-2.9 ± 0.3	1.6 ± 0.3	2.73 ± 0.08	2.84 ± 0.08	1.86 ± 0.04
Tm	2.9 ± 0.2	-0.3 ± 0.2	-0.56 ± 0.07	0.11 ± 0.04	1.80 ± 0.22
Dy	4.2 ± 0.4	-2.0 ± 0.5	0.72 ± 0.12	0.86 ± 0.06	0.49 ± 0.07

[a] with respect to structure 1J7O

[b] with respect to structure 1J7P

Figure S1. ~~Pre~~ PREs observed in the presence of a gadolinium(III) ion in the second binding loop of the N-terminal domain of N60D CaM, for~~of CaM~~ residues in the C-terminal domain at 298 (A) and 278 (B) K.

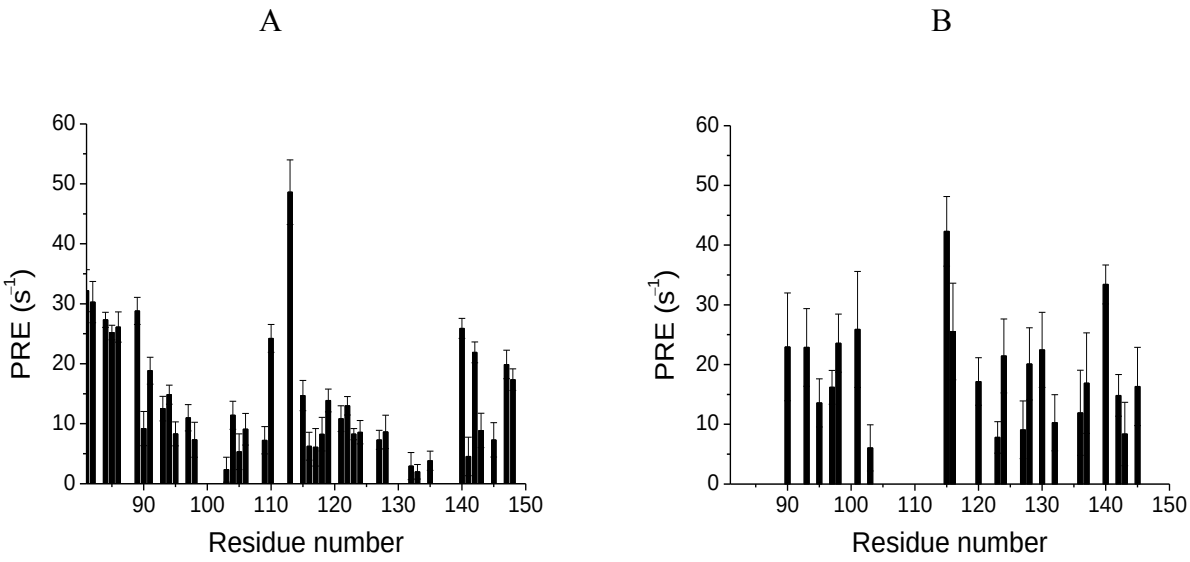


Figure S2. Agreement between the experimental ~~pre~~ PRE (A), ~~pcs~~ PCS (B) and ~~rdc~~ RDC (C) values of backbone amide protons at 298 K (A,B,C) and 278 K (D,E,F) and the averaged values calculated from the best fit families of conformations ~~calculated from the all sets of data~~. PRE-derived distances have been calculated using an effective correlation time of 6 and 10 ns at 298 and 279 K, respectively~~ns~~.

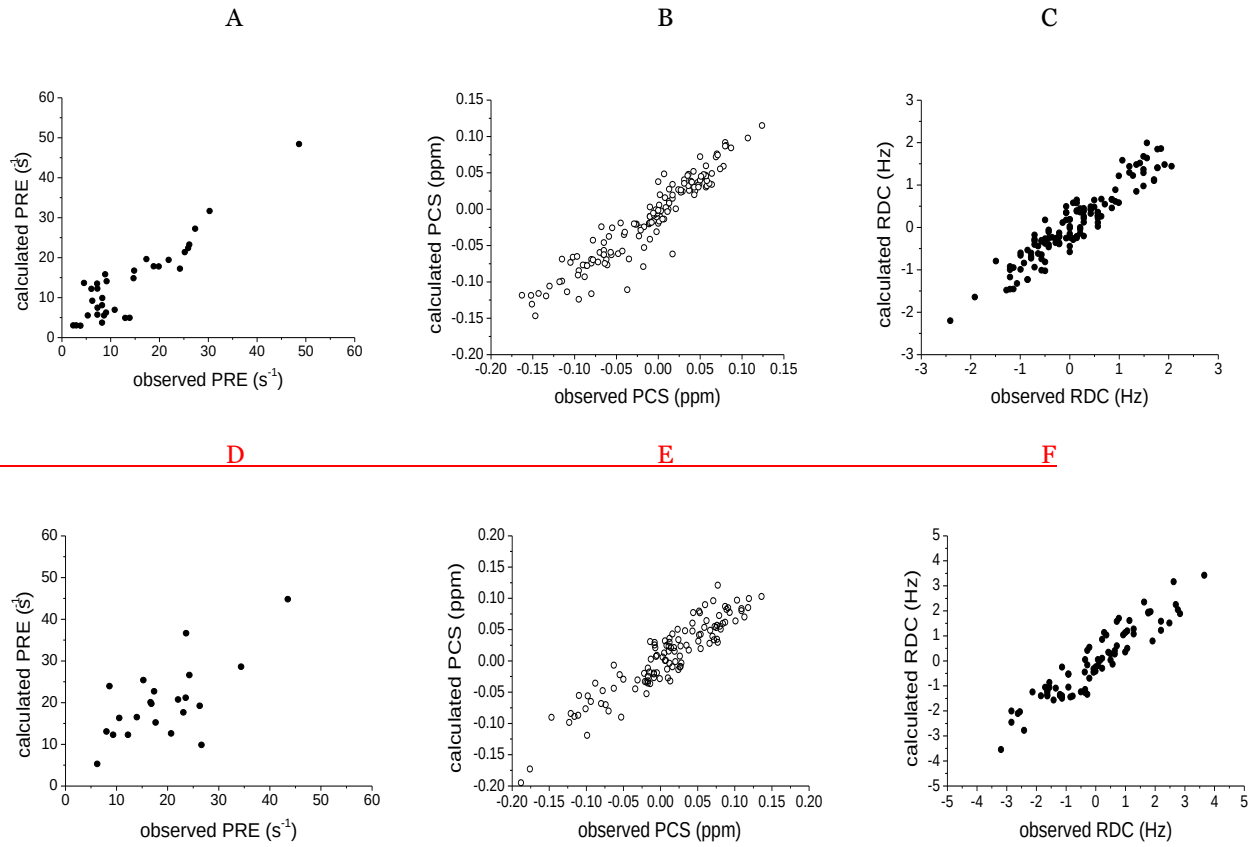
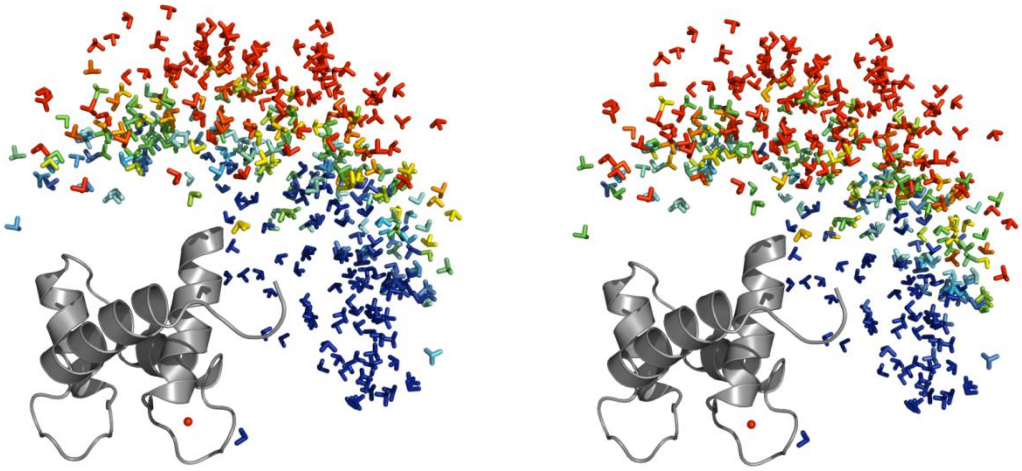


Figure S3. MO calculated in the slow exchange limit (range 0-1) at 298 K (A) and at 278 K (B) using PRE data only.



A B

Figure S4. Observed versus calculated ~~pcs~~ PCS values of backbone amide protons at 278 K of N-terminal domain nuclei for the three lanthanide-substituted CaM samples. Corresponding magnetic susceptibility anisotropy tensor parameters are reported in Table S1.

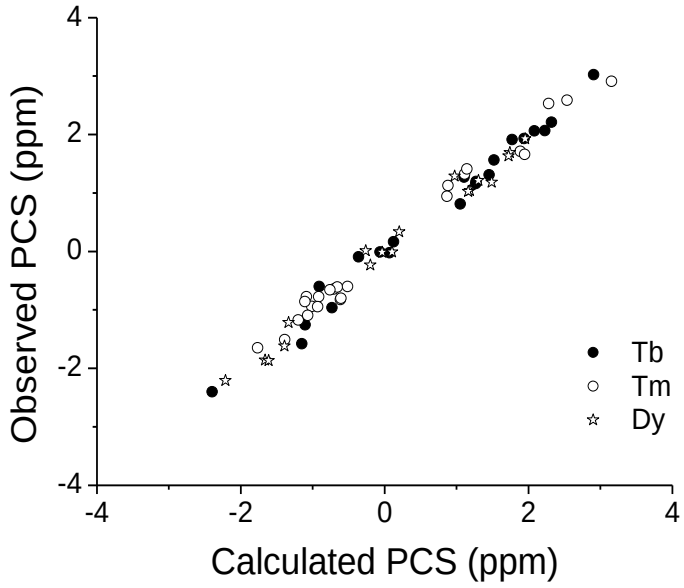


Figure S5. Observed versus calculated ~~rdc~~ RDC values of C-terminal domain nuclei for the three lanthanide-substituted CaM samples at 278 K. The fit has been performed using the structure 1J7P. Corresponding average tensor parameters are reported in Table S1. Since the RDCs do not depend on the position of the metal ion, RDCs can in fact be fitted to the protein structure even in the presence on interdomain motion, and the resulting tensors are average tensors with magnitude reduced with respect to the paramagnetic susceptibility tensors.

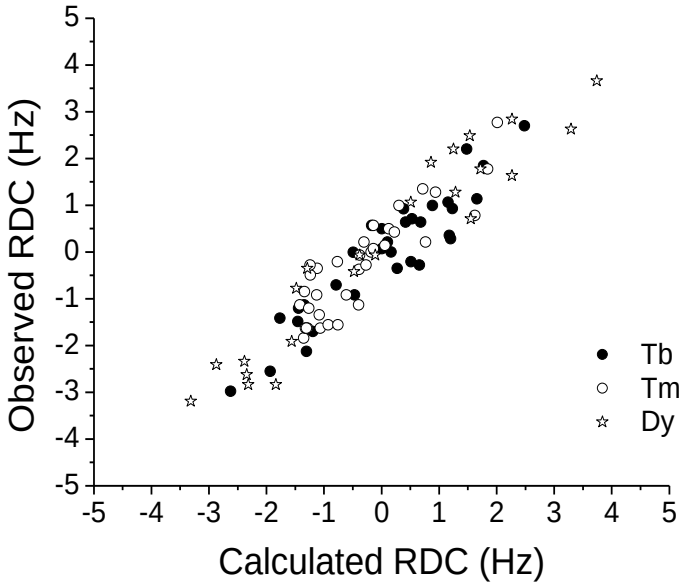


Figure S6. Comparison of the ~~pcs~~ PCS values of backbone amide protons for the three lanthanide metal ions substituted in the N-terminal domain of CaM at 278 K (red bars) and 298 K (empty bars).

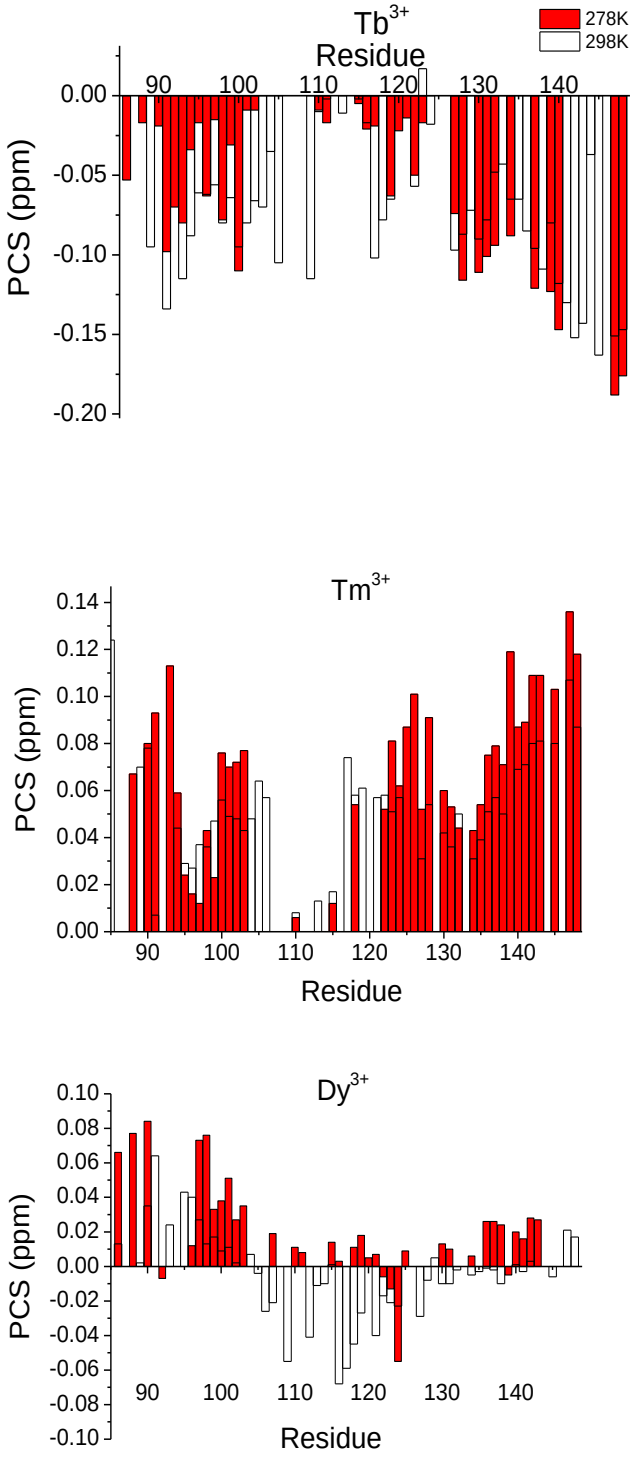


Table S2. Backbone amide proton ~~Pcs~~ PCS (in ppm) of N60D CaM ~~with~~ measured when Tb^{3+}, Tm^{3+} or Dy^{3+} ions are substituted to the calcium(II) ion in the second binding site of the N-terminal domain, at 278 K.

Residue Number	Tb	Tm	Dy
2	-	-0.705	-
3	0.81	-1.087	-
4	-	-1.01	1.691
5	1.16	-0.613	1.213
6	1.31	-0.606	1.183
7	1.196	-	1.038
8	1.27	-0.655	-
9	1.928	-	1.633
10	2.065	-1.199	-
11	1.565	-0.914	1.06
12	1.914	-1.108	1.288
13	3.024	-1.387	1.926
14	2.061	-1.068	1.026
15	-	-0.76	-
16	-	-	0.333
17	2.211	-	-
18	-	-0.512	-
19	0.166	-	-
21	-	-	-2.21
22	-0.603	-0.93	-
23	-	-1.763	-
25	-	-	-0.013
26	-0.026	-	-
29	-	-	-0.017
31	-	1.112	-
33	-	3.157	-
34	-	1.887	-
35	-	1.947	-
36	-2.403	2.282	-
38	-1.581	-	-1.861
40	-	0.881	-1.618
41	-	-	-1.868
42	-1.256	1.147	-
44	-0.964	-	0.009
45	-	0.868	-
48	-0.093	-	-
49	-	2.539	-

Residue Number	Tb	Tm	Dy
51	-	-	-0.236
60	-0.01	-	-
83	-	-	-0.099
86	-0.053	-	0.066
88	-0.017	0.067	0.077
90	-0.019	0.08	0.084
91	-0.098	0.093	-
92	-0.07	-	-0.007
93	-0.08	0.113	-
94	-0.034	0.059	-
95	-0.017	0.024	-
96	-0.063	0.016	0.012
97	-0.015	0.012	0.073
98	-0.078	0.043	0.076
99	-0.031	0.023	0.033
100	-0.11	0.076	0.038
101	-0.009	0.07	0.051
102	-0.009	0.072	0.027
103	-	0.077	0.035
107	-	-	0.019
110	-0.009	0.006	0.011
111	-0.017	-0.008	0.008
113	-	-0.001	-
115	-0.005	0.012	0.014
116	-0.021	-0.008	0.003
117	-0.019	-	-
118	-	0.054	0.011
119	-0.063	-	0.018
120	-0.022	-0.014	0.005
121	-0.014	-	0.007
122	-0.05	0.052	-0.006
123	-0.017	0.081	-0.013
124	-	0.062	-0.055
125	-	0.087	0.009
126	-	0.101	-
127	-0.074	0.052	-
128	-0.116	0.091	-
130	-0.111	0.06	0.013

Residue Number	Tb	Tm	Dy
131	-0.101	0.053	0.01
132	-0.094	0.044	-
134	-0.088	0.043	0.006
135	-	0.054	-
136	-	0.075	0.026
137	-0.121	0.079	0.026
138	-	0.071	0.024
139	-0.123	0.119	-0.005
140	-0.147	0.087	0.02
141	-	0.089	0.016
142	-	0.109	0.028
143	-	0.109	0.027
145	-	0.103	-
147	-0.188	0.136	-
148	-0.176	0.118	-

Table S3. N-^{H}N ~~Rdc~~ RDC (in Hz) of N60D CaM with Tb^{3+}, Tm^{3+} or Dy^{3+} substituted to calcium(II) in the second binding site of the N-terminal domain, at 278 K and 700 MHz.

C-terminal domain RDC			
Residue Number	Tb	Tm	Dy
88	-	0.497	-
90	-1.135	0.78	-
91	0.639	0	-
92	-0.007	-	-
93	-2.554	2.767	-
94	-	1.277	-
95	1.135	0.425	-
96	0.497	-1.845	-2.838
97	-	-0.372	-
98	0.355	-1.348	3.657
99	-	-0.355	-
100	2.199	-1.632	2.199
101	-	0.213	1.064
102	0.709	-1.135	1.774
103	-	-0.497	1.916
106	-	-0.213	-
110	-	-1.135	-
113	-	-0.284	-
115	0.639	-	-2.838
116	-0.213	0.142	-3.193
118	-	-	0.71
119	-1.419	-	2.838
121	-1.206	-	1.277
122	-	0.992	-
123	-2.129	-1.561	-
125	-	-	2.483
127	-0.922	-1.206	-
128	-1.703	-0.071	-
129	-	0.213	-
130	0.071	1.774	-0.78
131	1.845	0.568	1.632
132	-0.355	0.071	-
134	0.284	-1.561	-0.071
135	0.213	-0.851	-
136	1.064	-1.632	2.625
137	2.696	-0.922	-
140	0.926	-0.919	-2.625
141	0.993	-1.632	-2.412
143	-0.284	-1.632	-0.355
145	-0.284	-	-

Table S4. Amide proton ~~T_2~~ R_2 ~~pre~~ PRE (in s^{-1}) of N60D CaM with Gd^{3+} substituted to calcium(II) in the second binding site of the N-terminal domain, at 278 K and 298 K (500 MHz).

Residue Number	298K	278K
4	40.90	-
5	47.88	-
6	49.32	59.25
9	40.07	-
10	65.60	-
35	42.50	
39	33.77	-
40	53.31	-
41	22.74	-
42	54.04	68.3
44	54.36	72.7
45	63.81	-
77	-	57.57
78	-	40.74
83	30.28	-
84	27.32	-
85	25.20	-
86	26.11	-
90	9.18	22.95
91	18.83	-
93	-	22.87
94	14.84	-
95	8.31	13.57
97	-	16.19
98	7.31	23.57
101	-	25.88
103	2.33	6.05
105	5.32	-
106	9.07	-
109	7.23	-
110	24.21	-
113	48.60	-
115	14.70	42.30
116	6.25	25.54
117	6.07	-
118	8.25	-

Residue Number	298K	278K
120	-	17.16
121	10.83	-
122	12.98	-
123	8.26	7.80
124	-	21.44
127	7.30	9.07
128	8.64	20.10
130	-	22.44
132	2.94	10.25
135	3.82	-
136	-	11.91
137	-	16.88
140	25.90	33.41
141	4.53	-
142	21.89	14.83
143	8.87	8.37
145	7.30	16.34
147	19.87	-
148	17.34	-

119	13.88	-		