Supplementary information

Long-range distance measurements in proteins using Gd^{3+} spin labeling

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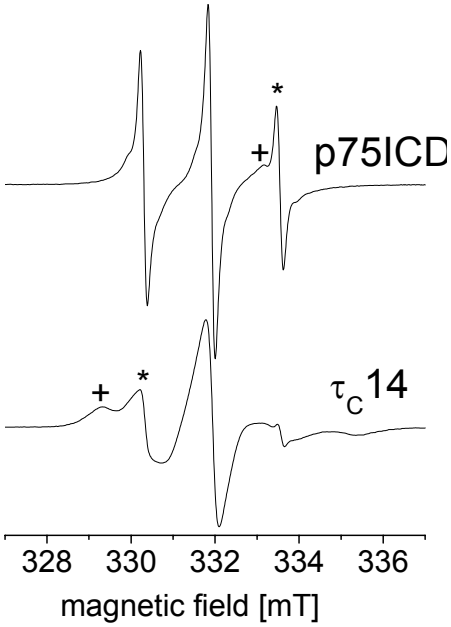

Fig. S1. X-band continuous wave EPR spectra of p75ICD and τ_C14 labeled with a pair of MTSL labels. Each spectrum features two components due to labels with different local order (mobility). The components due to labels with a large degree of disorder are marked with "*", the components due to labels tumbling in a more restricted manner are marked with "+".

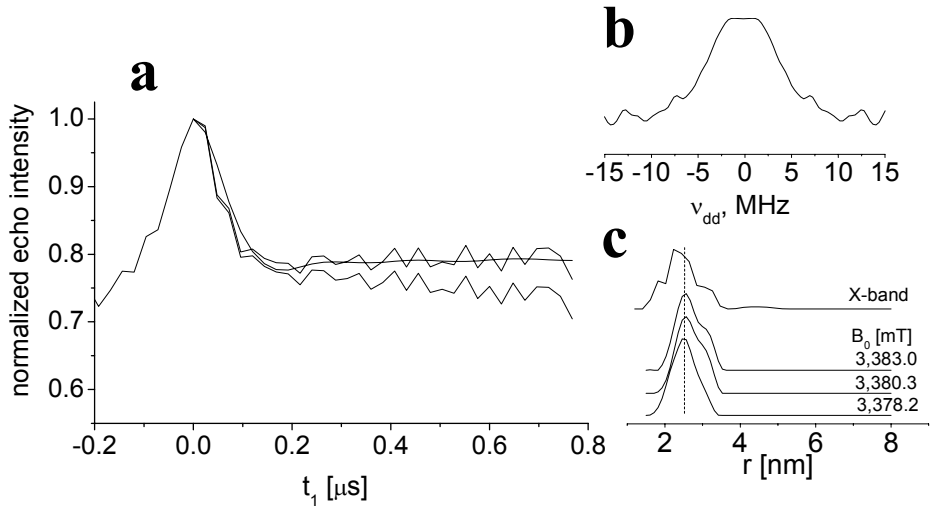

Fig. S2. X-band DEER measurements of p75ICD labeled with two nitroxides. a) Experimental raw DEER data, data after background subtraction and superimposition of the trace calculated from the distance distribution in (c). b) The Fourier transform of the DEER data after background subtraction. c) Distance distribution obtained using Tikhonov regularization compared with the W-band data (Fig. 1, main text). Experimental conditions: 50 K, t_{90} = 32 ns, t_{180} = 32 ns, t_{pump} = 32 ns, $\Delta\nu$ = 60 MHz. The amplitude of the observer π pulse was twice as large as that of the observer $\pi/2$ pulse. Proton modulations were removed by averaging over the τ interval.

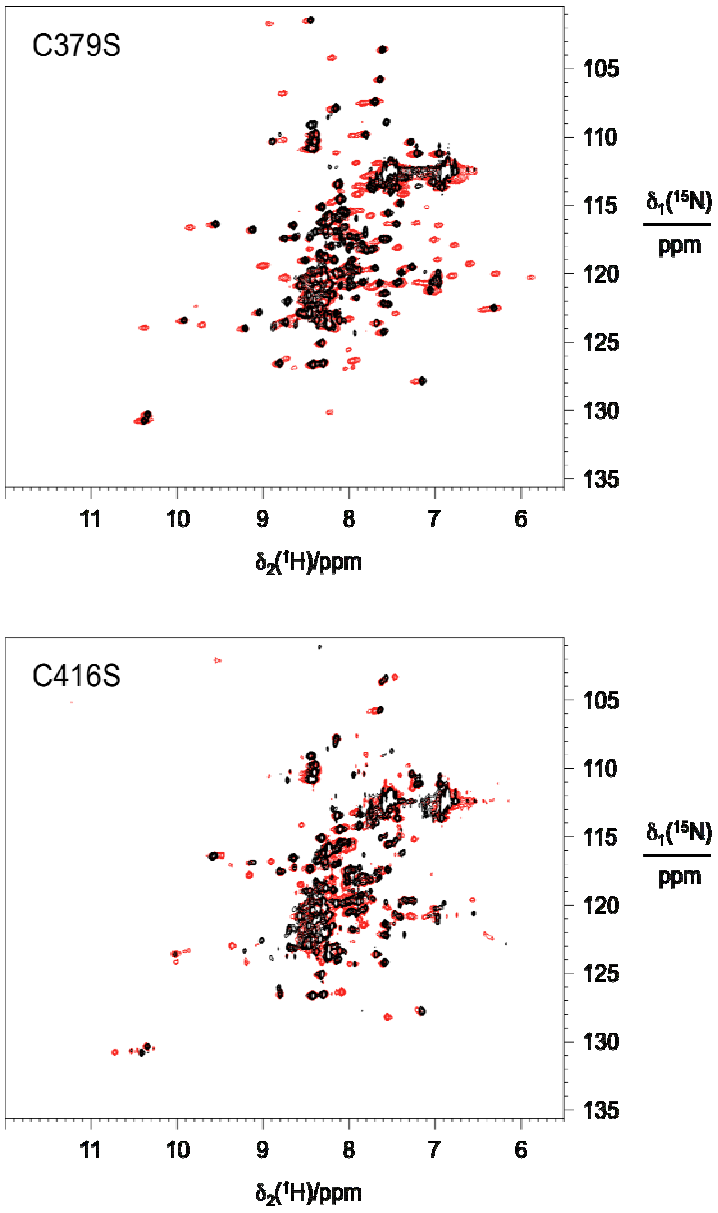

Fig. S3. PCSs in the C379S and C416S mutants of p75ICD ligated with 4MMDPA. The figure shows superimpositions of ^{15}N-HSQC spectra in the presence of Lu^{3+} (black) and a 1:1 mixture of Lu^{3+} and Tm^{3+} (red). The protein concentration was 0.2 mM in 20 mM HEPES buffer pH 7.0, 100 mM NaCl. The ratio of lanthanides to protein was 1.5:1. The spectra were recorded at 25 °C at a ^{1}H NMR frequency of 800 MHz.

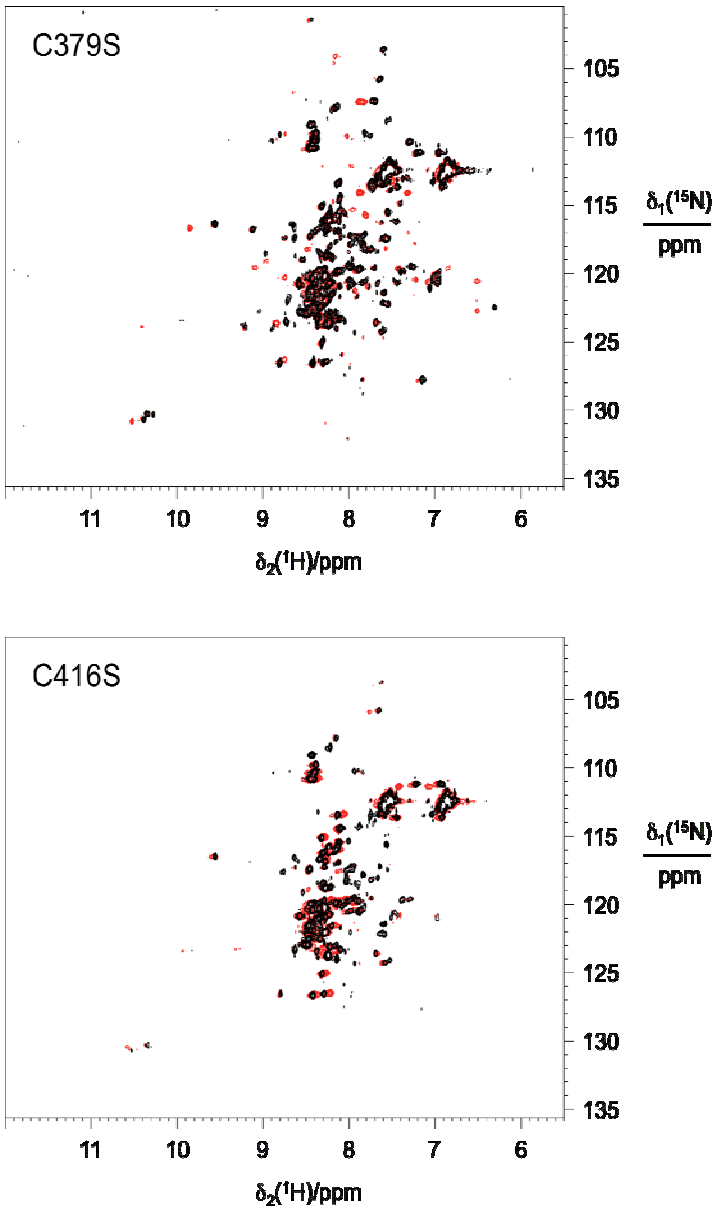

Fig. S4. PCSs in the C379S and C416S mutants of p75ICD ligated with 3MDPA. The figure shows a superimposition of ^{15}N-HSQC spectra in the presence of Lu^{3+} (black) and a 1:1 mixture of Lu^{3+} and Tm^{3+} (red). Except for ligation with 3MDPA in place of 4MMDPA, all parameters were the same as in Fig. S3. The small magnitude of the PCS observed for the C416S mutant was taken as evidence for flexibility of the tag attached to C379.

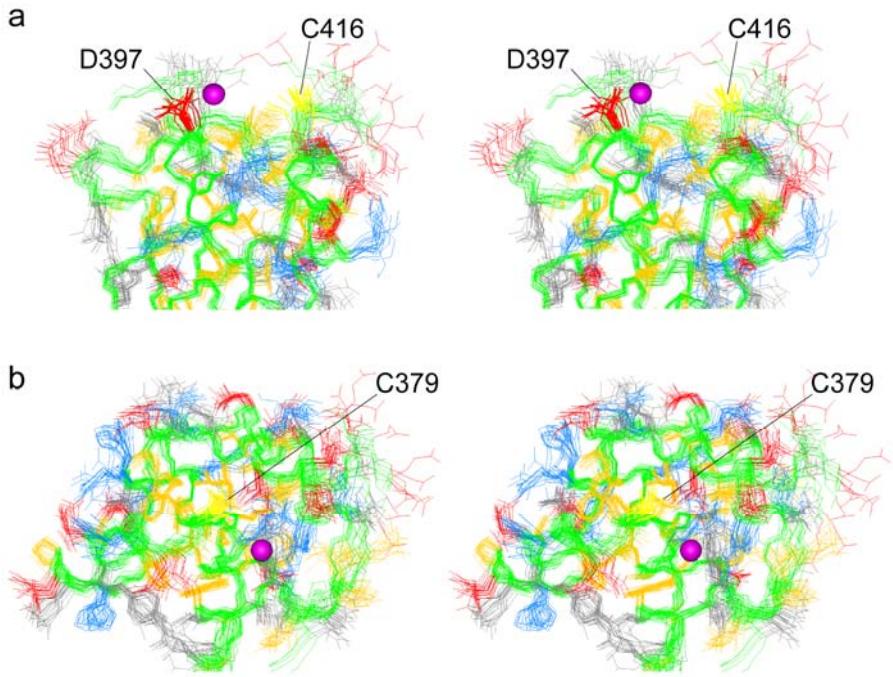

Fig. S5. Stereoviews of the NMR conformers of p75ICD (PDB accession code 1NGR), showing the positions of the lanthanide ions as magenta balls. The metal positions were determined from amide proton PCSs induced by Tb^{3+}, Tm^{3+}, and Yb^{3+} in the mutants C379S and C416S conjugated with 4MMDPA. The side chains of the cysteine residues carrying the 4MMDPA tags are shown in bright yellow. The side chain of D397 close to the lanthanide ion is marked. (a) C379S mutant. (b) C416S mutant

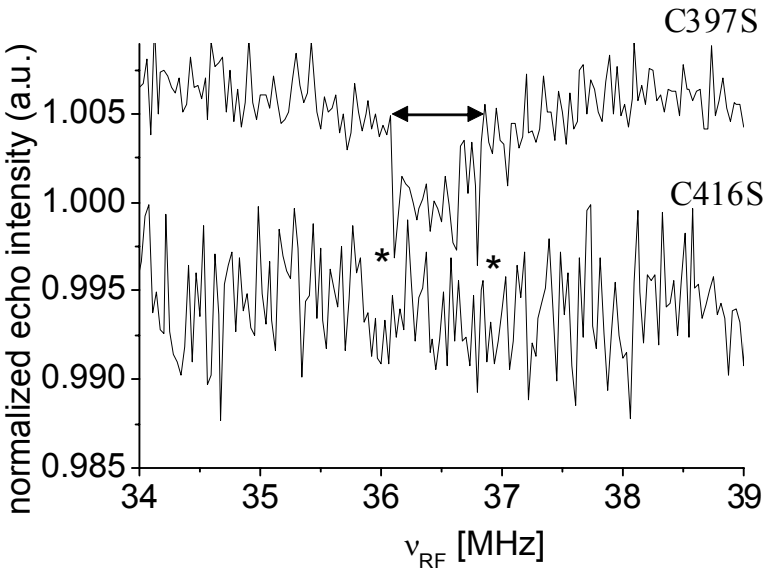

Fig. S6 W-band Mims ^{13}C-ENDOR spectra of the mutants C397S and C416S of p75ICD labeled with Gd^{3+}-4MMDPA. The spectra were recorded at the field set to the maximum of EPR spectrum, 3.404 T. Experimental parameters: microwave pulse durations t_{90} = 12.5 ns, τ = 300 ns and RF pulse duration t_{rf} = 60 μs. Measurement temperature ~ 8 K. The arrow marks a splitting corresponding to a hyperfine coupling of 0.7 MHz and the symbols * mark the tentative features of $A_{\perp}$.

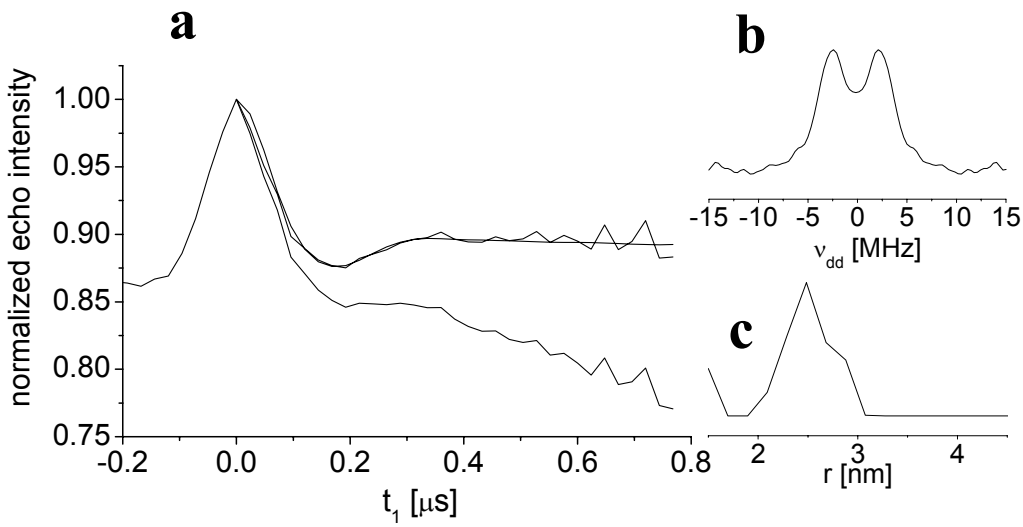

Fig. S7. X-band DEER measurement of τ_C14 labeled with two nitroxides. a) Experimental raw DEER data, data after background subtraction and superimposition of the trace calculated from the distance distribution in (c). b) The Fourier transform of the DEER data after background subtraction. c) Distance distribution obtained using Tikhonov regularization. Experimental conditions: 50 K, t_{90} = 32 ns, t_{180} = 32 ns, t_{pump} = 32 ns, $\Delta\nu$ = 60 MHz. The amplitude of the observer π pulse was twice as large as that of the observer $\pi/2$ pulse. Proton modulations were removed by averaging over the τ interval.

Table S1. PCSs (in ppm) of the backbone amide protons of the p75ICD mutants[a]

C379S				C416S			
Residue	Tm^{3+}	Tb^{3+}	Yb^{3+}	Residue	Tm^{3+}	Tb^{3+}	Yb^{3+}
342		0.33	-0.09	343	0.01	-0.02	
343	-0.32		-0.16	346	0.35		0.17
344	-0.65			353			0.12
345		0.47		356		-0.01	0.02
346		0.38		357		-0.04	0.06
347	-0.82		-0.30	361	0.12		
348	-1.19		-0.46	362		-0.08	
349	-0.77		-0.27	363			0.15
350		0.55		365	0.13	-0.23	0.09
351		0.79		367		-0.07	
352		0.85		369	-0.10	0.09	-0.03
353	-0.71		-0.20	373	-0.31	0.26	-0.07
354		0.38		375			-0.12
356	-0.19	0.25		387	0.97		
358		-0.06	0.12	388	0.80	-1.00	0.46
359		-0.03		389	1.20		0.58
360		-0.21		392			0.11
361	0.48		0.30	393			0.02
362	0.60		0.32	394	0.42	-0.48	0.18
365	1.15		0.58	396	0.35	-0.37	0.14
366	0.77			397	0.27		0.12
367	0.46	-0.42	0.26	402	0.25	-0.26	0.15
369	0.28	-0.21	0.15	403			0.18
370	0.29	-0.23	0.17	404		-0.34	0.11
372	0.22	-0.25		406	0.13		0.09
373	0.20		0.12	407	0.10	-0.36	
374		-0.05		410			0.10
375	0.13	-0.05	0.10	411			0.15
384	0.06						
387			0.22				
388		-0.35	0.35				
389	0.54	-0.54	0.40				
390	0.42	-0.47	0.24				
391		-0.65					
392			0.18				
406	0.85		0.35				
409	-0.79	1.08	-0.34				
410			-0.43				
411			-0.61				
412			-1.10				
413			-1.70				

[a] Tagging with 4MMDPA. The PCSs are reported as the chemical shift measured with each lanthanide minus that measured in the presence of diamagnetic Lu^{3+}.

Table S2. Fitted $\Delta\chi$ tensors[a]

	Mutant C416S		Mutant C379S	
Ln^{3+}	$\Delta\chi_{ax}$ (10^{-32} m^3)	$\Delta\chi_{rh}$ (10^{-32} m^3)	$\Delta\chi_{ax}$ (10^{-32} m^3)	$\Delta\chi_{rh}$ (10^{-32} m^3)
Tb^{3+}	-9.8	-6.0	-7.9	-4.2
Tm^{3+}	11.3	7.4	6.4	1.7
Yb^{3+}	4.9	2.4	3.2	1.4

[a] The $\Delta\chi$ tensors were fitted using the PCSs of Table S1. The fits simultaneously used all PCSs induced by Tm^{3+}, Tb^{3+}, and Yb^{3+}, with a common metal position. The sum of the squared deviations in fitting the PCSs of the C379S and C416S mutants was $3.2*10^{-5}$ and $1.0*10^{-4}$ ppm^2, respectively.