Supporting Information

Lanthanide-binding peptides for NMR measurements of residual dipolar couplings and paramagnetic effects from multiple angles

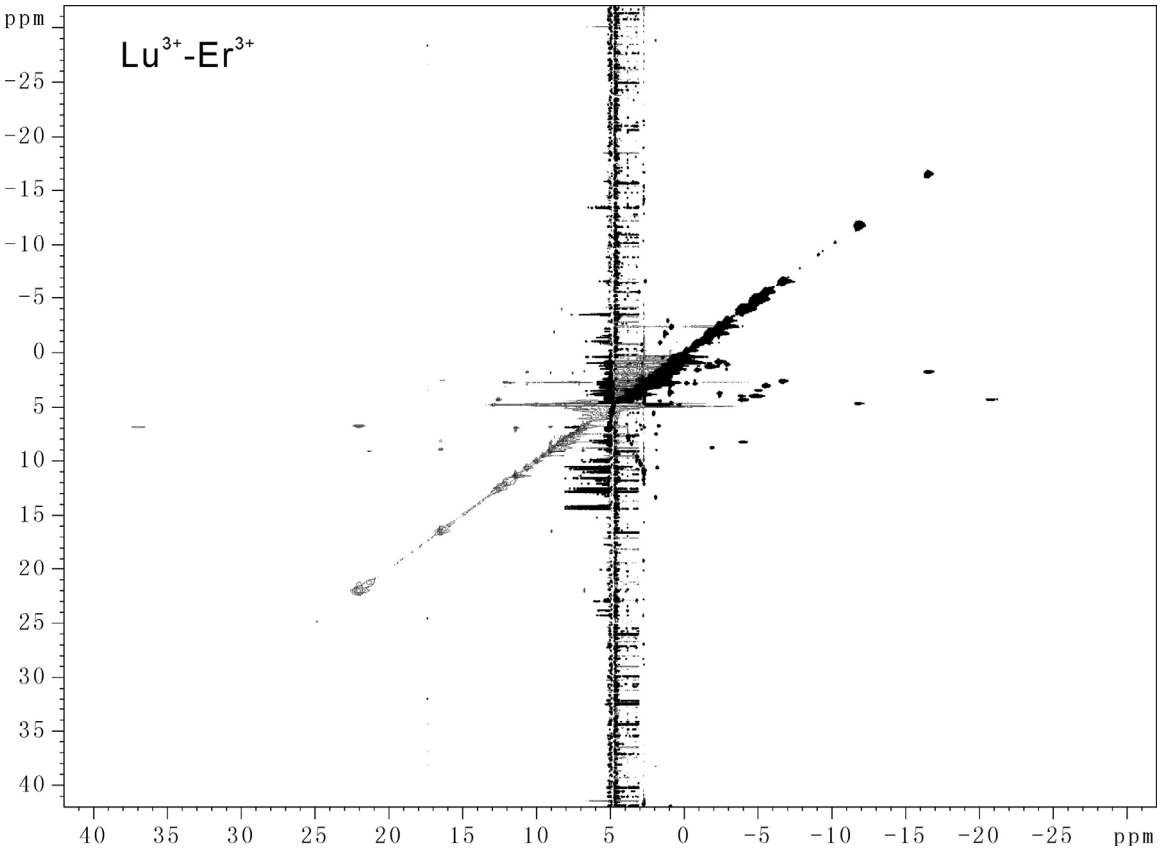
Xun-Cheng Su, Kerry McAndrew, Thomas Huber, Gottfried Otting

Table S1 ^{1}H chemical shifts of the LBT4-Lu^{3+} complex[a]

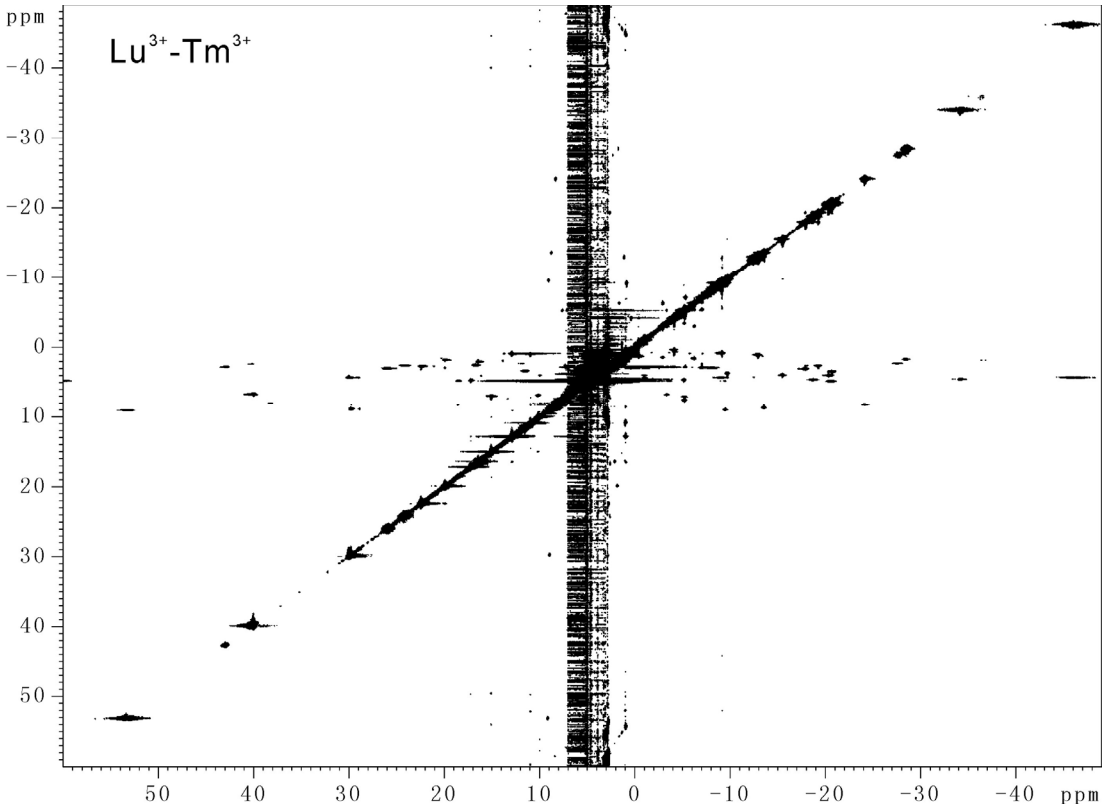
residue	H^N	H^α	H^β	others			
Cys 0							
Tyr 1		4.70	2.69 1.76	C^dH_2	6.92	$C^\varepsilon H_2$	6.76
Val 2	8.90 (8.90)	4.31	2.04	$C^\gamma H_3$	0.93 0.91		
Asp 3	9.10 (9.07)	4.87	2.81 2.24				
Thr 4	8.05 (7.93)	3.96	4.30	$C^\gamma H_3$	1.30		
Asn 5	8.37 (8.25)	4.85	3.34 2.92	$N^\delta H_2$	8.35 6.95		
Asn 6	8.01 (7.88)	4.31	3.05 2.58				
Asp 7	8.10 (7.93)	4.65	3.02 2.37				
Gly 8	9.56 (9.45)	4.04 3.49					
Ala 9	7.77 (7.61)	4.48	1.20				
Tyr 10	9.16 (9.10)	5.58	2.71 2.52	$C^\delta H_2$	6.89	$C^\varepsilon H_2$	6.73
Glu 11	8.54 (8.41)	4.67	2.33 1.81	$C^\gamma H_2$	2.39 1.92		
Gly 12	8.78 (8.72)	3.96 3.70					
Asp 13	9.01 (9.00)	4.58	2.85 2.80				
Glu 14	8.26 (8.14)	4.42	2.57 2.27	$C^\gamma H_2$	2.89 2.82		
Leu 15	7.06 (6.91)	4.02	1.59 1.38	$C^\gamma H$	1.06	$C^\delta H_3$	0.78 0.27

[a] In ppm at 25 °C. H^N chemical shifts at 10 °C are shown in brackets.

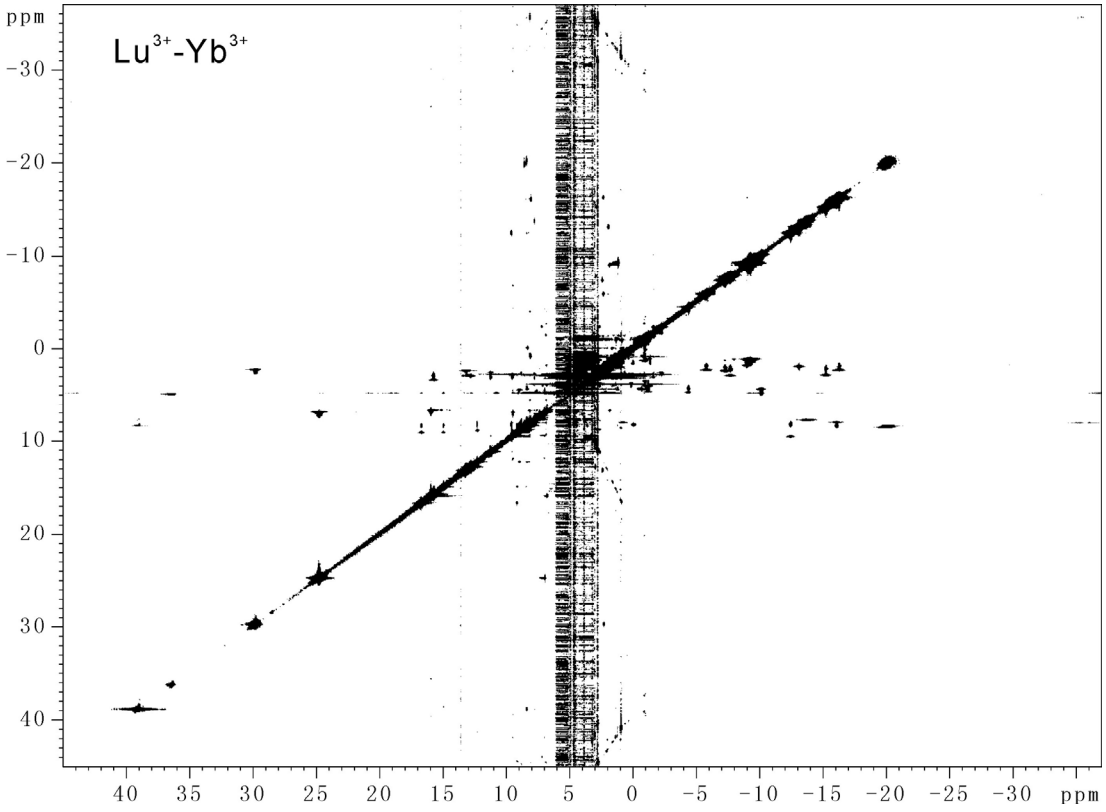
Figure S1. EXSY spectra of complexes of LBT4 with mixtures of Lu^{3+} and paramagnetic lanthanide ions at 25 ^{o}C. The peptide concentration was 1.5 mM in the presence of 4 mM glycine, 4 mM DTT, and 20 mM MES at pH 6.5. The mixing time was 5 ms. (a) [LBT4] : [Lu^{3+}] : [Er^{3+}] = 1 : 0.5 : 0.6; (b) [LBT4] : [Lu^{3+}] : [Tm^{3+}] = 1 : 0.5 : 0.6; (c) [LBT4] : [Lu^{3+}] : [Yb^{3+}] = 1 : 0.5 : 0.6.



(a)


Lu3+-Tm3+
ppm
-40
-30
-20
-10
0
10
20
30
40
50
50 40 30 20 10 0 -10 -20 -30 -40 ppm

(b)


Lu3+-Yb3+
ppm
-30
-20
-10
0
10
20
30
40
40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 -30 ppm

(c)

Table S2. Chemical shifts of backbone amide protons of LBT4 in complexes with Er^{3+}, Yb^{3+}, or Tm^{3+} [a]

	25 °C			10 °C	
residue	Er^{3+}	Yb^{3+}	Tm^{3+}	Yb^{3+}	Tm^{3+}
Val 2	16.48	12.24	29.72	12.79	33.41
Asp 3	21.35	14.91	53.18	15.84	61.26
Thr 4		0.74	38.30	0.50	
Asn 5		-20.32			
Asn 6		-16.14		-18.91	
Asp 7		-35.80			
Gly 8		-12.51		-14.28	
Ala 9		2.70			
Tyr 10		16.66		16.37	
Glu 11		-19.92		-22.56	
Gly 12	-1.90	2.54	-13.64	1.99	-16.34
Asp 13	1.84				-12.11
Glu 14	-3.96	-0.09	-24.21	-0.87	-28.88
Leu 15	2.87	4.39	-5.31	3.58	-7.30

[a] Chemical shifts in ppm.

Table S3. PCS of backbone amide protons of ArgN-LBT-Tm^{3+} complexes at 10 ^{o}C [a]

residue	ArgN-LBT2	ArgN-LBT3	ArgN-LBT4	ArgN-LBT5
Leu 10	0.29	-0.20	0.28	0.35
Phe 11		-0.23	0.34	
Lys 12	0.44		0.41	0.52
Ala 13	0.55	-0.23	0.46	0.61
Phe 14	0.60	-0.30	0.53	0.73
Lys 15	0.79	-0.41	0.74	0.97
Ala 16	0.87	-0.36	0.81	
Leu 17		-0.36	0.81	
Leu 18	1.17	-0.58	1.01	
Lys 19	1.41			
Ser 24		-0.21	0.51	
Ser 25	0.66			
Gln 26		-0.37		0.37
Gly 27	0.29	-0.26	0.21	0.33
Glu 28	0.42	-0.27	0.29	0.46
Ala 29	0.50	-0.29	0.38	0.63
Phe 30		-0.25	0.31	0.49
Ala 31	0.36	-0.14	0.32	0.40
Ala 32	0.41	-0.19	0.34	
Leu 33	0.41	-0.19	0.36	
Gln 34	0.32	-0.15	0.26	0.42
Glu 35	0.32	-0.12	0.28	
Gln 36	0.33	-0.12	0.24	0.42
Gly 37	0.28	-0.09	0.24	0.37
Phe 38	0.28	-0.11	0.19	0.36
Asp 39	0.27	-0.14	0.15	0.26
Asn 40		-0.12	0.18	0.22
Ile 41		-0.13	0.17	
Asn 42	0.22	-0.19	0.11	0.26
Gln 43	0.21			0.22
Ser 44	0.18	-0.19	0.14	0.18
Lys 45	0.23	-0.21	0.19	0.22
Val 46	0.33	-0.26	0.20	
Ser 47	0.35	-0.30	0.15	0.30
Arg 48	0.25	-0.31	0.24	0.24
Met 49	0.35	-0.38	0.26	0.36
Leu 50	0.57	-0.53	0.25	
Thr 51	0.48	-0.57	0.24	0.33
Lys 52	0.43	-0.55	0.50	
Phe 53	0.77	-0.74	0.52	
Gly 54	0.87	-0.98	0.35	0.43
Ala 55	1.29	-1.21		
Val 56	2.68			
Lys 62	1.05			
Met 63	0.97			
Met 65	0.74			
Val 66				1.27
Tyr 67	1.38			
Cys 68	2.55			
Leu 69	2.99			

[a] PCS values in ppm.

Table S4. RDCs of backbone amides measured for ArgN-LBT complexes with Tm^{3+} [a]

residue	ArgN-LBT2	ArgN-LBT3	ArgN-LBT4	ArgN-LBT5
Leu 10	-2.8	-6.8	-5.1	-0.7
Phe 11		-5.6	-6.2	
Lys 12	8.4	-9.6	1.7	6.7
Ala 13	0.8	-9.2	-1.5	4.2
Phe 14	-4.7	-4.6	-3.3	-1.7
Lys 15	-0.9	-5.0	-3.4	-2.7
Ala 16	8.4	-10.9	1.9	
Leu 17			-2.3	
Leu 18	-7.7	-4.0		
Ser 24	13.6			
Gly 27	2.2		2.8	4.8
Glu 28	22.6	-0.7	16.0	21.4
Ala 29	10.9	6.6	10.0	9.8
Phe 30		4.1		6.0
Ala 31	9.4		2.8	
Leu 33	8.7	5.2		
Gln 34	5.8	2.8	1.9	4.3
Glu 35	17.8	-5.2	12.1	17.8
Gln 36		4.5		10.4
Phe 38	-1.8	-8.6		2.6
Asp 39		0.3		-6.4
Asn 40	-2.3	0.6		-4.8
Asn 42		-1.9		-6.6
Gln 43				-3.2
Ser 44	7.4	-7.8	7.8	8.2
Lys 45	13.5	-4.5	12.6	7.9
Val 46	-2.5		-0.3	
Ser 47	3.6	-3.7	4.2	2.5
Arg 48	16.5	-7.5	13.7	13.3
Met 49	6.8	-2.6	11.0	
Leu 50	-3.4			-4.0
Thr 51	3.2	-5.4	4.5	4.1
Lys 52	12.5	-4.5		
Phe 53	-2.2	-1.0	0.3	-6.2
Gly 54	-8.8	0.1		-2.0
Met 65	-9.3			

[a] RDCs measured in Hz as the splitting observed for the Lu^{3+} complex minus the splitting observed for the Tm^{3+} complex at 10 ^{o}C and a ^{1}H NMR frequency of 800 MHz.

Table S5. $\Delta\chi$ tensors of ArgN-LBT-Tm^{3+} complexes obtained by fitting the PCS of the amide protons of ArgN (Table S3) with the solution structure of ArgN [a]

construct	χ_{ax}	χ_{rh}	χ_{xx}	χ_{yy}	χ_{zz}	tensor axis	coordinates of tensor axes		
ArgN-LBT2	23.1	14.4	-0.5	-14.9	15.4	x	-0.618	0.523	-0.587
						y	-0.173	0.638	0.751
						z	0.767	0.565	-0.304
ArgN-LBT3	-13.3	-2.7	3.1	5.8[b]	-8.9[b]	x	-0.235	0.584	-0.777
						y	-0.728	0.424	0.538
						z	0.644	0.693	0.326
ArgN-LBT4	22.8	13.6	-0.8	-14.4	15.2	x	-0.670	0.509	-0.540
						y	-0.049	0.696	0.717
						z	0.740	0.507	-0.442
ArgN-LBT5	22.0	5.9	-4.4	-10.3	14.7	x	-0.336	-0.239	-0.911
						y	-0.658	0.751	0.046
						z	0.674	0.615	-0.410

[a] The tensor parameters are in units of 10^{-32} m^3. The axes refer to conformer 8 of the NMR structure of ArgN (PDB code 1AOY).

[b] The y and z axes of this tensor are swapped compared to the alignment tensor (see Figures 4 and S2) to satisfy the condition $|\chi_{zz}| > |\chi_{yy}| > |\chi_{xx}|$.

Figure S2. Stereoview of a ribbon representation of ArgN illustrating the Δχ tensor axes of different ArgN-LBT-Tm^{3+} complexes. The tensor axes are centered at the positions of the metal ions as determined by best fitting. The x, y, and z-axes (as defined in Table S5) are shown in cyan, blue, and red, respectively. The numbers refer to the LBT numbering in Table 1.

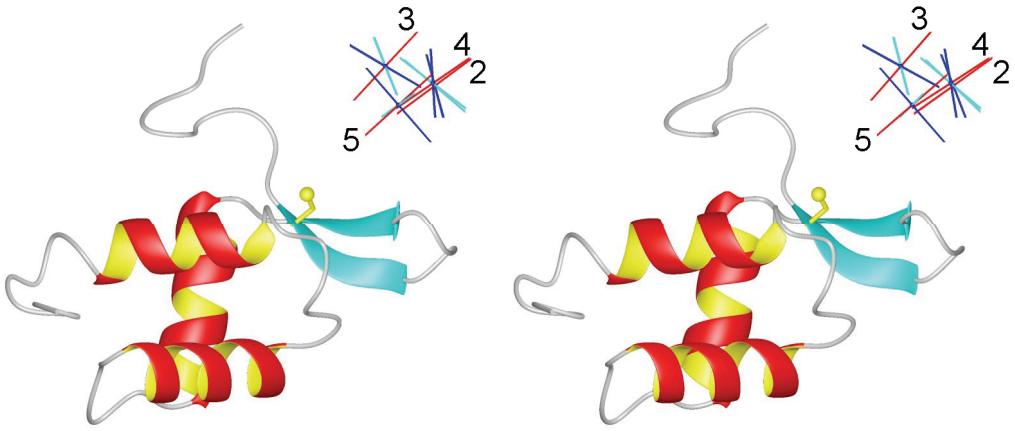
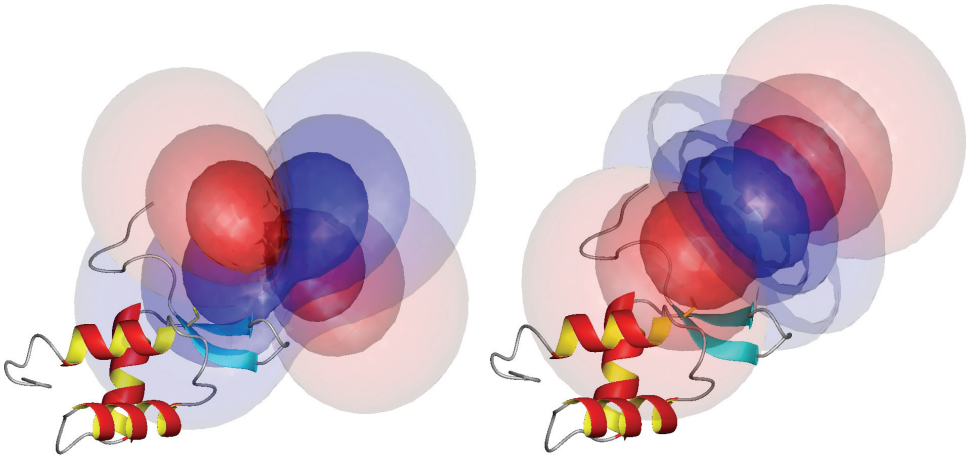


Figure S3. Isosurfaces of the PCS of Tm^{3+} complexes of (a) ArgN-LBT2 and (b) ArgN-LBT3. The isosurfaces were calculated for PCS of ±3, ±1 and ±0.3 ppm. Blue and red surfaces identify positive and negative PCS, respectively.



(a) (b)