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Supporting Information

4,4′-Dithiobis-dipicolinic Acid: A Small and Convenient Lanthanide Binding Tag for Protein NMR Spectroscopy

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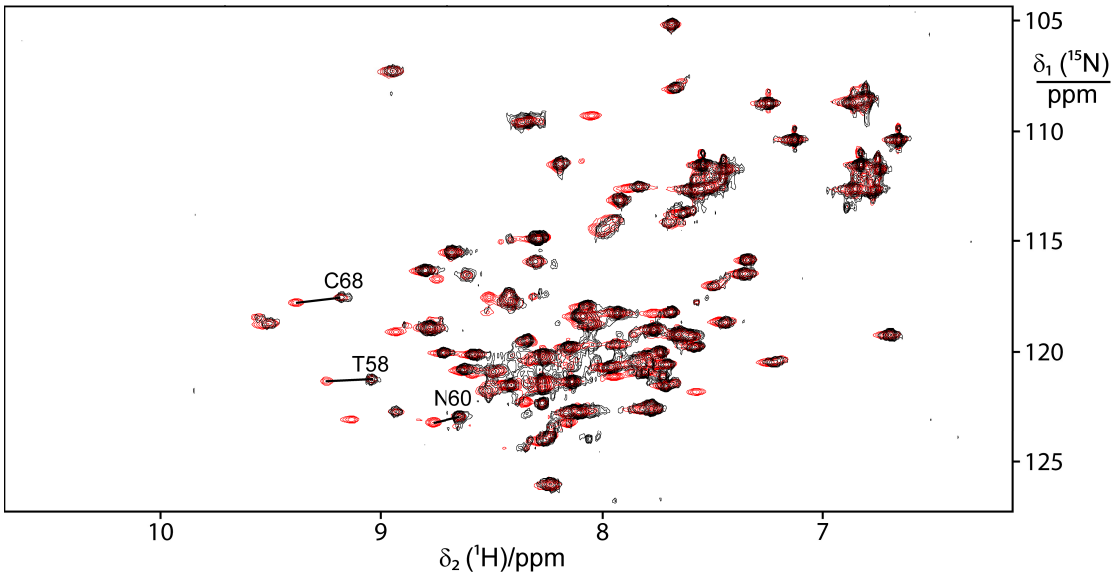

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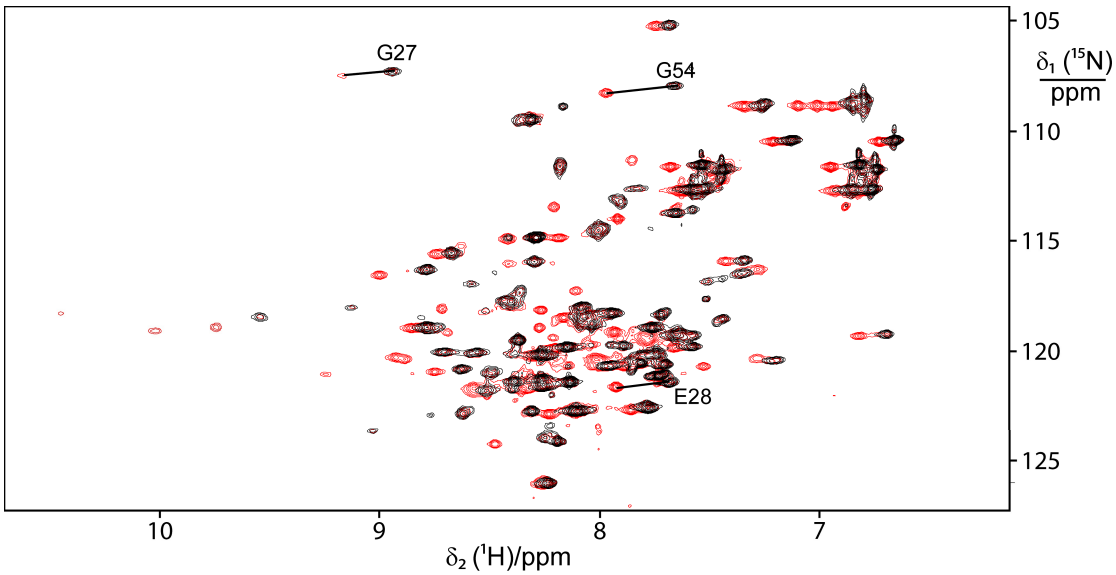


Figure S1. Superimposition of ^{15}N-HSQC spectra of ArgN-4MDPA in complex with paramagnetic and diamagnetic metal ions. The spectra were acquired of a 0.15 mM solution of ArgN-4MPDA in 20 mM MES buffer, pH 6.5, at 25 ^{o}C using a Bruker 600 MHz NMR spectrometer. A) Spectra recorded in the presence of Y^{3+} (black) and a 1:0.8 mixture of Y^{3+} and Ce^{3+} (red). Lines connecting paramagnetic and diamagnetic cross-peaks highlight the PCSs of Thr58, Asn60 and Cys68. B) Same as A, except with Zn^{2+}(black) and a 1:1 mixture of Zn^{2+} and Co^{2+} (grey). Lines connecting the paramagnetic and diamagnetic cross-peaks of Gly27, Gly54 and Glu28 present examples of PCSs.

Table S1. PCSs (in ppm) observed for backbone amide protons of ArgN-4MDPA in complex with Tb^{3+}, Tm^{3+}, Yb^{3+}, Ce^{3+} and Co^{2+}. [a]

Residue	Tb^{3+}	Tm^{3+}	Yb^{3+}	Ce^{3+}	Co^{2+}
L10					0.078
K12	-0.093	0.044	0.030		0.153
A13	-0.100	0.037	0.023		0.131
F14		-0.104	-0.081		0.225
L17				0.067	
G27	0.416	-0.385	-0.164		0.221
E28	0.411	-0.402	-0.170		0.244
I29	0.455	-0.461	-0.200		0.310
V30	0.329	-0.325	-0.140		0.237
A31	0.206	-0.209	-0.099		0.136
A32	0.161	-0.191	-0.073		0.177
L33	0.132	-0.167	-0.07		0.179
Q34	0.090	-0.109	-0.046		0.130
Q36					0.079
G37					0.059
F38	-0.084				0.072
N40	0.055	-0.055	-0.028		0.066
I41	0.087	-0.088	-0.026		0.082
N42	0.166	-0.162	-0.075		0.124
S44	0.203	-0.179	-0.082		0.118
K45	0.197	-0.188	-0.089		0.209
V46	0.273	-0.257	-0.118		0.172
S47	0.360	-0.326	-0.152		0.211
R48	0.313	-0.281	-0.133		0.177
M49	0.346	-0.313	-0.154		0.234
L50	0.554	-0.488	-0.234		0.336
T51	0.524	-0.451	-0.219		0.267
K52	0.379	-0.318	-0.156	0.021	0.225
F53	0.459	-0.489	-0.205	0.020	0.306
G54	0.565	-0.442	-0.222	0.025	0.314
A55	1.122	-0.909	-0.436		0.618
T58				0.205	

N60		0.121	
M63			-0.326
E64		0.036	
V66		0.148	
C68		0.197	

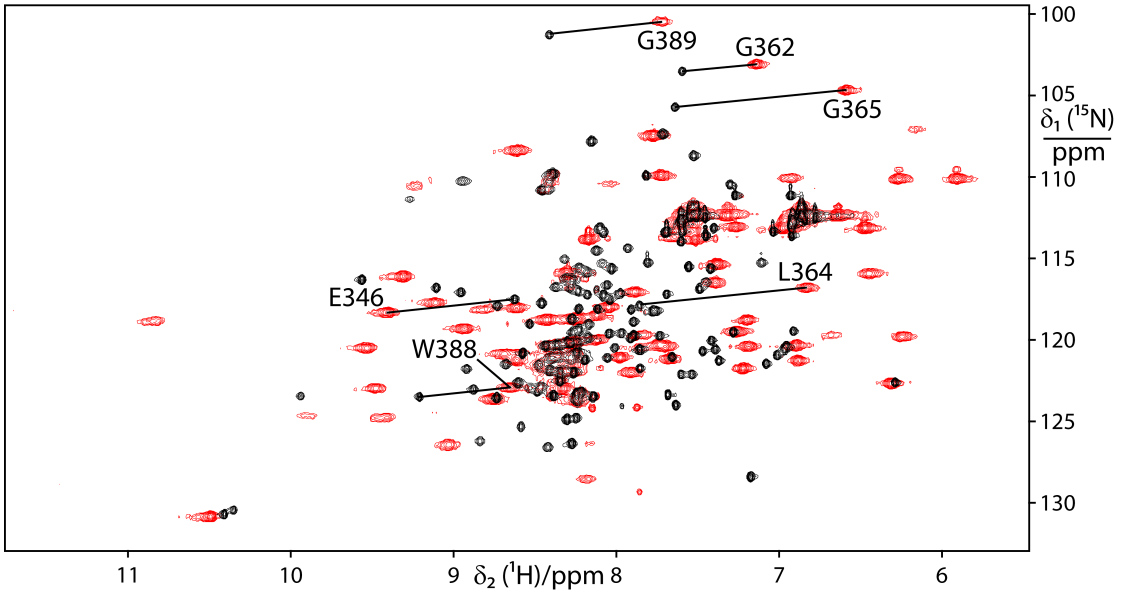
[a] PCSs measured in 20 mM MES buffer (pH 6.5) at 25 °C at a ^{1}H NMR frequency of 600 MHz, using the Y^{3+} complex as the diamagnetic reference. For some residues, no PCS data were measured because of spectral overlap, excessive PRE (e.g. for amides of residues 56-68 which are close to the attachment site of the 4MDPA at Cys68) or for residues of the flexible N- and C-terminal polypeptide segments.

Table S2. $\Delta\chi$ tensors of different metal ions bound to ArgN-4MDPA.[a]

Metal ion	$\Delta\chi_{ax}$ /10^{-32} m^3	$\Delta\chi_{rh}$ /10^{-32} m^3	Tensor axis	Coordinates of tensor axes		
Ce^{3+}	-0.8(0.1)	-0.3(0.1)	x	0.991	0.053	-0.119
			y	0.012	0.869	0.495
			z	0.130	-0.492	0.861
Tb^{3+}	11.3 (0.5)	4.4(0.6)	x	0.996	0.055	0.077
			y	-0.091	0.768	0.634
			z	-0.024	-0.638	0.769
Tm^{3+}	-9.2(0.7)	-5.4(1.0)	x	0.947	-0.151	0.284
			y	-0.028	0.841	0.540
			z	-0.320	-0.519	0.792
Yb^{3+}	-4.2(0.3)	-1.7(0.5)	x	0.997	-0.042	0.070
			y	-0.009	0.794	0.607
			z	-0.081	-0.606	0.791
Co^{2+}	5.3(0.2)	2.5(0.7)	x	0.937	0.032	-0.348
			y	0.183	0.804	0.566
			z	0.298	-0.594	0.747

[a] PCSs (Table S1) were measured at 25 ºC and pH 6.5 in 20 mM MES buffer. The tensors are listed in their unique tensor representation (UTR)[1] as obtained by fitting of the PCSs to the first conformer of the PDB structure 1AOY of ArgN,[2] using all PCSs of all metal ions simultaneously and a common metal position. To take into account the covalent structure of the 4MDPA tag, the 4MDPA tag was crafted onto Cys68 of the NMR conformer 1 with the metal ion positioned in the plane of the DPA moiety and the dihedral angles of the side chain of Cys68 of the bonds with the sulfurs were systematically varied as described in the Experimental Section. The orientations of the tensor axes are given as unit vectors with respect to the origin (0, 0, 0). Standard deviations (shown in brackets) were determined by repeating the fits 100 times following random removal of 20% of the data. The coordinates of the common metal position were (8.372, 11.910, 5.038) with a standard deviation of about 0.4 Å in each of the dimensions.

A)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G362
G365
L364
E346
W388
100
105
110
115
120
125
130
δ1 (15N)
ppm
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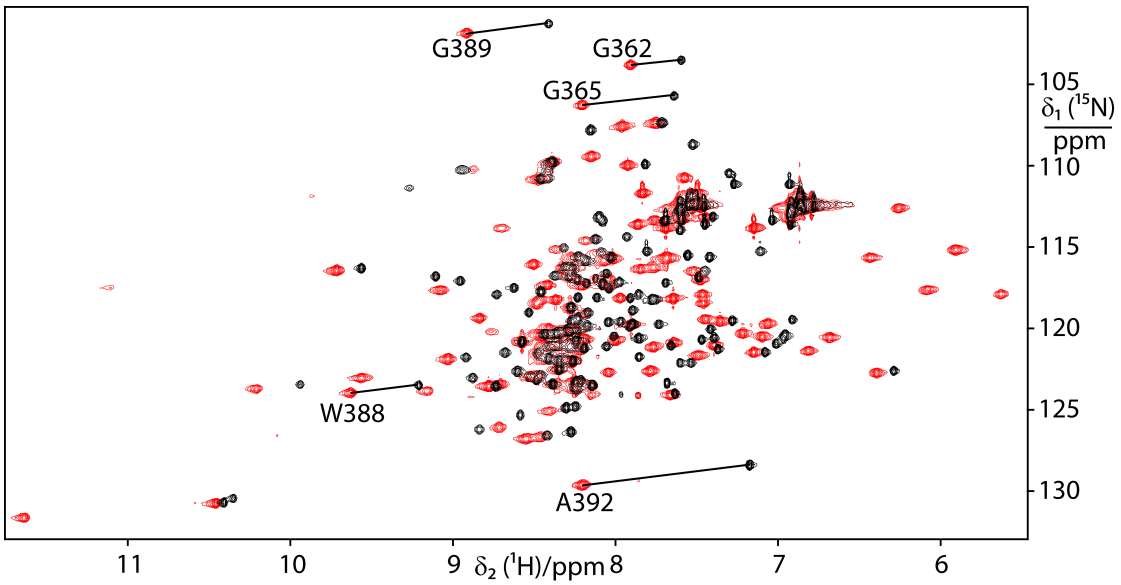
10
9
δ2 (1H)/ppm
8
7
6

B)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δ2 (1H)/ppm
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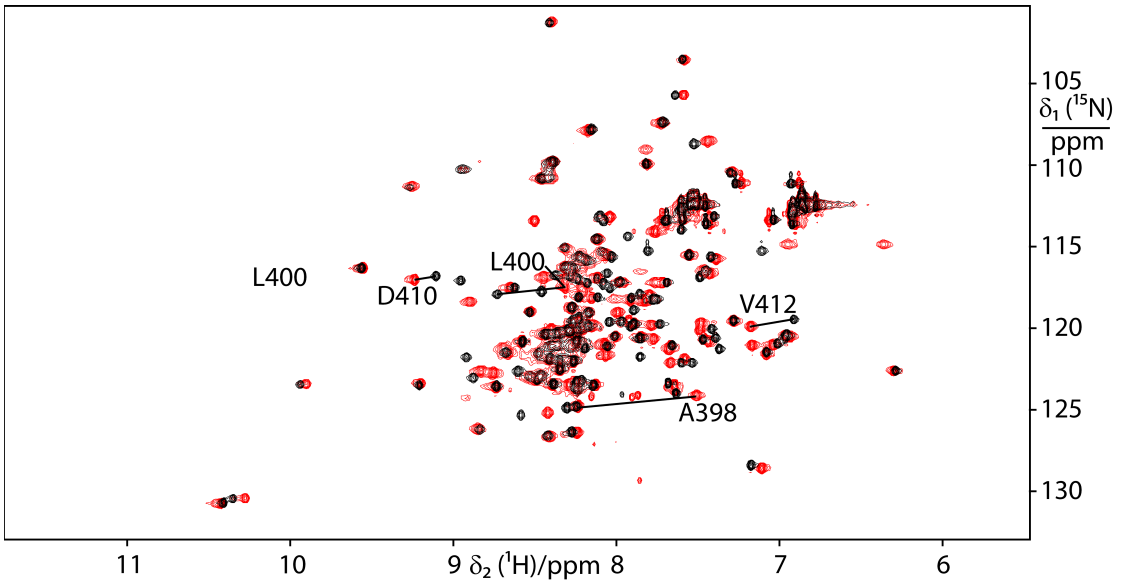
Figure S2, continued

C)



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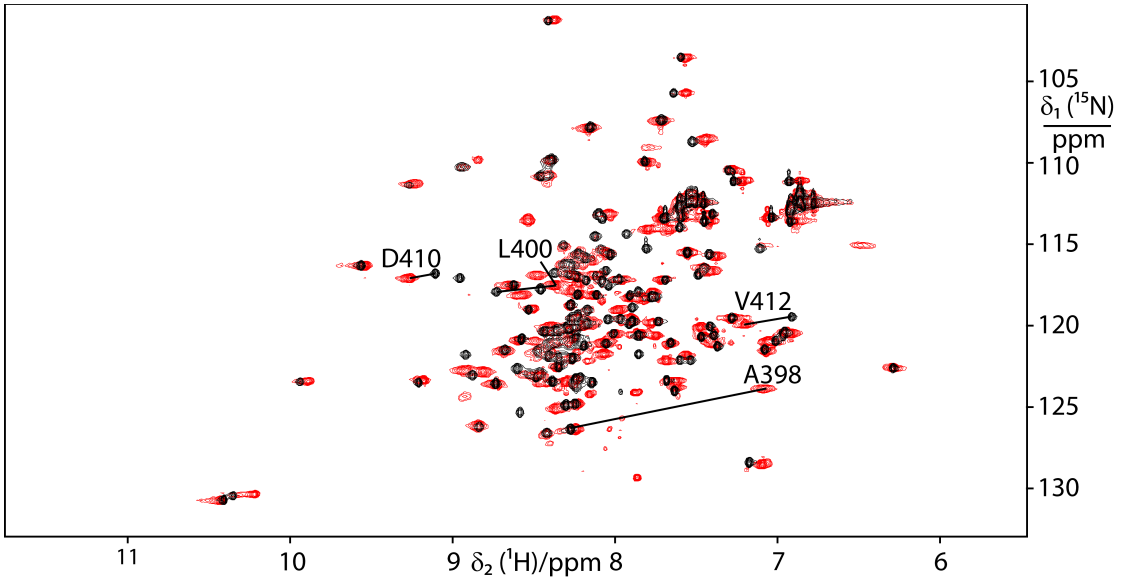


Figure S2, continued

E)

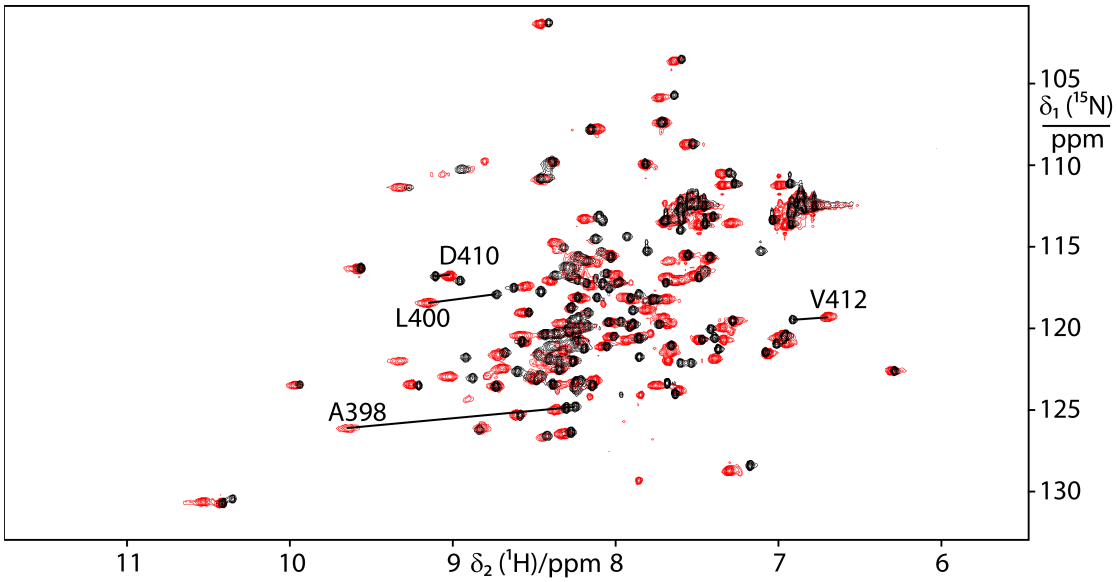


Figure S2. Superimposition of ^{15}N-HSQC spectra of p75ICD-4MDPA in complex with paramagnetic and diamagnetic metal ions. The spectra were acquired of a 0.2 mM solution of p75ICD-4MPDA in a buffer containing 20 mM HEPES buffer, pH 7.0, and 100 mM NaCl at 25 ^{o}C using a Bruker 800 MHz NMR spectrometer. A) Spectra recorded in the presence of Y^{3+} (black) and Tb^{3+} (red). Lines connecting paramagnetic and diamagnetic cross-peaks highlight the PCSs of selected residues. B, C, D, E) Same as A, except with, respectively, Yb^{2+}, Ce^{3+}, Nd^{3+}, Eu^{3+} instead of Tb^{3+}.

Table S3. PCSs (in ppm) measured for backbone amide protons of p75ICD-4MDPA in the presence of different metal ions.[a]

Residue	Tb^{3+}	Tm^{3+}	Yb^{3+}	Ce^{3+}	Nd^{3+}	Eu^{3+}
L342	0.193	-0.070	-0.123			
T343	0.458	-0.305	-0.194	0.021	0.010	-0.033
K344	0.898	-0.616	-0.329	0.031	0.024	-0.061
R345	0.952	-0.535	-0.289	0.050	0.041	-0.054
E346	0.780	-0.463	-0.206	0.028	0.015	-0.069
E347	0.942	-0.824	-0.352	0.070	0.059	-0.075
V348	1.639	-1.080	-0.433	0.098	0.083	-0.088
E349	1.053	-0.582	-0.211			
K350	0.922	-0.575	-0.210	0.028	0.025	-0.057
L351	1.180	-0.787	-0.290	0.074	0.071	-0.061
L352	0.993	-0.536	-0.174			
N353	0.651	-0.387	-0.131			
T356	0.274	-0.099	-0.011			
R358	0.022	0.138	0.106	0.011		0.012
H359	-0.057	0.211	0.141			0.017
L360	-0.124	0.343	0.231			
A361	-0.412	0.505	0.302	-0.009	-0.029	0.033
G362	-0.456	0.560	0.313	-0.012	-0.031	0.047
E363	-0.719	0.813	0.440			
L364	-1.023	1.138	0.628			
G365	-1.052	1.038	0.566	-0.052	-0.077	0.092
Y366	-0.769	0.802	0.443			
Q367	-0.461	0.494	0.270	-0.037	-0.050	0.025
E369	-0.254	0.295	0.152	-0.009	-0.021	0.015
H370	-0.281	0.308	0.171	-0.01	-0.012	0.026
I371	-0.276	0.318	0.178	-0.004	-0.011	0.028
D372	-0.185	0.245	0.138	-0.009	-0.012	0.006
S373	-0.174	0.223	0.127			0.014
F374	-0.156	0.244	0.141			
T375	-0.092	0.181	0.107	-0.005	-0.013	0.002
H376	-0.058	0.127	0.070	0.012	0.007	0.015
E377	-0.025	0.114	0.073		-0.008	
A378	0.020	0.066	0.045			
V381					0.027	0.018
R382	0.142	0.073	0.070			
A383	0.011	0.169	0.107			
L384	-0.080	0.277	0.169	0.017	0.007	0.026
L385	-0.088	0.389	0.234	0.005	-0.014	0.012
A386	-0.163	0.413	0.223	0.009	-0.010	0.015
S387	-0.326	0.506	0.268		-0.016	0.028
W388	-0.559	0.769	0.420	-0.010	-0.032	0.046
G389	-0.696	0.980	0.504	-0.015	-0.037	0.053
A390	-0.719	0.858	0.428	-0.027	-0.045	0.035
Q391	-1.041	1.080	0.556	-0.061	-0.077	0.037
D392	-0.977	0.664	0.353			
S393	-1.362	1.074	0.624			

A394		1.962	1.030			
T395			3.177			
A402		4.095	2.191	-0.203	-0.309	0.299
R405	-1.176	1.248		-0.087	-0.108	0.079
I406	-0.378	0.553	0.276	-0.011	-0.022	0.050
Q407		0.185	0.069			
R408	0.622	-0.324	-0.133			
A409	1.197	-0.908	-0.429			
D410	1.721	-1.347	-0.603	0.133	0.158	-0.085
I411	2.334	-1.750	-0.723	0.132	0.148	-0.146
V412		-3.058	-1.284	0.264	0.291	-0.215
E413		-5.405	-2.135			
L415		-4.777	-1.963			
S417			-1.644			

[a] PCSs measured in 20 mM HEPES buffer (pH 7.0) and 100 mM NaCl at 25 ^{o}C at a ^{1}H NMR frequency of 800 MHz, using the Y^{3+} complex as the diamagnetic reference. All PCSs in this table were used simultaneously to compute a new structure of p75ICD by PCS-Rosetta.

Table S4. Δχ-tensor of different metal ions bound to p75ICD-4MDPA.[a]

Metal ion	$\Delta\chi_{ax}$ /10^{-32} m^3	$\Delta\chi_{rh}$ /10^{-32} m^3	Tensor axis	Coordinates of tensor axes		
Ce^{3+}	1.1(0.1)	0.3(0.1)	x	0.471	0.701	-0.536
			y	-0.807	0.588	0.060
			z	0.357	0.404	0.842
Nd^{3+}	1.2(0.1)	0.2(0.1)	x	0.685	0.535	-0.494
			y	0.676	-0.720	0.157
			z	-0.272	-0.441	-0.855
Eu^{3+}	-0.7(0.1)	-0.3(0.1)	x	0.921	-0.200	-0.334
			y	0.060	0.920	-0.387
			z	0.385	0.337	0.859
Tb^{3+}	15.8(0.1)	2.4(0.2)	x	0.792	0.333	-0.511
			y	-0.461	0.876	-0.143
			z	0.400	0.349	0.847
Tm^{3+}	-14.8(0.3)	-3.9(0.2)	x	0.538	0.556	-0.634
			y	-0.764	0.639	-0.088
			z	0.355	0.532	0.769
Yb^{3+}	6.2(0.3)	2.9(0.4)	x	0.422	0.442	0.792
			y	-0.431	0.866	-0.254
			z	-0.798	-0.234	0.556

[a] At 25 °C and pH 7.0 in 20 mM HEPES, 100 mM NaCl. UTR tensors were determined as described in Table S2, i.e. the 4MDPA-metal complex was crafted onto Cys416 of the best structure calculated with PCS-Rosetta, the dihedral angles between the C^{α} atom of Cys416 and the DPA moiety were varied, and Δχ-tensors were fitted using all PCSs of all metal ions simultaneously with a common metal position (see the Experimental Section). Between the 20 conformers with the best PCS fit, the coordinates of mean metal position were (-7.329, 7.848, -10.996; see http://rsc.anu.edu.au/~go/coordinates/ for the coordinates of the p75ICD structure determined by PCS-Rosetta) varied with a standard deviation of about 0.6 Å in each dimension.

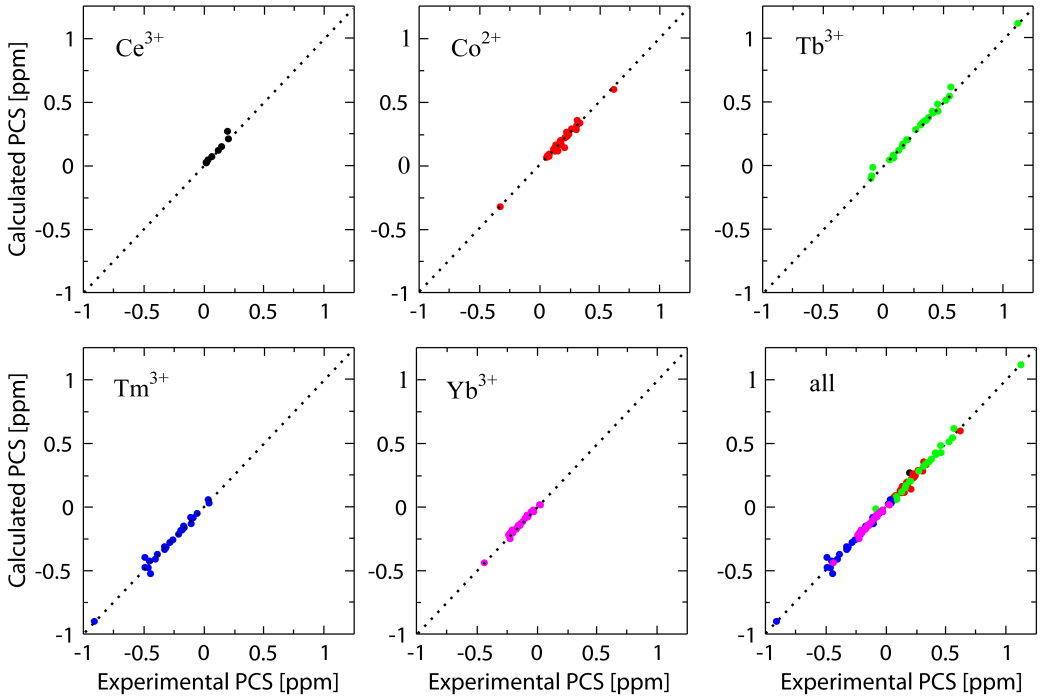
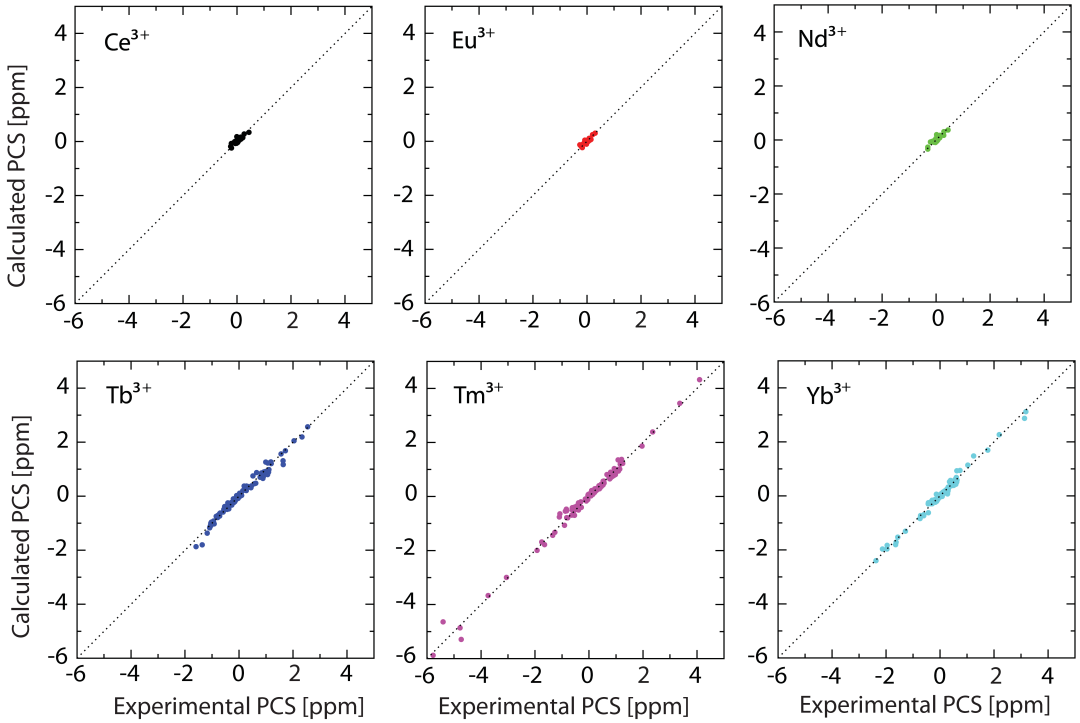


Figure S3. Correlation between experimental and back-calculated amide proton PCSs of ArgN-4MDPA in complex with different metal ions. The experimental PCSs are listed in Table S1. The back-calculated PCSs were obtained from a best fit of the experimental PCSs to the first NMR conformer of ArgN (PDB ID 1AOY) as described in the main text.

A)



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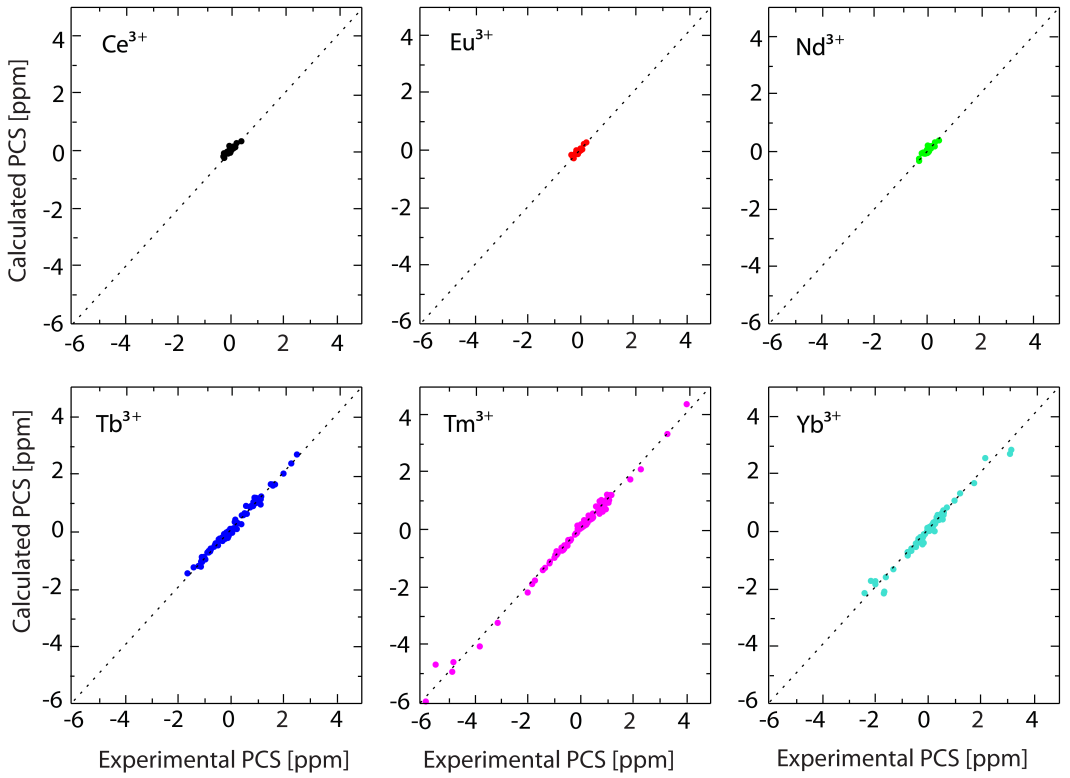

Figure S4. Correlation between experimental and back-calculated backbone amide PCSs of p75ICD-4MDPA in complex with different metal ions (experimental PCSs listed in Table S2). (A) Using the first conformer of the structure calculated from NOEs (PDB ID 1NGR). (B) Using the structure calculated with PCS-Rosetta.

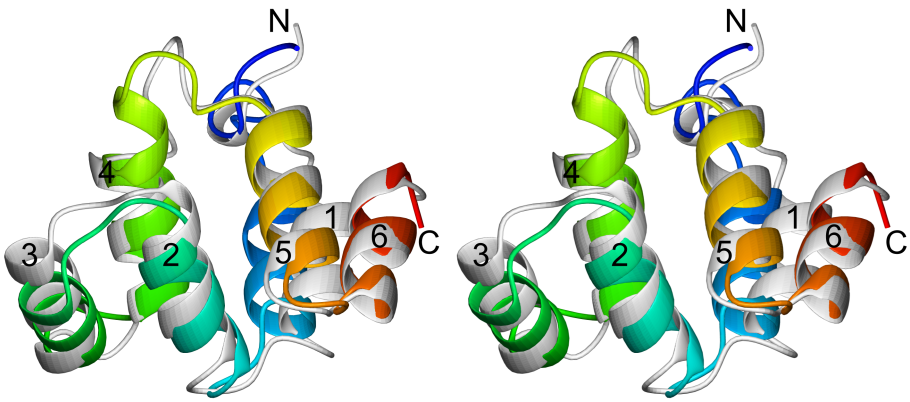

Figure S5. Comparison of the 3D structures of p75ICD determined by NOEs and PCS-Rosetta. The figure shows a stereo-view of the superimposition of the first conformer of the NMR structure (PDB ID 1NGR; drawn with a coloured ribbon) and of the structure obtained from PCS-Rosetta calculations using the PCSs of Table S2 as experimental restraints (ribbon drawn in grey). The structures are displayed in an orientation closely similar to that of Figure 5B, with the helices of the death-domain fold numbered 1-6.

The shift of helix 1 relative to helices 5 and 6 (the site of the metal ion) in the PCS-Rosetta structure improved the fit of the PCSs. PCS-Rosetta minimizes the sum of the square of the differences between experimental and back-calculated pseudocontact shifts ($\Delta\delta^{PCS}$(exp) and $\Delta\delta^{PCS}$(calc), respectively) for all metal ions. Using the first conformer of the NMR structure 1NGR, a best fit of $\Sigma[\Delta\delta^{PCS}(exp) - \Delta\delta^{PCS}(calc)]^2$ (in ppm^2) yielded 0.78 for helix 1 (residues 343-350), 0.14 for helix 5 (residues 396-405) and 0.75 for helix 6 (residues 409-416). The respective values for the PCS-Rosetta structure were 0.11, 0.30 and 0.82.

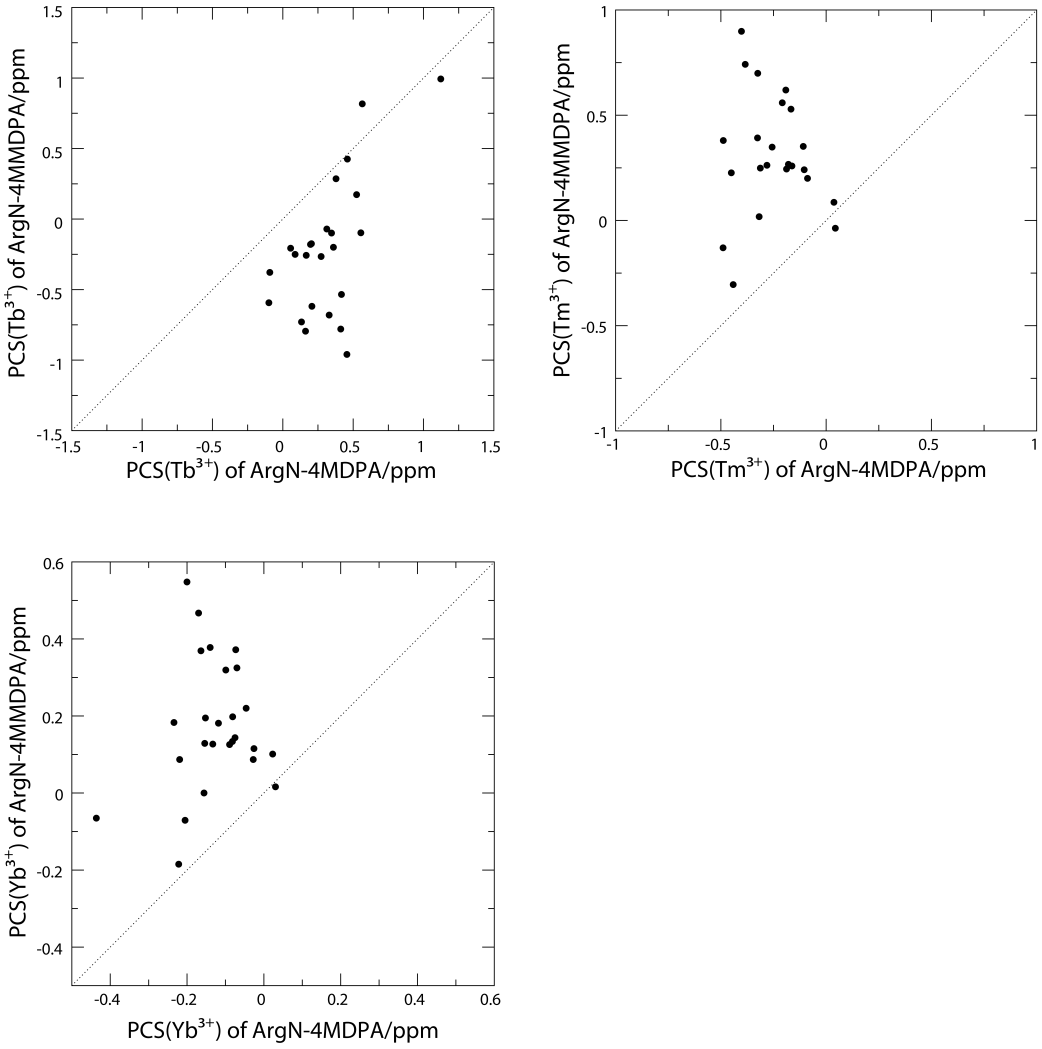

Figure S6. Correlation of PCSs observed for amide protons of ArgN tagged with 4MMDPA and 4MDPA and in complex with different metal ions. Data with 4MMDPA are those reported in ref. [3]. Data with 4MDPA are those of Table S1.

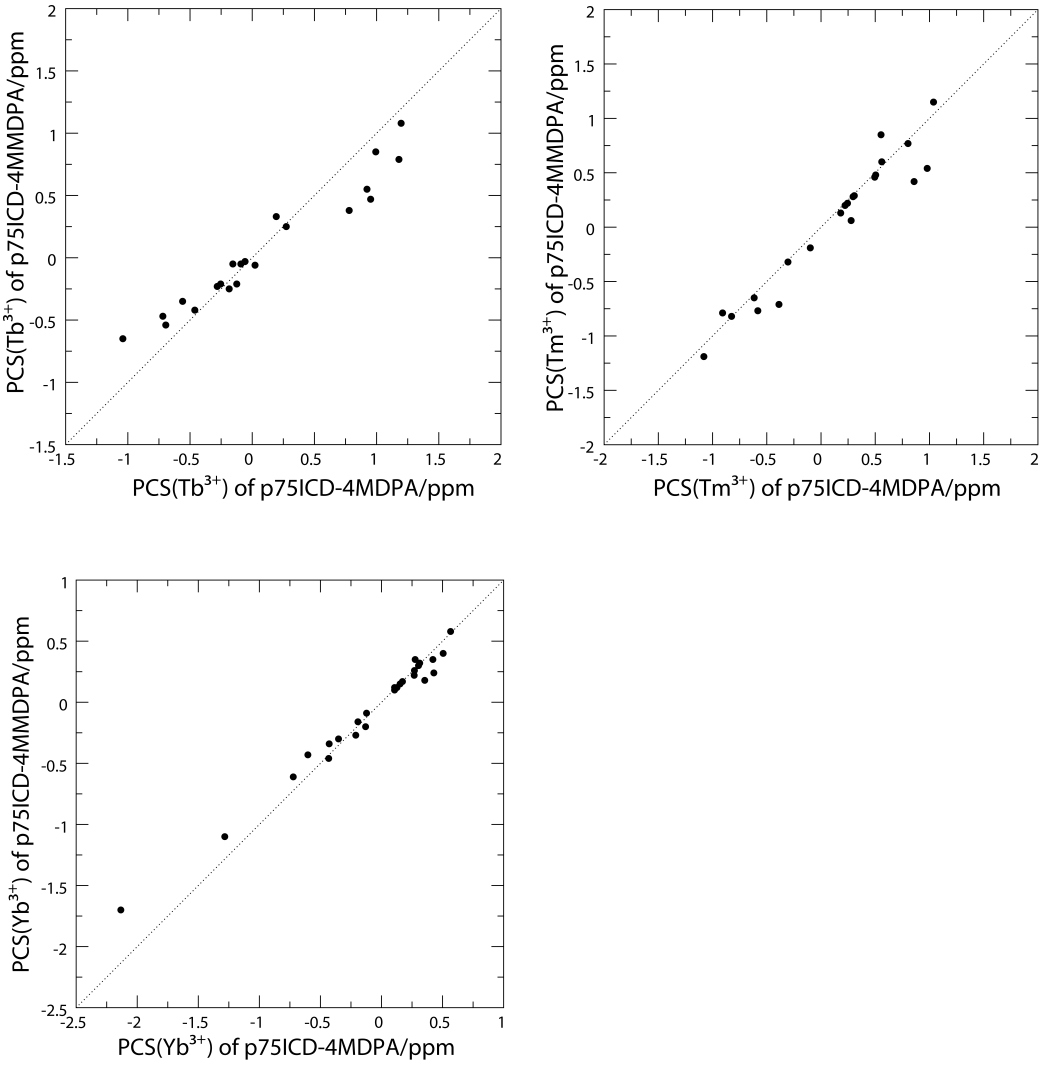

Figure S7. Correlation of PCSs observed for amide protons of p75ICD tagged with 4MMDPA and 4MDPA and in complex with different metal ions. Data with 4MMDPA are those reported in ref. [4]. Data with 4MDPA are those of Table S3.

References:

[1] C. Schmitz, M. J. Stanton-Cook, X. C. Su, G. Otting, T. Huber, *J. Biomol. NMR* **2008**, *41*, 179-189.

[2] M. Sunnerhagen, M. Nilges, G. Otting, J. Carey, J. *Nat. Struct. Biol.* **1997**, *4*, 819-826.

[3] X. C. Su, B. Man, S. Beeren, H. Liang, S. Simonsen, C. Schmitz, T. Huber, B. A. Messerle, G. Otting, *J. Am. Chem. Soc.* **2008**, *130*, 10486-10487.

[4] A. Potapov, H. Yagi, T. Huber, S. Jergic, N. E. Dixon, G. Otting, D. Goldfarb, *J. Am. Chem. Soc.* **2010**, *132*, 9040-9048.