

Supporting Information

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Tunable paramagnetic relaxation enhancements by $[\text{Gd}(\text{DPA})_3]^{3-}$ for protein structure analysis by NMR spectroscopy

Cloning of the GCN4 leucine zipper

The GCN4 leucine zipper was expressed as a fusion protein with His₆-ubiquitin followed by proteolysis with deubiquitinating enzyme (DUB) to yield an authentic N-terminus of the leucine zipper (1).

A synthetic gene of the GCN4 leucine zipper was ordered from GenScript (USA) using the most abundant codons for each of the 33 residues (RMKQLEDKVEELLSKNYHLENEVARLKKLVGER). The nucleotide sequence included restriction sites for *SacII* and *EcoRI* at the 5' and at the 3'-end, respectively.

The His₆-tagged ubiquitin from expression vector pHUE (1) was used to construct the fusion protein of ubiquitin and leucine zipper. The leucine zipper was fused to the C-terminus of ubiquitin using the *SacII* and *EcoRI* restriction sites. As the ubiquitin sequence from vector pHUE contains the *SacII* restriction site in the codons for residues 73-75 of ubiquitin, the DNA sequence of the leucine zipper had been designed with the additional nucleotides TGGT between the *SacII* site and the codon of its first residue (Arg) to restore the C-terminal residues of ubiquitin (Gly75 and Gly76) required for recognition by DUB. The final construct encoding the His₆-tagged fusion protein in vector pRSET-6d (2) was pKL1421.

For expression *in vivo*, the fusion construct was cloned into vector pETMCSI (3). Site-directed mutagenesis by overlap extension (4) was used to eliminate the internal *NdeI* site in the ubiquitin gene, using the forward primers (a)
5'-TTTTTT**CATAT**GGGCAGCAGGCCATCATC and reverse primers (c)
5'-GCGGCAGCCACATGCAGATCTTGTC and (b)
5'-AGATCTGCATGTGGCTGCCGCGCGG and (d)
5'-TTTGAATTCTAACGTTCACCAACCAGTTTC (bold – *NdeI* site; underlined – *EcoRI* site). The PCR product was digested with *NdeI* and *EcoRI*. Ligation with double-digested pETMCSI yielded pKL1426 and transformation of pKL1426 into *E. coli* BL21(DE3)*recA* yielded RSC2929.

Expression and purification

10 ml of culture containing strain RSC2929, 50 µg/l thymine and 100 mg/l ampicillin in LB medium were grown at 37 °C overnight. The overnight culture was used to inoculate 1 l of minimal

auto-induction medium consisting of 1 g $^{15}\text{NH}_4\text{Cl}$, 4.5 g glucose, 6.8 g KH_2PO_4 , 7.1 g Na_2HPO_4 , pH 6.8, 5 g glycerol, 2 g α -lactose, 1 mM MgSO_4 , trace metal mixture (5), and 100 mg/l ampicillin. The culture was grown at 37 °C for 2 days. After harvesting the cells at 7200 x g at 4 °C for 15 min, the pellet was suspended in 30 ml ice-cold buffer A (25 mM HEPES, pH 7.0, 300 mM NaCl, 15 mM imidazole, 10% glycerol) + 0.1 mM AEBSF. The cell suspension was lysed using a French press at 12,000 psi. The lysate was centrifuged at 38700 x g at 4 °C for 45 min. The supernatant was filtered (Sartorius 0.45 µm) before loading onto a HisTrap HP column (GE Healthcare, 5 ml) equilibrated with buffer A. After washing the column with 25 ml buffer A at 1 ml/min, a gradient from 15-500 mM imidazole was applied within 60 min. The His₆-tagged fusion protein eluted at about 210 mM imazole. The fractions containing the desired fusion protein were pooled and His₆-tagged DUB¹ at a enzyme:substrate ratio of 1:1000 was added. The mixture was dialyzed against 2 x 2 l of buffer A containing 1 mM β -mercaptoethanol at pH 7.5 (Spectra/Por membrane, MWCO 3500). After dialysis, the sample was spun at 38700 x g at 4 °C for 20 min. The supernatant was loaded at 1 ml/min onto a HisTrap HP column (GE Healthcare, 5 ml) equilibrated with buffer A and the flow-through was collected. Washing the column with 25 ml buffer A at the same flow rate, remaining leucine zipper was eluted in the flow-through. The combined fractions yielded 14 mg of highly-purified and ^{15}N -labeled leucine zipper which was desalting by dialysis against 3 x 2 l Milli-Q water at 4 °C followed by concentrating in a centrifugal filter device (Millipore, MWCO 3000).

References:

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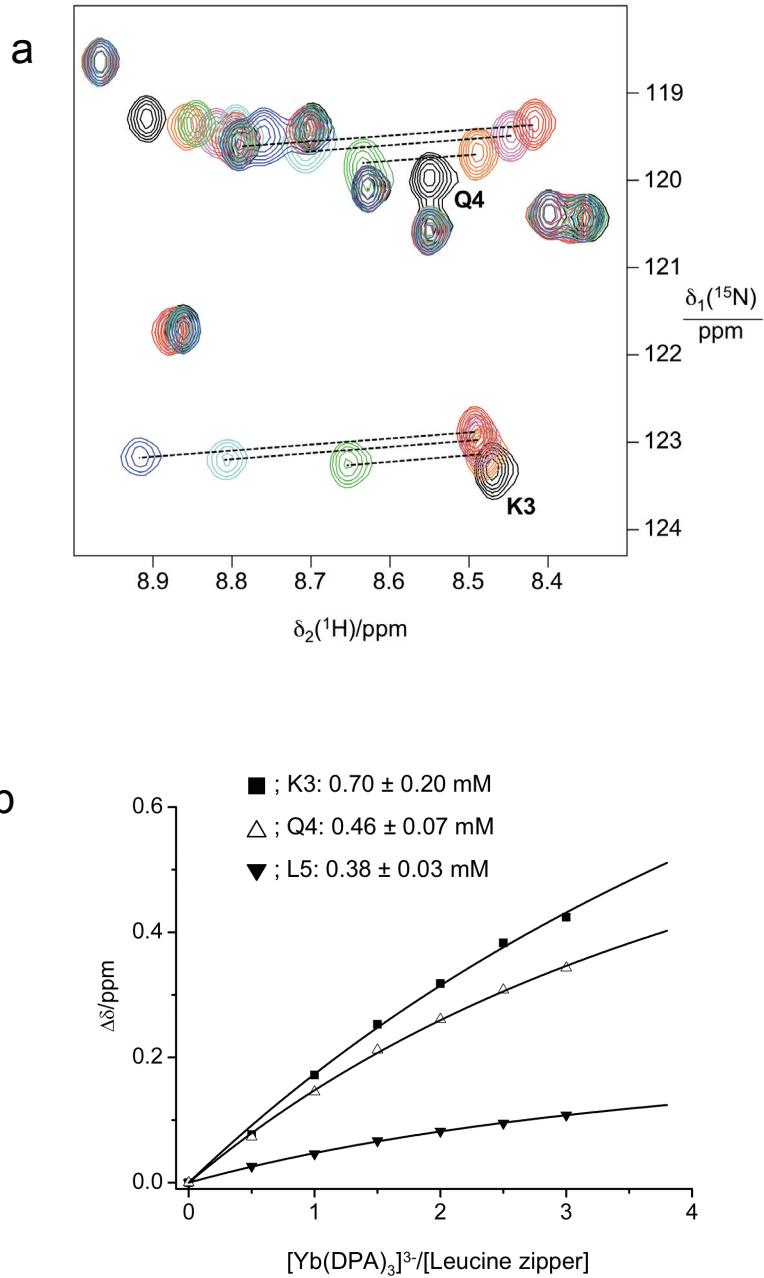


Figure S1. Binding affinity of $[\text{Yb}(\text{DPA})_3]^{3-}$ to the GCN4 leucine zipper. (a) PCSs induced in the GCN4 leucine zipper by $[\text{Yb}(\text{DPA})_3]^{3-}$. The figure shows a superimposition of ^{15}N -HSQC spectra of 0.1 mM (monomer) uniformly ^{15}N -labeled GCN4 leucine zipper. The black spectrum was recorded in the absence of $[\text{Ln}(\text{DPA})_3]^{3-}$. The green, cyan, and blue spectra were recorded in the presence of 0.1, 0.2 and 0.3 mM $[\text{Yb}(\text{DPA})_3]^{3-}$, respectively. The orange, purple, and red spectra were recorded in the presence of 0.1, 0.2 and 0.3 mM $[\text{Y}(\text{DPA})_3]^{3-}$, respectively, demonstrating the much smaller influence of the diamagnetic Y^{3+} complex on the peptide chemical shifts. (b) Changes in chemical shifts $\Delta\delta$ of selected amide proton resonances plotted versus the molar ratio of $[\text{Yb}(\text{DPA})_3]^{3-}$ to leucine zipper. Solid lines show the fits of the data using the dissociation constant indicated for each curve. The chemical shifts were measured in ^{15}N -HSQC spectra of uniformly ^{15}N labeled sample.

Table S1. Transverse ^1H PRE rates (Γ_2) of 0.3 mM GCN4 leucine zipper at various concentrations of $[\text{Gd(DPA)}_3]^{3-}$ ^a

Residue	$[\text{Gd(DPA)}_3]^{3-}$ concentration (mM)				
	0.006	0.015	0.03	0.06	0.12
3	90 ± 7	280 ± 20 ^b	640 ± 50 ^b	1480 ± 120 ^b	3400 ± 300 ^b
4	68 ± 4	210 ± 10 ^b	486 ± 30 ^b	1120 ± 70 ^b	2570 ± 160 ^b
5	49 ± 3	153 ± 9 ^b	353 ± 20 ^b	810 ± 50 ^b	1860 ± 110 ^b
6	22 ± 1	92 ± 9	155 ± 20 ^b	360 ± 50 ^b	820 ± 100 ^b
7	9.5 ± 0.7	29 ± 1	66 ± 2	150 ± 6 ^b	350 ± 13 ^b
8	7.3 ± 0.6	26 ± 1	55 ± 2	130 ± 20	310 ± 40 ^b
9	6.3 ± 0.5	18 ± 1	43 ± 1	97 ± 4	220 ± 10 ^b
10	2.6 ± 0.6	10 ± 1	23 ± 1	50 ± 2	105 ± 9
11	0.7 ± 0.5	4.0 ± 0.6	14 ± 1	33 ± 1	79 ± 3
12	0.3 ± 0.6	5.0 ± 0.7	15 ± 1	28 ± 1	54 ± 2
13	4.3 ± 0.5	7.7 ± 0.6	13 ± 1	28 ± 1	62 ± 2
14	0.3 ± 0.5	4.6 ± 0.5	11 ± 1	25 ± 1	60 ± 2
15	0.7 ± 0.5	4.3 ± 0.6	11 ± 1	24 ± 1	54 ± 2
16	-0.3 ± 0.6	2.1 ± 0.6	6.1 ± 0.5	14 ± 1	38 ± 1
17	-0.4 ± 0.6	2.5 ± 0.7	6.6 ± 0.6	18 ± 1	43 ± 1
18	0.6 ± 0.6	4.5 ± 0.7	12 ± 1	29 ± 1	71 ± 3
19	3.1 ± 0.7	4.8 ± 0.7	7.9 ± 0.6	17 ± 1	37 ± 1
20	1.5 ± 0.5	2.9 ± 0.6	4.8 ± 0.5	11 ± 1	25 ± 1
21	0.1 ± 0.5	4.0 ± 0.5	6.2 ± 0.4	14 ± 1	34 ± 1
22	4.7 ± 0.6	6.3 ± 0.6	7.1 ± 0.5	18 ± 1	36 ± 1
23	2.5 ± 0.5	3.0 ± 0.5	3.9 ± 0.4	8.6 ± 0.5	19 ± 1
24	0.9 ± 0.4	2.5 ± 0.5	3.9 ± 0.4	8.8 ± 0.4	20 ± 1
25	0.5 ± 0.5	3.0 ± 0.5	3.9 ± 0.4	9.3 ± 0.5	22 ± 1
26	2.2 ± 0.6	3.2 ± 0.7	4.4 ± 0.5	8.8 ± 0.5	19 ± 1
27	-0.2 ± 0.5	-0.1 ± 0.6	2.6 ± 0.5	4.9 ± 0.5	10 ± 1
28	4.0 ± 0.4	5.6 ± 0.5	4.4 ± 0.4	7.3 ± 0.4	17 ± 0
29	0.3 ± 0.5	1.5 ± 0.5	2.1 ± 0.4	4.6 ± 0.4	11 ± 0
30	1.9 ± 0.5	2.1 ± 0.5	1.7 ± 0.4	3.1 ± 0.4	7.9 ± 0.5
31	1.1 ± 0.5	2.4 ± 0.5	2.2 ± 0.4	4.8 ± 0.4	11 ± 1
32	-0.7 ± 0.7	3.0 ± 0.4	1.9 ± 0.3	4.2 ± 0.3	9.5 ± 0.4
33	5.3 ± 0.3	7.7 ± 0.3	8.0 ± 0.2	13 ± 0	27 ± 0

^a Relaxation rates are in s^{-1} . Data recorded at 25 °C, pH 6, in 20 mM MES buffer at a ^1H NMR frequency of 800 MHz.

^b Value determined by extrapolation from data at lower $[\text{Gd(DPA)}_3]^{3-}$ concentration because of excessive line broadening due to PRE. The extrapolated value was calculated by multiplying the Γ_2 value of the same residue observed at a lower concentration of $[\text{Gd(DPA)}_3]^{3-}$ by a factor which was determined by the average Γ_2 ratio of several residues observed at the $[\text{Gd(DPA)}_3]^{3-}$ concentration of interest and the next lower concentration. Data at 0.015 (0.03, 0.06, 0.012) mM $[\text{Gd(DPA)}_3]^{3-}$ concentration were extrapolated using a factor of 3.1 (2.3, 2.3, 2.3) determined from residues 7-9 (8-13, 9-19, 11-26).

Table S2. Transverse ^1H PRE rates (Γ_2) of 1.5 mM GCN4 leucine zipper at various concentrations of $[\text{Gd(DPA)}_3]^{3-}$ ^a

Residue	$[\text{Gd(DPA)}_3]^{3-}$ concentration (mM)				
	0.006	0.015	0.03	0.06	0.12
3	64 ± 2	192 ± 6 ^b	442 ± 14 ^b	970 ± 30 ^b	1940 ± 60 ^b
4	45 ± 1	135 ± 3 ^b	310 ± 6 ^b	680 ± 13 ^b	1370 ± 30 ^b
5	35 ± 1	104 ± 2 ^b	239 ± 5 ^b	525 ± 12 ^b	1050 ± 25 ^b
6	15 ± 0	63 ± 1	144 ± 3 ^b	318 ± 6 ^b	635 ± 12 ^b
7	7.0 ± 0.2	20 ± 0	49 ± 1	108 ± 1 ^b	216 ± 3 ^b
8	6.1 ± 0.2	17 ± 0	42 ± 1	92 ± 2	183 ± 4 ^b
9	3.6 ± 0.2	12 ± 0	31 ± 0	67 ± 1	134 ± 2 ^b
10	2.4 ± 0.2	7.2 ± 0.2	18 ± 0	41 ± 1	81 ± 2
11	2.4 ± 0.2	6.0 ± 0.2	14 ± 0	29 ± 0	55 ± 1
12	1.5 ± 0.2	6.2 ± 0.2	12 ± 0	26 ± 0	44 ± 1
13	0.8 ± 0.2	5.6 ± 0.2	11 ± 0	25 ± 0	50 ± 1
14	1.2 ± 0.2	4.9 ± 0.2	9.9 ± 0.2	24 ± 0	49 ± 1
15	4.1 ± 0.2	5.6 ± 0.1	11 ± 0	25 ± 0	52 ± 1
16	0.7 ± 0.2	5.0 ± 0.3	10 ± 0	18 ± 0	35 ± 0
17	0.2 ± 0.2	4.0 ± 0.2	8.1 ± 0.2	19 ± 0	41 ± 1
18	2.0 ± 0.2	7.1 ± 0.2	14 ± 0	34 ± 0	72 ± 1
19	0.5 ± 0.2	3.7 ± 0.2	7.4 ± 0.2	17 ± 0	34 ± 0
20	1.4 ± 0.2	2.8 ± 0.2	5.5 ± 0.2	13 ± 0	24 ± 0
21	0.7 ± 0.2	3.3 ± 0.2	6.7 ± 0.2	15 ± 0	31 ± 0
22	1.5 ± 0.2	3.7 ± 0.2	7.4 ± 0.2	15 ± 0	30 ± 0
23	1.4 ± 0.2	2.2 ± 0.2	4.3 ± 0.2	8.4 ± 0.2	16 ± 0
24	0.6 ± 0.1	5.7 ± 0.2	11 ± 0	9.7 ± 0.2	19 ± 0
25	0.5 ± 0.2	2.4 ± 0.2	4.7 ± 0.2	11 ± 0	23 ± 0
26	0.5 ± 0.2	1.9 ± 0.2	3.7 ± 0.2	8.2 ± 0.2	17 ± 0
27	-0.3 ± 0.3	1.5 ± 0.3	3.0 ± 0.2	5.5 ± 0.2	12 ± 0
28	0.1 ± 0.1	1.3 ± 0.2	2.7 ± 0.2	8.0 ± 0.2	17 ± 0
29	-0.2 ± 0.3	0.9 ± 0.2	1.7 ± 0.2	6.2 ± 0.2	13 ± 0
30	-0.2 ± 0.2	1.2 ± 0.2	2.0 ± 0.2	4.4 ± 0.2	10 ± 0
31	0.3 ± 0.2	1.4 ± 0.2	2.7 ± 0.2	7.6 ± 0.2	15 ± 0
32	0.3 ± 0.1	1.4 ± 0.1	2.8 ± 0.1	6.6 ± 0.2	13 ± 0
33	3.0 ± 0.1	5.3 ± 0.1	11 ± 0	19 ± 0	34 ± 0

^a Relaxation rates are in s^{-1} . Sample conditions as in footnote ^a of Table S1.

^b Value determined by extrapolation as in Table S1. Data at 0.015 (0.03, 0.06, 0.012) mM $[\text{Gd(DPA)}_3]^{3-}$ concentration were extrapolated using a factor of 3.0 (2.3, 2.2, 2.0) determined from residues 7-9 (8-13, 9-19, 11-26).

Table S3. Longitudinal ^1H PRE rates (Γ_1) of 0.3 mM GCN4 leucine zipper at various concentrations of $[\text{Gd(DPA)}_3]^{3-}$ ^a

Residue	$[\text{Gd(DPA)}_3]^{3-}$ concentration (mM)				
	0.006	0.015	0.03	0.06	0.12
3	0.3 ± 0.0	1.0 ± 0.1 ^b	2.0 ± 0.2 ^b	3.9 ± 0.4 ^b	7.8 ± 0.7 ^b
4	0.3 ± 0.0	0.9 ± 0.1 ^b	1.8 ± 0.1 ^b	3.7 ± 0.3 ^b	7.4 ± 0.5 ^b
5	0.2 ± 0.0	0.6 ± 0.0 ^b	1.2 ± 0.0 ^b	2.5 ± 0.1 ^b	5.0 ± 0.2 ^b
6	0.2 ± 0.0	0.5 ± 0.0	1.0 ± 0.1 ^b	2.0 ± 0.1 ^b	3.9 ± 0.3 ^b
7	0.1 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	1.2 ± 0.2 ^b	2.4 ± 0.3 ^b
8	0.08 ± 0.01	0.2 ± 0.0	0.5 ± 0.0	1.0 ± 0.1	2.0 ± 0.2 ^b
9	0.06 ± 0.01	0.2 ± 0.0	0.4 ± 0.0	0.7 ± 0.1	1.4 ± 0.1 ^b
10	0.04 ± 0.01	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.7 ± 0.1 ^b
11	0.03 ± 0.00	0.09 ± 0.00	0.2 ± 0.0	0.4 ± 0.0	0.7 ± 0.1
12	0.02 ± 0.00	0.08 ± 0.00	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
13	0.02 ± 0.00	0.07 ± 0.00	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.0
14	0.02 ± 0.00	0.08 ± 0.00	0.2 ± 0.0	0.3 ± 0.0	0.6 ± 0.0
15	0.02 ± 0.00	0.07 ± 0.00	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0
16	0.01 ± 0.00	0.05 ± 0.00	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.0
17	0.01 ± 0.00	0.05 ± 0.00	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
18	0.02 ± 0.00	0.07 ± 0.00	0.2 ± 0.0	0.3 ± 0.0	0.7 ± 0.0
19	0.02 ± 0.00	0.06 ± 0.00	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
20	0.02 ± 0.00	0.05 ± 0.00	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
21	0.01 ± 0.00	0.05 ± 0.00	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
22	0.02 ± 0.00	0.06 ± 0.00	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
23	0.01 ± 0.00	0.04 ± 0.00	0.09 ± 0.00	0.2 ± 0.0	0.3 ± 0.0
24	0.00 ± 0.00	0.03 ± 0.00	0.09 ± 0.00	0.2 ± 0.0	0.4 ± 0.0
25	0.02 ± 0.00	0.06 ± 0.00	0.1 ± 0.0	0.3 ± 0.0	0.6 ± 0.0
26	0.01 ± 0.00	0.01 ± 0.00	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
27	0.01 ± 0.00	0.04 ± 0.00	0.09 ± 0.00	0.2 ± 0.0	0.3 ± 0.0
28	0.01 ± 0.00	0.04 ± 0.00	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
29	0.00 ± 0.00	0.03 ± 0.00	0.08 ± 0.00	0.2 ± 0.0	0.3 ± 0.0
30	0.00 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	0.1 ± 0.0	0.2 ± 0.0
31	0.00 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	0.1 ± 0.0	0.3 ± 0.0
32	0.01 ± 0.00	0.04 ± 0.00	0.08 ± 0.00	0.1 ± 0.0	0.3 ± 0.0
33	0.03 ± 0.00	0.08 ± 0.00	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0

^a Relaxation rates in s^{-1} . Sample conditions as in footnote ^a of Table S1.

^b Value determined by extrapolation as in Table S1. Data at 0.015 (0.03, 0.06, 0.012) mM $[\text{Gd(DPA)}_3]^{3-}$ concentration were extrapolated using a factor of 3.0 (2.0, 2.0, 2.0) determined from residues 7-8 (8-11, 9-19, 11-26).

Table S4. Summary of transverse (Γ_2) and longitudinal (Γ_1) ^1H PRE rates of GCN4 leucine zipper at different concentrations in the presence of 0.12 mM [Gd(DPA)₃]^{3-a}

Residue	Γ_2	Γ_2	$\Gamma_{2,\text{bound}}^{\text{b}}$	Γ_1
	leucine zipper concentration			
	0.3 mM	1.5 mM	0.3 mM	0.3 mM
3	3400 ± 300	1940 ± 60	2590 ± 510	7.8 ± 0.7
4	2570 ± 160	1370 ± 30	2130 ± 290	7.4 ± 0.5
5	1860 ± 110	1050 ± 25	1440 ± 200	5.0 ± 0.2
6	820 ± 100	635 ± 12	860 ± 190	3.9 ± 0.3
7	350 ± 13	216 ± 3	236 ± 24	2.4 ± 0.3
8	310 ± 40	183 ± 4	219 ± 72	2.0 ± 0.2
9	220 ± 10	134 ± 2	156 ± 18	1.4 ± 0.1
10	105 ± 9	81 ± 2	43 ± 16	0.7 ± 0.1
11	79 ± 3	55 ± 1	41 ± 5	0.7 ± 0.1
12	54 ± 2	44 ± 1	18 ± 3	0.5 ± 0.0
13	62 ± 2	50 ± 1	21 ± 4	0.6 ± 0.0
14	60 ± 2	49 ± 1	19 ± 3	0.6 ± 0.0
15	54 ± 2	52 ± 1		0.6 ± 0.0
16	38 ± 1	35 ± 0		0.5 ± 0.0
17	43 ± 1	41 ± 1		0.5 ± 0.0
18	71 ± 3	72 ± 1		0.7 ± 0.0
19	37 ± 1	34 ± 0		0.5 ± 0.0
20	25 ± 1	24 ± 0		0.4 ± 0.0
21	34 ± 1	31 ± 0		0.4 ± 0.0
22	36 ± 1	30 ± 0		0.4 ± 0.0
23	19 ± 1	16 ± 0		0.3 ± 0.0
24	20 ± 1	19 ± 0		0.4 ± 0.0
25	22 ± 1	23 ± 0		0.6 ± 0.0
26	19 ± 1	17 ± 0		0.4 ± 0.0
27	10 ± 1	12 ± 0		0.3 ± 0.0
28	17 ± 0	17 ± 0		0.4 ± 0.0
29	11 ± 0	13 ± 0		0.3 ± 0.0
30	7.9 ± 0.5	10 ± 0		0.2 ± 0.0
31	11 ± 1	15 ± 0		0.3 ± 0.0
32	9.5 ± 0.4	13 ± 0		0.3 ± 0.0
33	27 ± 0	34 ± 0		0.5 ± 0.0

^a Relaxation rates are in s⁻¹. Sample conditions as in footnote ^a of Table S1.

^b The column labeled $\Gamma_{2,\text{bound}}$ contains the PRE^{bound} values used to fit the metal position.

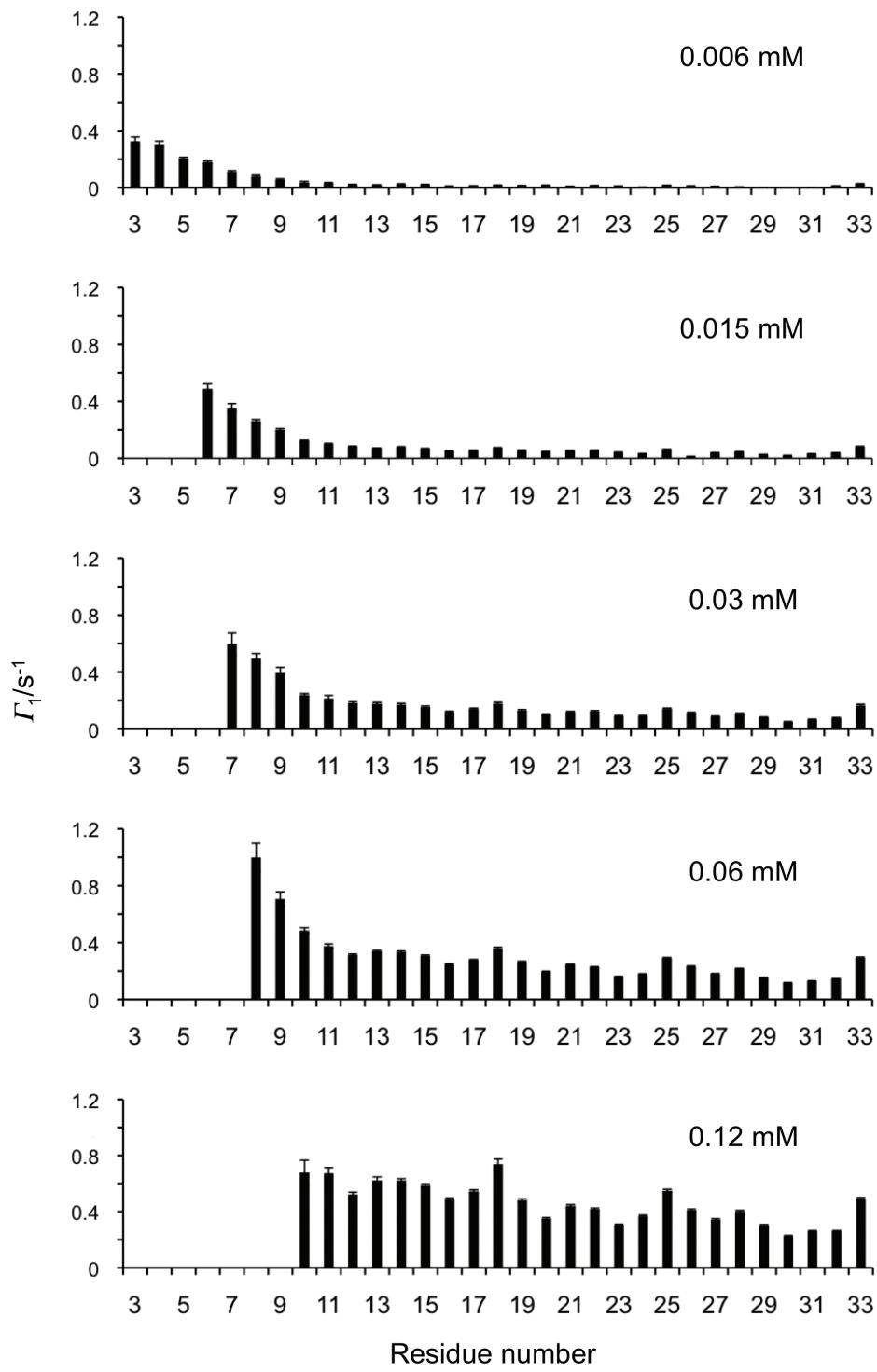


Figure S2. Amide proton longitudinal PREs of an 0.3 mM GCN4 leucine zipper at different concentrations of $[Gd(DPA)_3]^{3-}$.

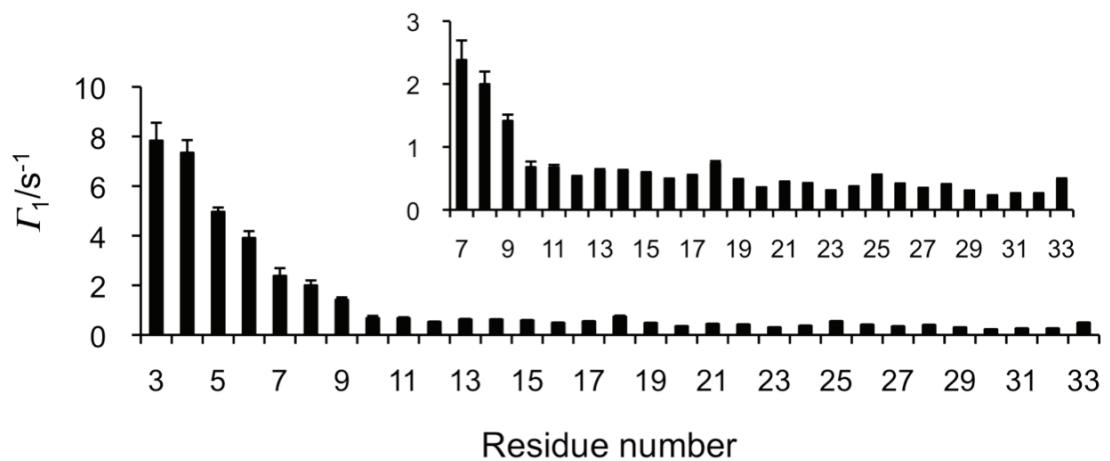


Figure S3. Overall profiles of longitudinal ^1H P弛豫 rates of the amide protons of GCN4 leucine zipper. The insert shows the P弛豫 rates of residues 7-33 on an expanded scale. The plot reports P弛豫 rates for 0.3 mM leucine zipper and 0.12 mM $[\text{Gd(DPA)}_3]^{3-}$. Values for residues near the N-terminus were extrapolated from data measured at lower $[\text{Gd(DPA)}_3]^{3-}$ concentrations.

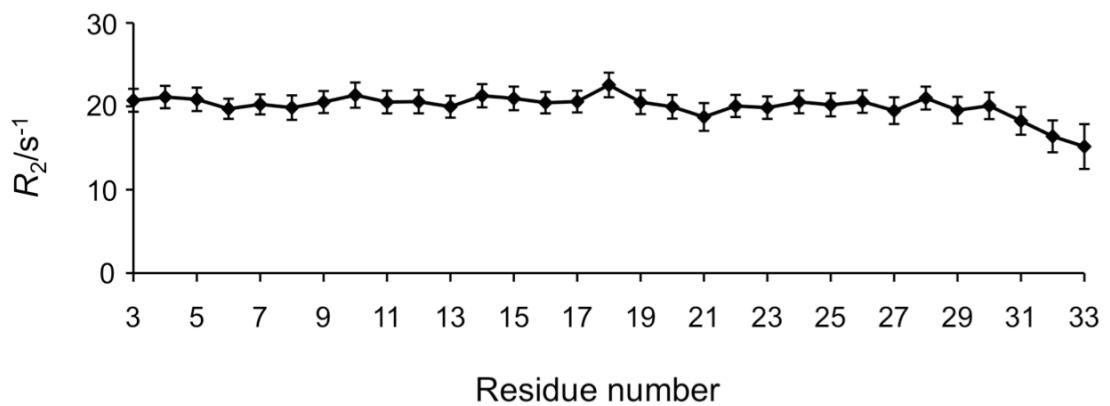


Figure S4. Transverse ^{15}N relaxation rates of the amides of 0.3 mM GCN4 leucine zipper.

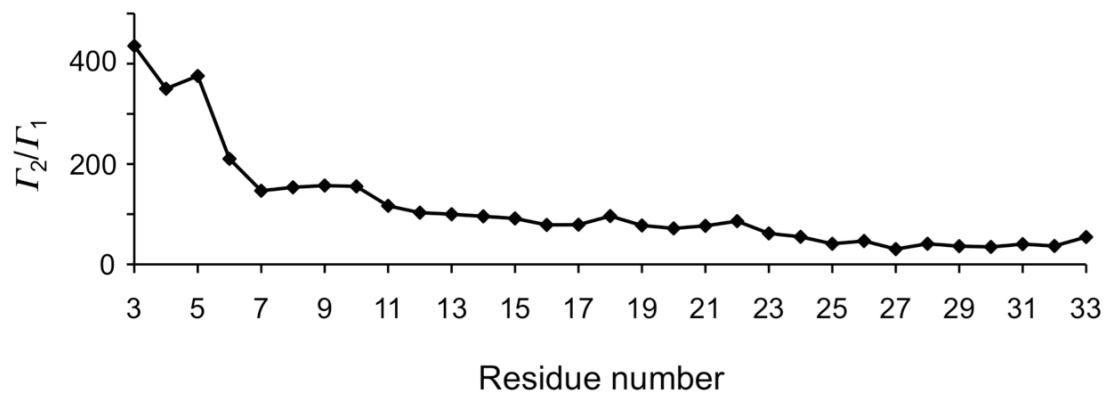


Figure S5. Ratio of transverse and longitudinal ^1H PREs (T_2/T_1) obtained for 0.3 mM GCN4 leucine zipper in the presence of 0.12 mM $[\text{Gd(DPA)}_3]^{3-}$ (Table S4).

Table S5. PCSs (in ppm) of the backbone amide protons of the 0.1 mM GCN4 leucine zipper at different molar ratios of $[\text{Ln}(\text{DPA})_3]^{3-}$ -to-peptide ^a

Residue	$[\text{Tm}(\text{DPA})_3]^{3-}$			$[\text{Tb}(\text{DPA})_3]^{3-}$			$[\text{Yb}(\text{DPA})_3]^{3-}$	
	1.0	3.0	5.0	1.0	3.0	5.0	1.0	3.0
K3	0.39	0.93 ^c	1.27 ^c	n.o.	n.o.	n.o.	0.17	0.42
Q4	0.34	0.81 ^c	1.10 ^c	n.o.	n.o.	n.o.	0.15	0.34
L5	0.12	0.29 ^c	0.39 ^c	n.o.	n.o.	n.o.	0.05	0.11
E6	-0.03	-0.08	-0.10	0.06	0.13	0.19 ^c	-0.01	-0.03
D7	s	0.01	0.01	-0.04	-0.09	-0.12	s	s
K8	s	s	s	-0.01	-0.03	-0.04	s	-0.01
V9	-0.04	-0.08	-0.10	0.05	0.10	0.12	-0.02	-0.03
E10	-0.02	-0.06	-0.08	0.03	0.07	0.10	-0.01	-0.03
E11	-0.01	-0.03	-0.04	0.01	0.03	0.04	-0.01	-0.01
L12	-0.02	-0.04	-0.05	0.02	0.04	0.06	-0.01	-0.02
L13	-0.02	-0.04	-0.06	0.03	0.06	0.08	-0.01	-0.02
S14	-0.01	-0.03	-0.04	0.02	0.05	0.06	s	-0.01
K15	s	-0.02	-0.02	0.01	0.02	0.03	s	-0.01
N16	s	-0.02	-0.02	0.01	0.02	0.03	s	-0.01
Y17	s	-0.02	-0.02	0.01	0.02	0.03	s	-0.01
H18–R33	s	s	s	s	s	s	s	s

^a Sample conditions as in footnote ^a of Table S1.

^b The PCSs are reported as the chemical shift measured with each $[\text{Ln}(\text{DPA})_3]^{3-}$ complex minus that measured in the presence of diamagnetic $[\text{Y}(\text{DPA})_3]^{3-}$. Fields labeled n.o. indicate that the respective amide proton signal was broadened beyond detection due to PRE. Fields labeled s identify PCSs less than ± 0.01 ppm. This situation applied to all residues from His18 to Arg33.

^c Value determined by extrapolation from data at lower $[\text{Ln}(\text{DPA})_3]^{3-}$ concentration because of excessive line broadening due to PRE. Extrapolation involved multiplication of the PCS value observed at a $[\text{Ln}(\text{DPA})_3]^{3-}$ -to-peptide ratio of 1.0 by a factor which was determined by the actual concentration of $[\text{Ln}(\text{DPA})_3]^{3-}$ -peptide complex present in solution. Under the conditions used and for $K_D = 0.5$ mM, 14.6%, 34.6%, and 47.5% of the peptide was bound to $[\text{Ln}(\text{DPA})_3]^{3-}$ at $[\text{Ln}(\text{DPA})_3]^{3-}$ concentrations of 0.1 mM, 0.3 mM, and 0.5 mM, respectively. Therefore, data at 0.3 and 0.5 mM $[\text{Ln}(\text{DPA})_3]^{3-}$ concentration were extrapolated using factors of 34.6/14.6 and 47.5/14.6, respectively.