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Supporting Information

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3-Mercapto-2,6-Pyridinedicarboxylic Acid: A Small Lanthanide-Binding Tag for Protein Studies by NMR Spectroscopy

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A)

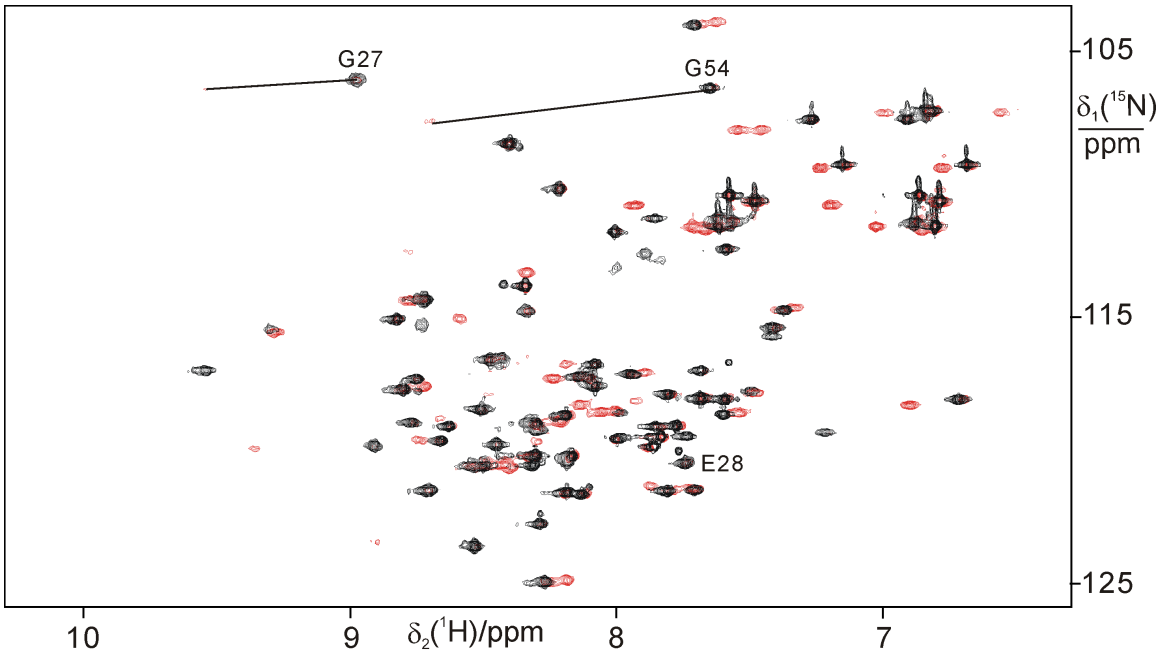
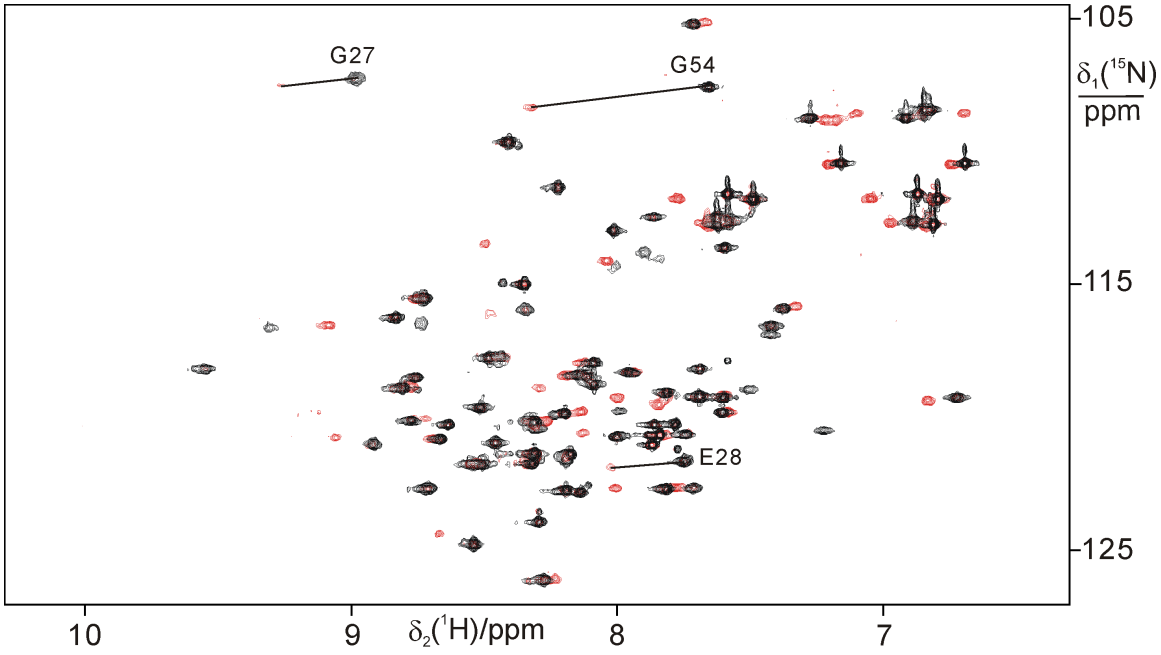


Figure S1 continued

B)



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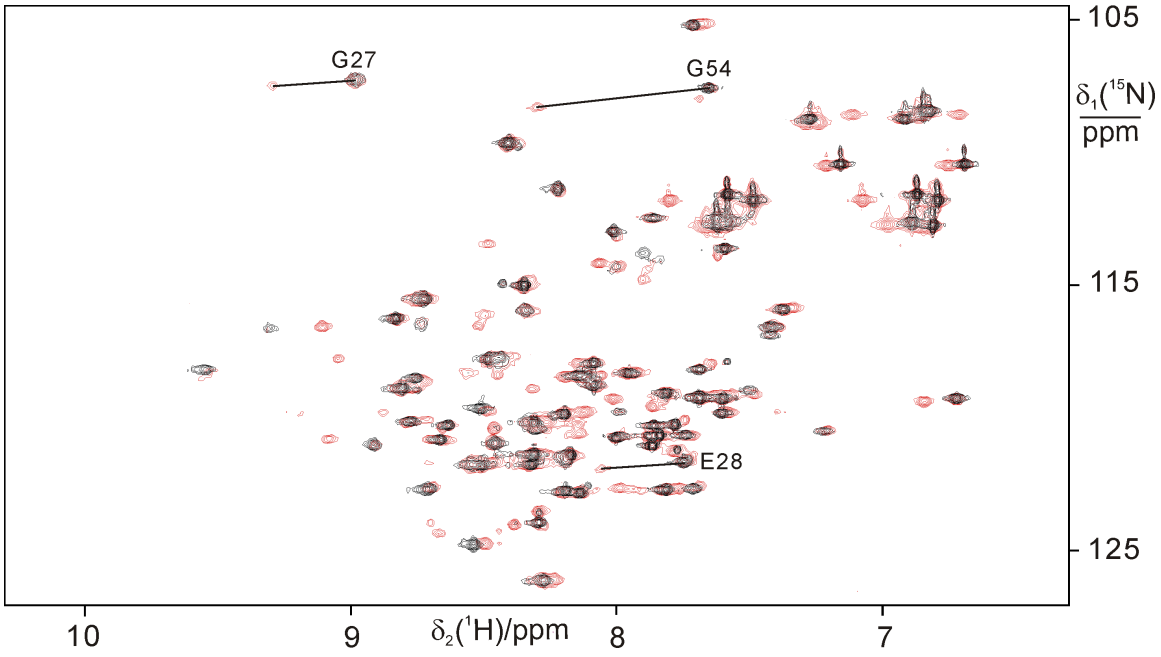
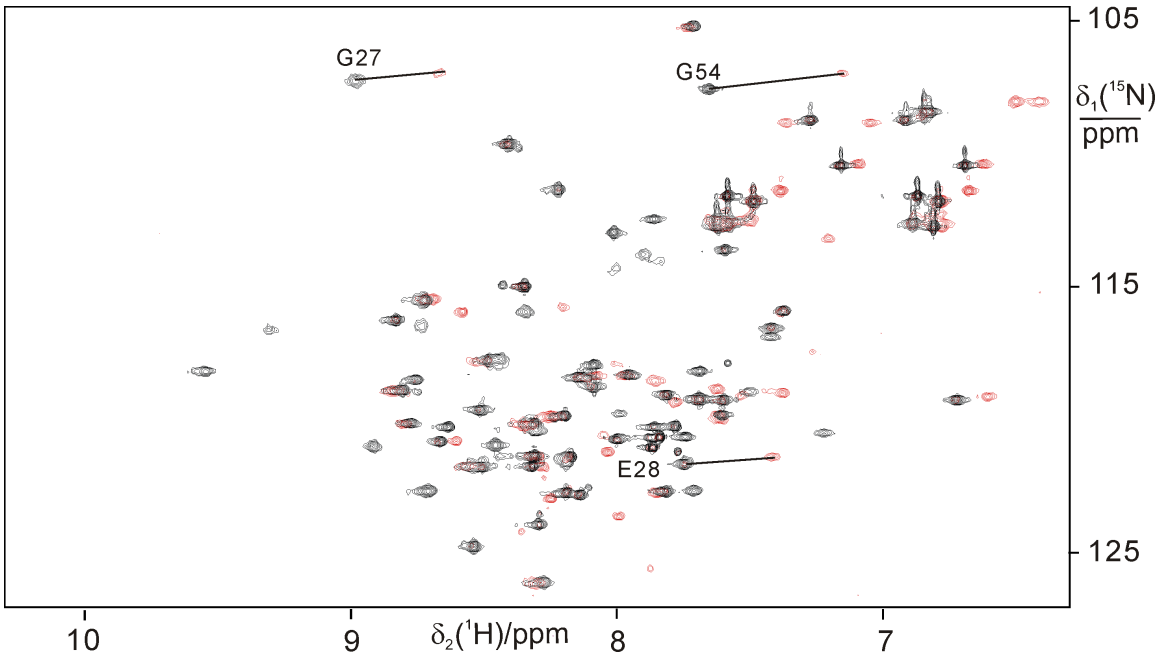


Figure S1, continued

D)



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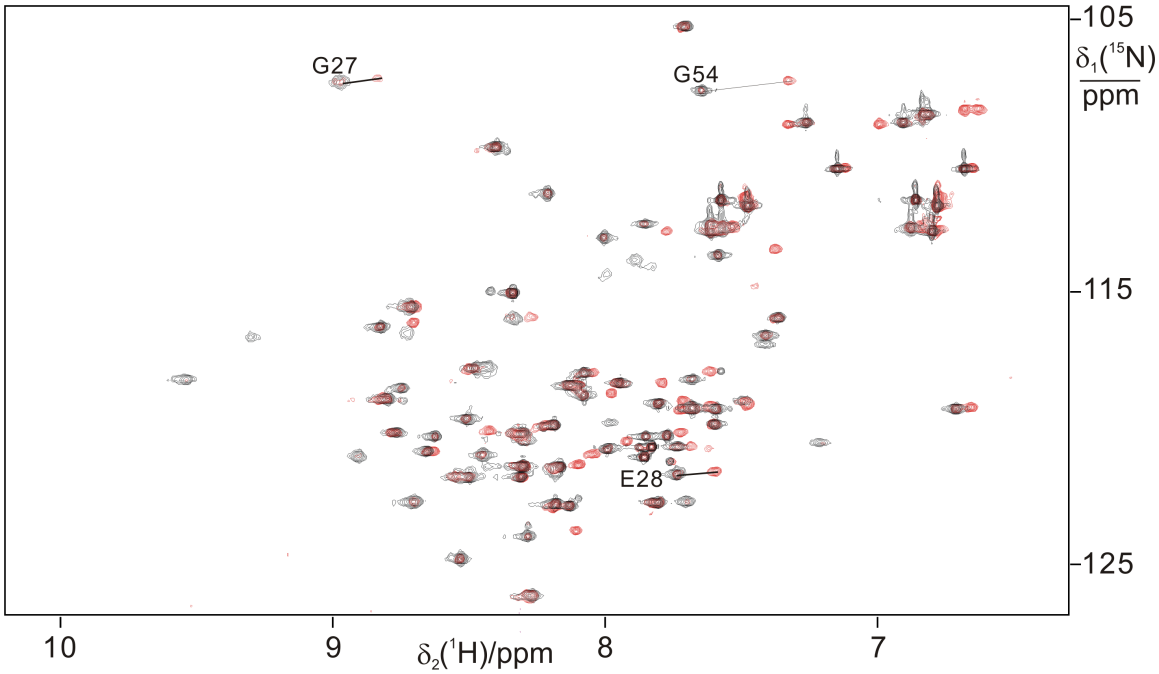

Figure S1, continued

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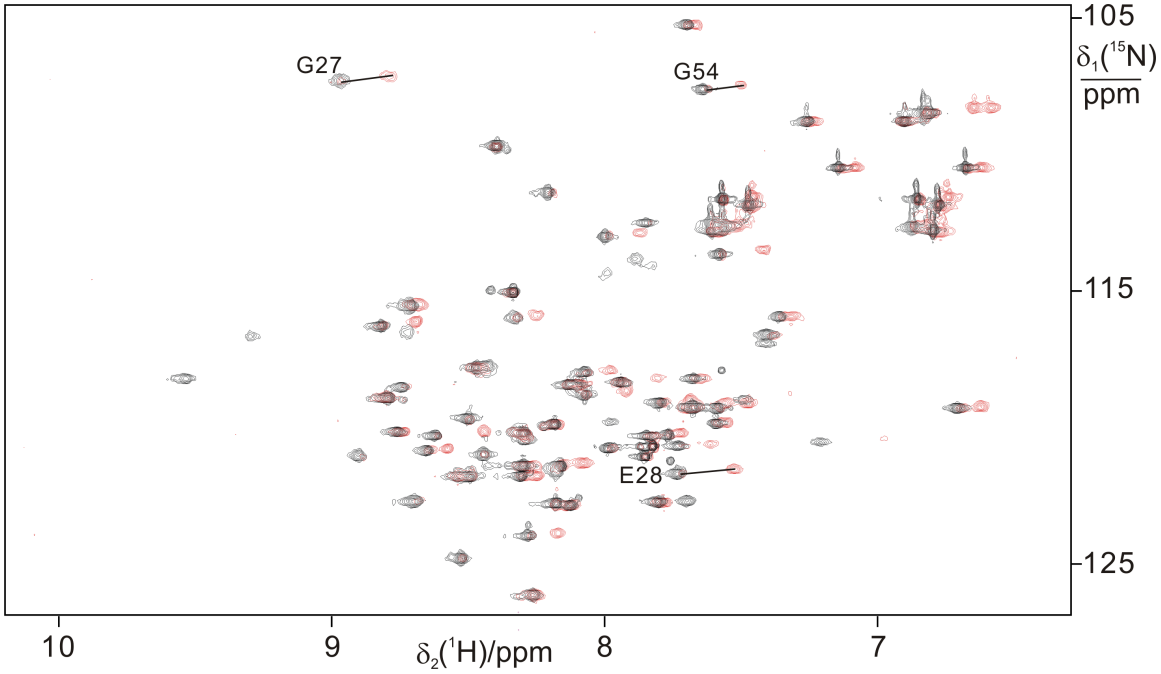

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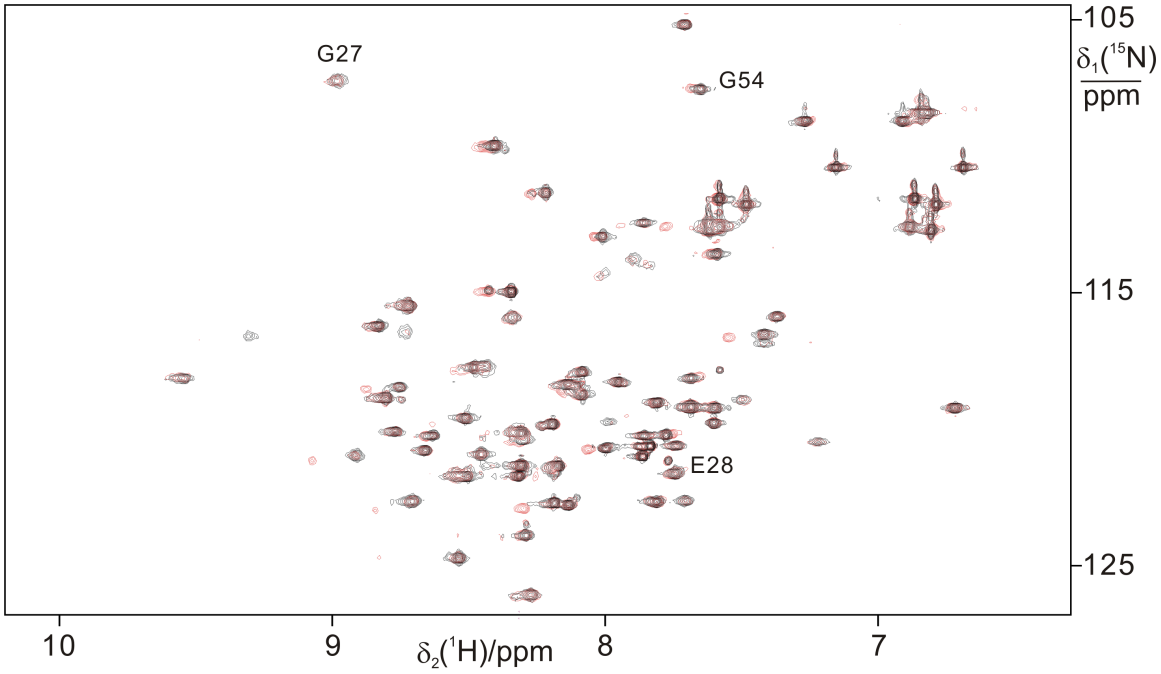

Figure S1. ^{15}N-HSQC spectra of ArgN-3MDPA in complex with (A) Dy^{3+}, (B) Ho^{3+}, (C) Tb^{3+}, (D) Tm^{3+} (E) Yb^{3+}, (F) Er^{3+} and (G) Ce^{3+}. In all cases, Y^{3+} was present together with the paramagnetic lanthanide in a ratio of 0.8:1. Each spectrum is superimposed by a spectrum recorded with Y^{3+} alone (black). The spectra were recorded at 25°C in a buffer of 20 mM MES (pH 6.5) on a Bruker 800 MHz NMR spectrometer, using a protein concentration of about 0.15 mM. Selected pairs of cross-peaks from the diamagnetic and paramagnetic molecules are connected by lines and labelled with their assignment. Some additional peaks appear in Fig. S1C due to protein without metal binding tag.

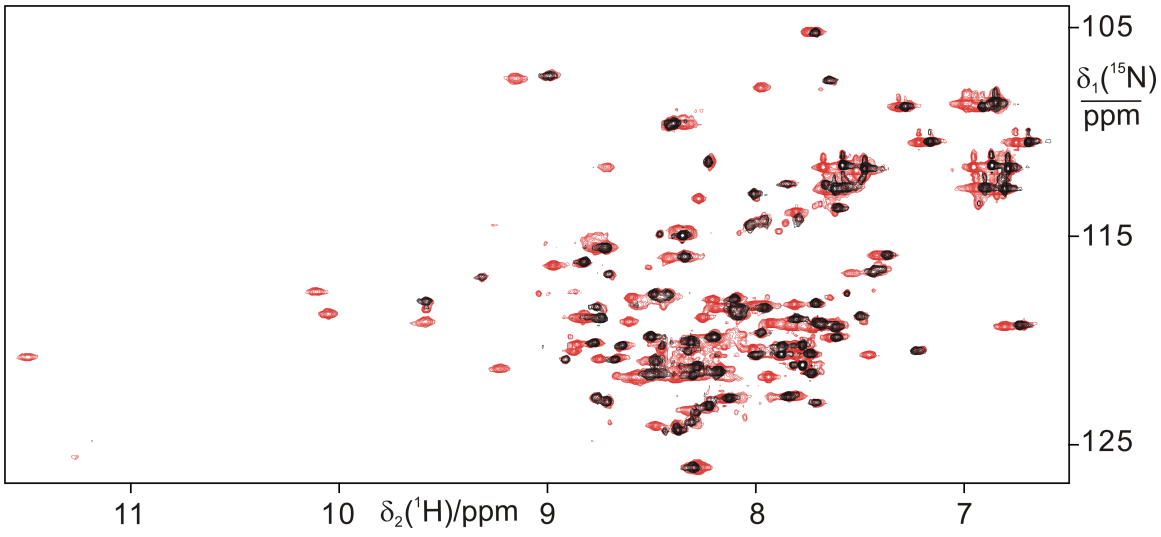

Figure S2. Colour version of Figure 1B, showing a superimposition of ^{15}N-HSQC spectra of ArgN-3MDPA in complex with Zn^{2+} (black) and a mixture of Co^{2+}/Zn^{2+} (red).

Table S1. PCSs (in ppm) observed for backbone amide protons of ArgN-3MDPA in complex with different metal ions[1]

Residue number	Ce^{3+}	Tb^{3+}	Dy^{3+}	Ho^{3+}	Er^{3+}	Tm^{3+}	Tb^{3+}	Co^{2+}
L10		-0.05	-0.07	-0.08		0.02		0.03
V11			-0.05					
K12		-0.11	-0.19	-0.10		0.08	0.04	0.03
A13		-0.15	-0.24	-0.19		0.11	0.06	0.03
F14		-0.06	-0.09	-0.10		0.02	0.02	0.09
K15			-0.06	-0.06		0.06	0.02	0.12
A16	-0.02	-0.38			0.15	0.27	0.15	
L17	-0.02					0.17		0.13
L18	0.00						0.09	0.34
G27		0.31	0.57	0.29	-0.18	-0.31	-0.14	0.17
E28		0.31	0.40	0.28	-0.19	-0.31	-0.14	0.20
I29				0.32	-0.23		-0.13	0.24
V30		0.23		0.19	-0.15	-0.22	-0.10	0.17
A31		0.14		0.14		-0.15	-0.06	0.14
A32		0.06		0.06	-0.10		-0.05	0.14
L33		-0.03	0.11	0.04	-0.08	-0.09	-0.03	0.12
Q34				0.03	-0.06	-0.06	-0.02	0.10
E35					-0.04			0.07
Q36			-0.07	-0.08				0.05
G37		-0.04	-0.05	-0.07	0.00			0.04
F38				-0.03				0.04
D39								0.04
N40		0.03	0.05	0.02			0.07	0.05
I41			0.14					0.09
N42		0.12	0.20	0.11	-0.06	-0.12	-0.06	
S44		0.16	0.26	0.14	-0.07	-0.14	-0.07	0.08
K45		0.14	0.27	0.14	-0.07	-0.15	-0.07	0.09
V46		0.20	0.35	0.19	-0.10	-0.19	-0.09	0.12
S47		0.28	0.46	0.27	-0.12	-0.25	-0.12	0.15
R48		0.26	0.41	0.24		-0.22	-0.11	0.13
M49		0.28	0.47	0.27		-0.24	-0.13	0.16
L50	0.03	0.45	0.74	0.42			-0.20	0.25
T51	0.01	0.47	0.76	0.44	-0.17	-0.39	-0.21	0.22
K52		0.38	0.63	0.38	-0.15	-0.30	-0.18	0.19
F53	0.01	0.47	0.79	0.49	-0.10	-0.40	-0.23	0.27
G54	0.03	0.65	1.10	0.67	-0.11	-0.46	-0.32	0.33
A55	0.05	1.09		1.02	-0.12	-0.81	-0.31	
V56								
T58	0.18							

R59		
N60	0.09	
N60	0.16	
N60	0.30	
K62	0.13	
M63	0.07	0.50
E64	0.08	
M65		
V66	0.12	
Y67	0.07	1.17
C68	0.18	

[1] Measurements in 20 mM MES buffer (pH 6.5) at 25°C and a ^{1}H NMR frequency of 800 MHz. PCS data are not listed when pronounced paramagnetic relaxation enhancements prevented observation of cross-peaks in the presence of a paramagnetic metal ion (e.g. for residues 56-68, as the 3MDPA tag was attached to Cys68), when the large magnitude of PCSs (of spins near the paramagnetic ion) made their assignment ambiguous, or for residues closer to the C-terminus for which flexibility prevented interpretation of their PCSs with a single conformer.

A)

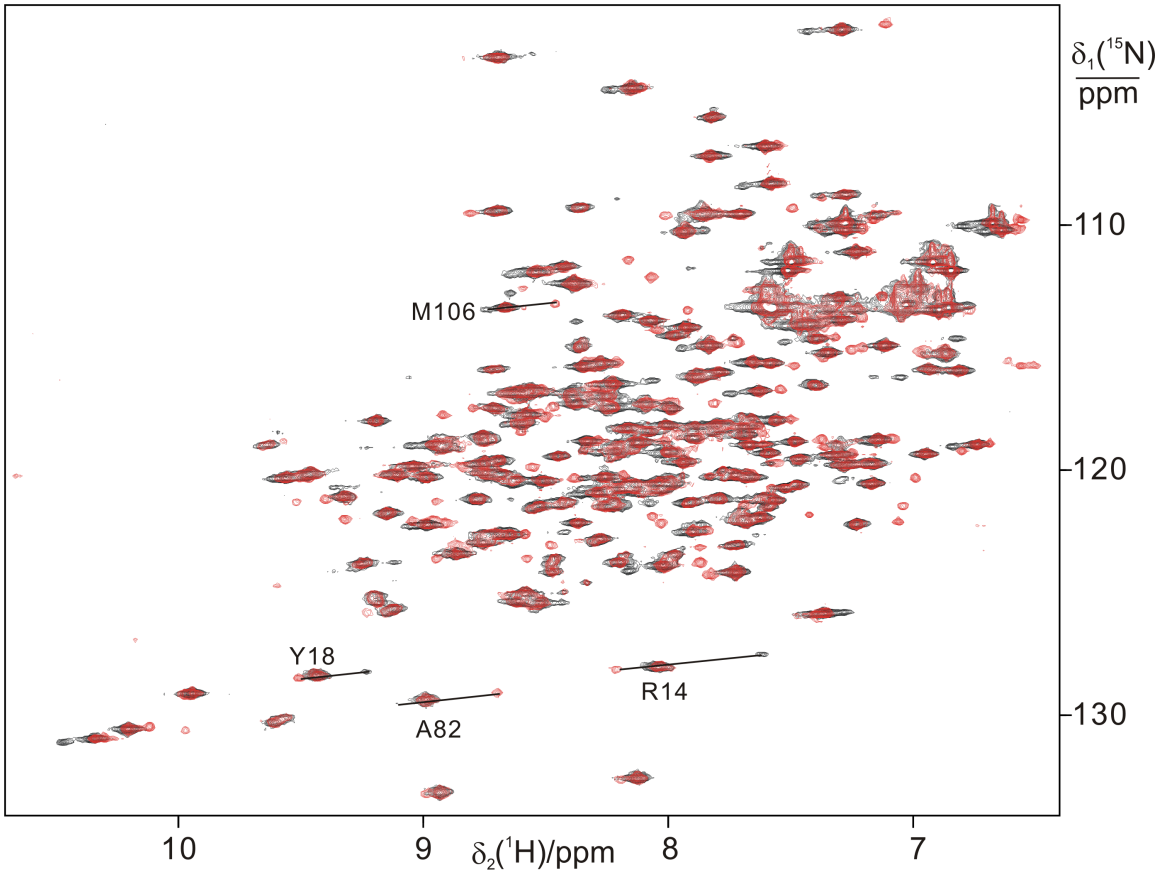

Figure S3, continued

B)

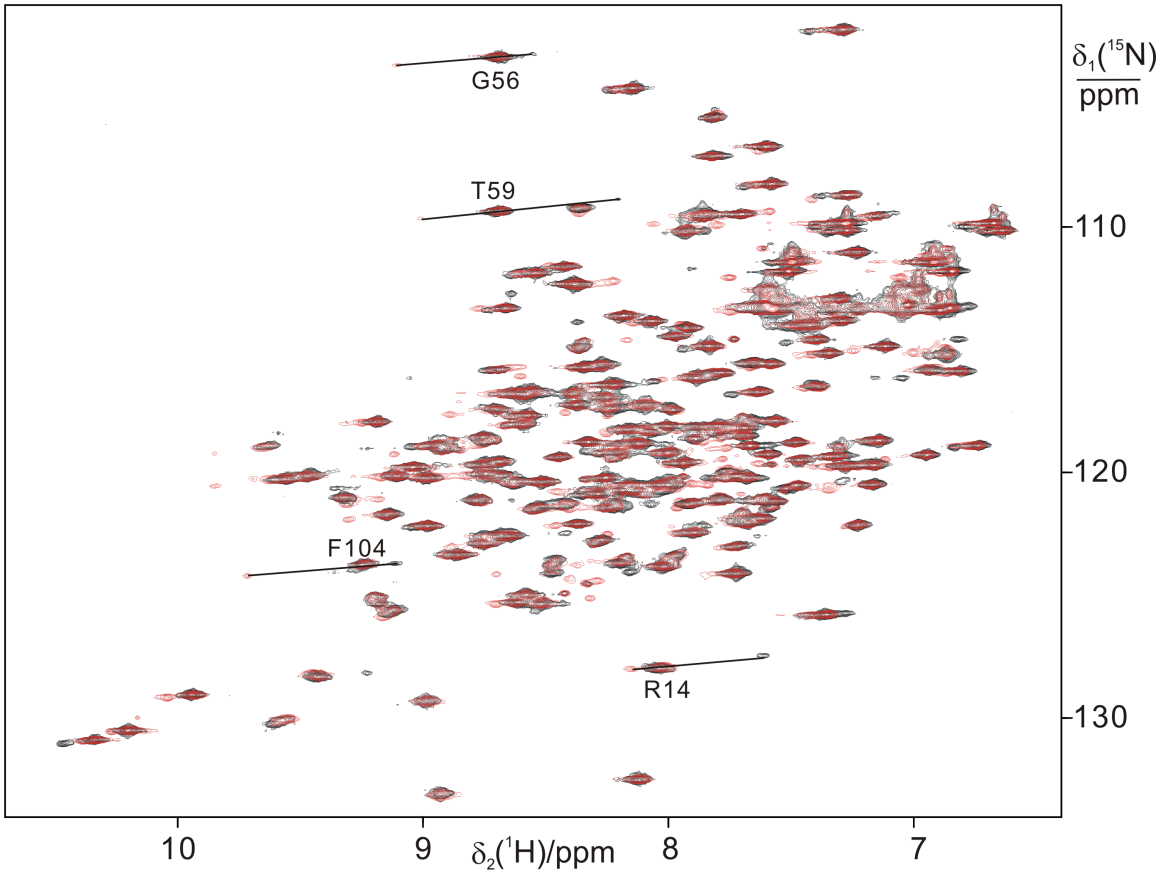

Figure S3. ^{15}N-HSQC spectra of the C54T/C97A/Q69C triple mutant of T4 lysozyme derivatized with 3MDPA and in complex with Tm^{3+}, Lu^{3+} and Co^{2+}. Selected pairs of cross-peaks from the diamagnetic and paramagnetic molecules are connected by lines and labelled with their assignment. (A) Superimposition of the spectrum with Co^{2+} (red) onto the spectrum recorded with a 1:0.8 mixture of Tm^{3+} and Lu^{3+} (black). (B) Superimposition of spectra recorded with 1:0.8 mixtures of Tm^{3+}:Lu^{3+} of the construct with 3MDPA (black) and the construct with 4MMDPA (red). The spectra were recorded at 25°C in a buffer of 20 mM MES (pH 6.5) on a Bruker 800 MHz NMR spectrometer, using a protein concentration of about 0.15 mM.

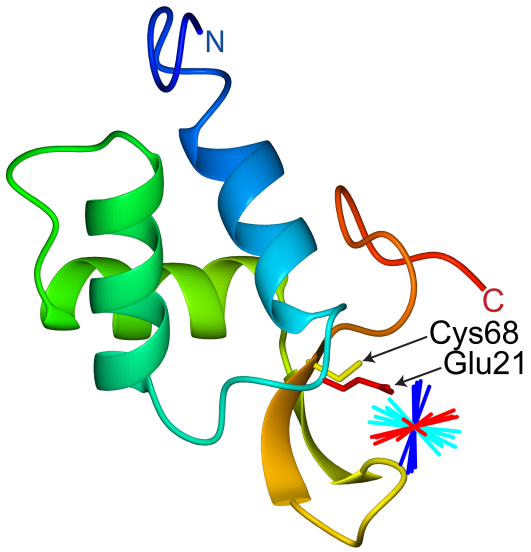

Figure S4. Colour version of Figure 2, showing the Δχ tensors of eight different metal ions bound to the ArgN-3DPA adduct. The x-, y- and z-axes of the Δχ tensors are coloured red, cyan and blue, respectively.

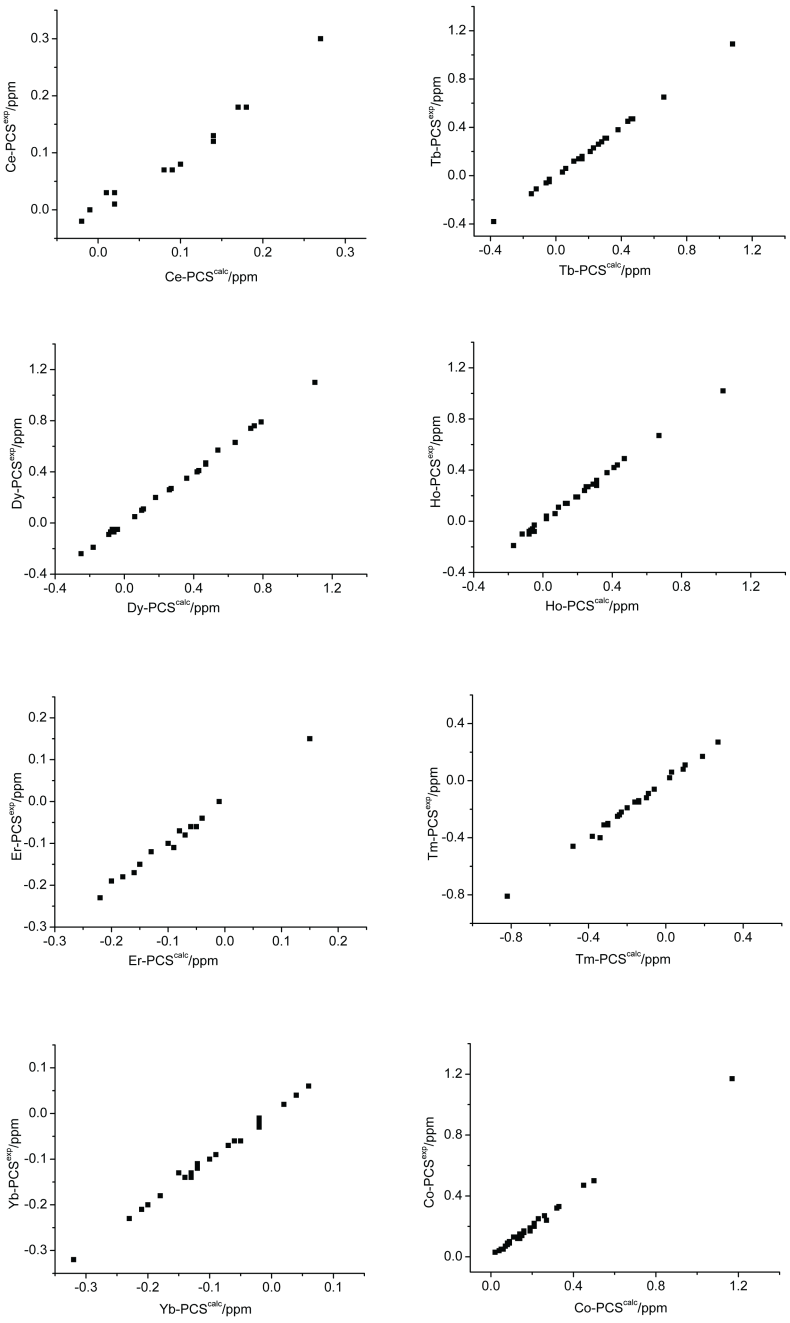

Figure S5. Correlation of experimental versus back-calculated PCSs from the fit of the $\Delta\chi$ tensors of eight different metal ions bound to the ArgN-3DPA adduct. All PCSs were simultaneously included in the fit using a single metal position.

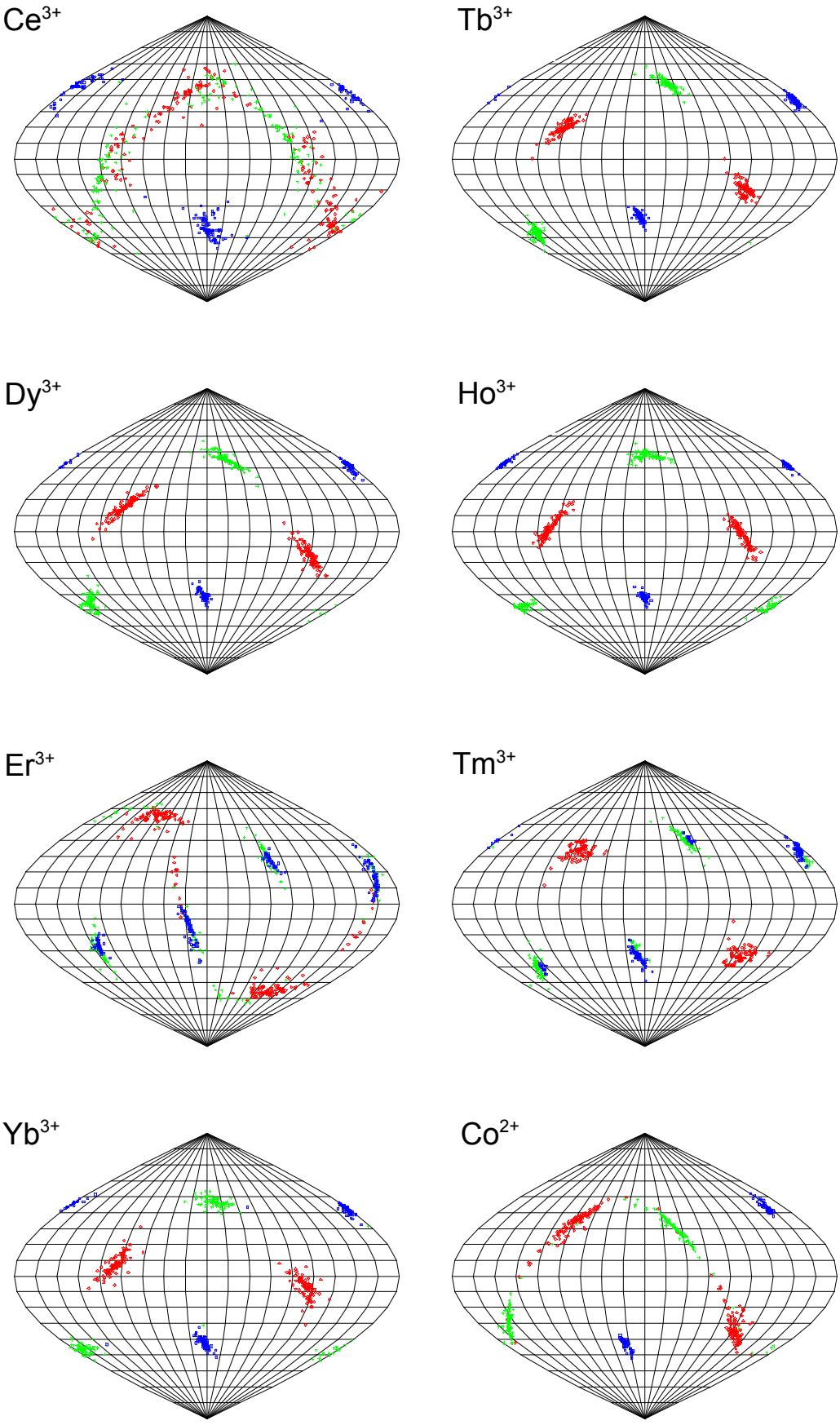
Ce^{3+}
Tb^{3+}
Dy^{3+}
Ho^{3+}
Er^{3+}
Tm^{3+}
Yb^{3+}
Co^{2+}

Figure S6. Sanson-Flamsteed plots of $\Delta\chi$-tensor stability analysis. $\Delta\chi$ tensors were reoptimized using 100 subsets of PCS data from 36 randomly selected amides (equal to 80% of the total of 45 amides). Colour code: blue: z axis, green: y axis, red: x axis.