Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Analysis of the solution conformations of T4 lysozyme by paramagnetic NMR spectrosocopy

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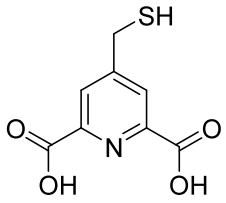

Figure S1. Chemical structure of the lanthanide binding tag 4MMDPA

Table S1. PCSs of backbone amide protons of T4-L-4MMDPA complexed with Tb^{3+}, Tm^{3+}, and Yb^{3+} [a]

Residue	PCS(Tb)/ppm	PCS(Tm)/ppm	PCS(Yb)/ppm
N2	-0.07	0.06	0.03
I3	-0.06	0.05	0.03
F4	-0.10	0.08	0.04
E5	-0.11	-	0.05
M6	-	0.07	0.03
L7	-0.12	0.10	0.05
R8	-0.14	0.14	0.06
I9	-0.14	0.11	0.06
D10	-0.11	0.11	0.05
E11	-0.13	0.14	0.08
G12	-	0.17	0.09
L13	-	0.23	0.11
R14	-0.36	0.37	0.19
L15	-0.29	0.33	0.16
K16	-0.32	0.39	0.21
I17	-0.32	0.49	0.28
Y18	-0.45	0.56	0.31
K19	-0.38	0.22	0.14
D20	-	0.38	0.19
T21	-	0.22	-
E22	-	0.22	-
G23	-	0.20	0.09

Y24	-	0.35	-
Y25	-	0.67	-
T26	-	0.74	0.39
I27	-	1.02	0.52
G28	-0.59	0.62	0.31
I29	-0.51	0.48	0.25
G30	-	0.43	0.20
H31	-0.67	0.69	0.34
L32	-	0.82	0.44
L33	-	-	0.98
S38	-	-	-0.57
N40	-	-	-0.70
I50	-	-	0.97
G51	-	1.80	0.61
R52	-1.45	1.27	0.43
N53	-0.53	-	0.18
G56	-0.19	0.58	0.31
V57	-0.30	0.53	0.26
I58	-0.45	-	-
T59	-0.53	0.50	0.22
K60	-0.31	0.29	0.13
D61	-0.33	0.29	0.13
E62	-0.48	0.41	0.18
A63	-0.46	0.47	0.22
E64	-0.43	0.35	0.16
K65	-0.49	0.36	0.17
L66	-0.71	-	-
F67	-0.49	0.39	0.19
N68	-0.31	0.20	0.11
Q69	-0.29	0.14	0.09
D70	-0.23	0.13	0.10
V71	-0.08	0.04	0.04
D72	0.05	-0.09	0
A73	-	-0.23	-0.07
A74	0.20	-0.16	-0.05
V75	0.18	-0.15	-0.06
R76	0.29	-0.25	-0.09
G77	0.36	-0.28	-0.10
I78	0.25	-0.12	-0.06
L79	0.24	-0.19	-0.07
R80	0.28	-0.22	-0.09
N81	0.24	-0.19	-0.07
A82	0.20	-0.12	-0.05
K83	0.15	-0.09	-0.04
L84	0.14	-0.09	-0.03

K85	0.15	-0.11	-0.04
V87	0.09	-0.06	-0.02
Y88	0.09	-0.06	-0.02
D89	0.09	-0.07	-0.02
S90	0.06	-0.05	0
R96	-	0	-
A97	0	0	0
A98	0	0	0
L99	0.02	0	0
I100	0.02	0	0
N101	0.01	0.01	0.01
M102	0	0.01	0.01
F104	-	0.02	0.02
Q105	-	0.06	0.03
M106	0.01	0.04	0.02
E108	0.22	-0.11	-0.05
T109	0.19	-0.09	-0.05
G110	0.10	-0.03	-0.02
V111	0.09	-0.03	0
A112	0.11	-0.03	-0.01
G113	0.07	-0.02	0
F114	-	-	0
T115	0.04	0	0
S117	0.02	-	0
L118	0.04	-0.01	0
R119	0.04	-0.02	0
M120	0.03	0	0
L121	0.03	-	-
Q122	0.03	-	-
Q123	0.03	0	0
A129	0	0	0
A130	0	0	0
V131	0	0	-
N132	0	0	0
L133	0	0	0
A134	0	0	0
W138	-0.01	-	0
Y139	-0.02	0.03	0.02
N140	-0.03	0.03	0.02
Q141	-0.04	0.04	0.02
N144	-0.05	0.05	0.04
R145	-0.06	0.06	0.03
A146	-0.04	0.05	0.03
K147	-0.04	0.04	0.02
R148	-0.04	0.05	0.03

V149	-0.03	0.05	0.03
I150	-0.02	0.02	0.01
T151	-0.01	0.02	0.02
T152	-0.01	0.02	0.02
F153	0	0	0
R154	0	-	0
T155	0	0	0
D159	-0.02	0.02	0.02
A160	-0.03	0.03	0.03
Y161	-0.04	0.03	0.02
K162	-0.04	0.04	0.03

[a] From ^{15}N-HSQC spectra recorded in 20 mM MES buffer, pH 6.5, at 25 ^{o}C and a ^{1}H NMR frequency of 600 MHz. PCSs were measured as the chemical shift of the paramagnetic samples minus the chemical shift of the diamagnetic reference with Y^{3+}. Dashes indicate cross-peaks vanishing due to PRE.

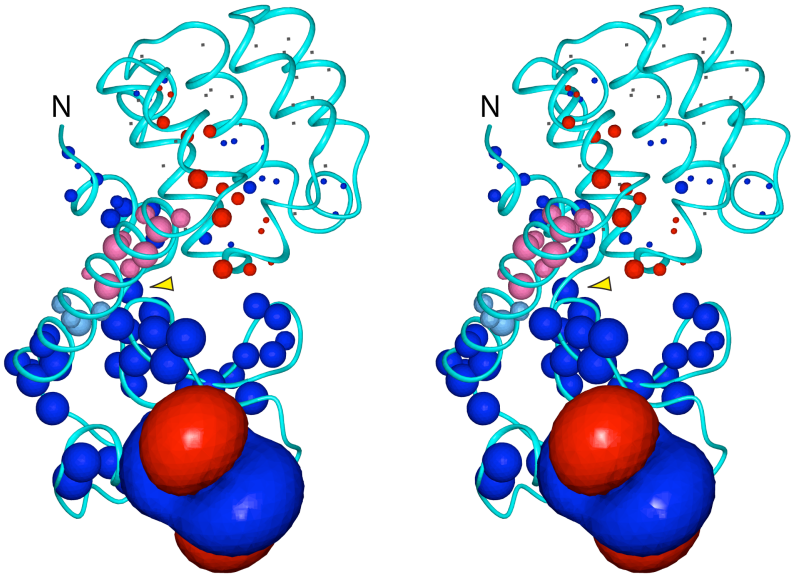

Fig. S2 $\Delta\chi$ tensor generated by Tm^{3+} plotted on a ribbon representation of the crystal structure 2LZM of wild-type T4-L. The stereo view represents the $\Delta\chi$ tensor by PCS isosurfaces (blue: +5 ppm, red: -5 ppm) and T4-L by a ribbon representation. Amide protons with positive and negative PCSs are shown as blue and red spheres, respectively, with larger spheres corresponding to larger PCSs. Amide protons for which the PCS was small (up to ±0.02 ppm) are shown as small grey spheres. The N-terminus is marked. The amide protons of residues 66-81 in the helical segment connecting the N- and C-domains are shown in lighter colours. PCSs from this segment were not used in the back-calculations to avoid biasing the structure selection. The yellow arrow marks the amide of residue 13. The N-terminal helix up to residue 13 was considered to be part of the C-domain.

Table S2. RDCs of backbone amides measured for T4-L-4MMDPA complexed with Tb^{3+} and Tm^{3+} [a]

Residue	RDC (Tm^{3+}) / Hz	Estimated error / Hz	RDC (Tb^{3+}) / Hz	Estimated error / Hz
N2	-4.4	0.8	4.1	0.9
I3	-3.3	0.8	4.0	0.8
F4	-1.3	0.9	-1.1	0.8
E5	-4.3	0.8	2.2	0.7
M6	-2.7	1.0	4.4	1.0
R8	-2.2	1.3	1.0	1.0
D10	-3.8	1.0		
E11	-1.6	2.0		
R14	-0.9	1.4	0.6	0.9
L15	0.9	1.1	-0.9	0.8
K16	2.1	0.8	-0.5	0.8
Y24	-0.2	1.6	0.9	0.5
Y25	-0.5	1.7		
I27	-3.9	2.0		
G28	-2.6	1.3	4.7	1.5
R52	1.4	2.2		
N53	1.3	1.8		
T54	-0.9	1.5		
G56	-1.2	2.2		
T59	-0.3	0.9	-1.2	0.8
K60	1.0	0.9	0.9	0.8
D61	-1.5	0.4	3.2	0.8
E62	-2.1	1.0	5.1	0.7
E64	1.7	0.8	2.4	0.8
K65			5.7	1.0
A82	-0.7	1.1	-2.8	2.0
K83	-3.5	0.8		
L84	0.0	0.6	-2.6	0.7
K85	2.3	0.7	-4.9	0.8
V87	1.3	0.6	-3.3	0.5
Y88	3.4	0.7	-4.7	0.6
D89	2.7	0.7	-5.5	0.6
S90	0.9	0.7	-2.1	0.6
L91	0.4	0.7	-2.7	0.8
D92	1.1	0.5	-1.7	0.4
R96	-0.7	0.6	-1.2	0.5
A97	0.2	0.4	-2.9	0.4
A98	-1.9	0.5	1.9	0.6

L99	-1.2	0.5	-0.1	0.6
I100	0.3	0.7	-0.2	0.8
N101	0.0	1.0	-1.2	1.0
G107	2.9	0.8	-3.1	1.4
E108	1.3	0.8	0.6	0.9
T109	-0.8	0.7	3.9	0.7
G110	0.2	0.7	1.9	0.9
V111	1.6	0.6	0	0.7
G113	-2.0	0.5	5.2	0.6
T115	-0.2	0.5	-1.7	0.7
S117			0.4	0.9
L118	0.7	0.6	-2.3	0.6
R119	-0.6	0.4	-2.6	0.4
M120	-2.6	0.7	1.1	0.5
L121			-2.6	0.3
Q123	-0.9	0.5	1.3	0.5
K124	-2.1	0.7	-0.4	0.9
W126	-0.9	0.3	0.3	0.4
E128	-2.6	0.4		
A129	-1.2	0.4	0.4	0.5
A130	-1.6	0.4	0.2	0.3
V131	-3.0	0.3	3.2	0.4
L133			-0.3	0.6
A134	-1.9	0.5		
K135	-4.1	0.8	2.3	0.8
W138	-0.5	0.7		
Y139	-0.8	0.9	3.3	0.8
N140			0.1	0.4
Q141	-3.8	0.7	4.0	0.5
T142	-4.0	0.6	4.9	0.6
N144	-3.2	1.0		
A146	-4.1	0.7	2.0	0.5
K147	-4.6	0.8		
R148	-5.1	0.8	3.5	0.6
V149	-3.8	0.8	2.0	0.7
I150	-3.6	0.7	1.9	0.6
T151	-5.9	0.5	5.7	0.5
T152	-2.3	0.9	1.4	0.6
F153	-2.6	0.5	1.6	0.4
R154	-3.0	0.7	4.8	0.5
T155	-4.1	0.7	3.9	0.5
G156	-1.0	0.6	-0.7	0.4
T157	-0.2	0.5	-1.2	0.4
W158	2.1	0.7		

D159	-4.2	1.0	5.8	0.7
A160			1.0	0.6
Y161	-4.4	0.7	4.2	0.6
K162	-1.2	0.8	3.7	0.7
N163	-0.7	0.7		
L164	-3.3	0.5	3.2	0.7

[a] Measured at a ^{1}H NMR frequency of 600 MHz. Uncertainties were estimated according to the protocol in ref. 1.

Table S3. PDB structures of lysozyme used to analyse the paramagnetic NMR data. The four-letter PDB accession codes are given followed by an underscore and the chain identifier.

102L_A,103L_A,104L_A,104L_B,107L_A,108L_A,109L_A,110L_A,111L_A,
112L_A,113L_A,114L_A,115L_A,118L_A,119L_A,120L_A,122L_A,123L_A,
125L_A,126L_A,127L_A,128L_A,129L_A,130L_A,131L_A,137L_A,137L_B,
138L_A,139L_A,140L_A,141L_A,142L_A,143L_A,144L_A,145L_A,146L_A,
147L_A,148L_E,149L_A,150L_A,150L_B,150L_C,150L_D,151L_A,152L_A,
155L_A,156L_A,157L_A,158L_A,159L_A,160L_A,161L_A,162L_A,163L_A,
164L_A,165L_A,166L_A,167L_A,167L_B,168L_A,168L_B,168L_C,168L_D,
168L_E,169L_A,169L_B,169L_C,169L_D,169L_E,170L_A,171L_A,172L_A,
173L_A,174L_A,174L_B,175L_A,175L_B,176L_A,176L_B,177L_A,178L_A,
180L_A,180L_B,181L_A,182L_A,183L_A,184L_A,185L_A,186L_A,187l_A,
188L_A,189L_A,190L_A,191L_A,192L_A,195L_A,196L_A,197L_A,198L_A,
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3LZM_A,4LZM_A,5LZM_A,6LZM_A,7LZM_A

References

1 G. Kontaxis, G. M. Clore and A. Bax, *J. Magn. Reson*., 2000, **143**, 184–196.