Supporting Information

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Engineering $[Ln(DPA)_3]^{3-}$ binding sites in proteins: a widely applicable method for tagging proteins with lanthanide ions

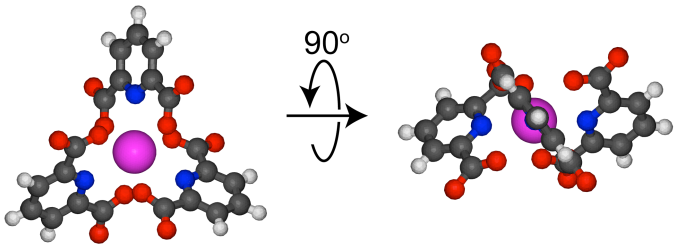



**Fig. S1** Structure of the $[Lu(DPA)_3]^{3-}$ complex (Harrowfield JM, Kim Y, Skelton BW, White AH (1995) Aust J Chem 48:807-823). The view on the left is along the C3 symmetry axis and the view on the right is after rotation by 90°. Magenta: $Lu^{3+}$ ion; black: carbon; blue: nitrogen; red: oxygen; white: hydrogen.

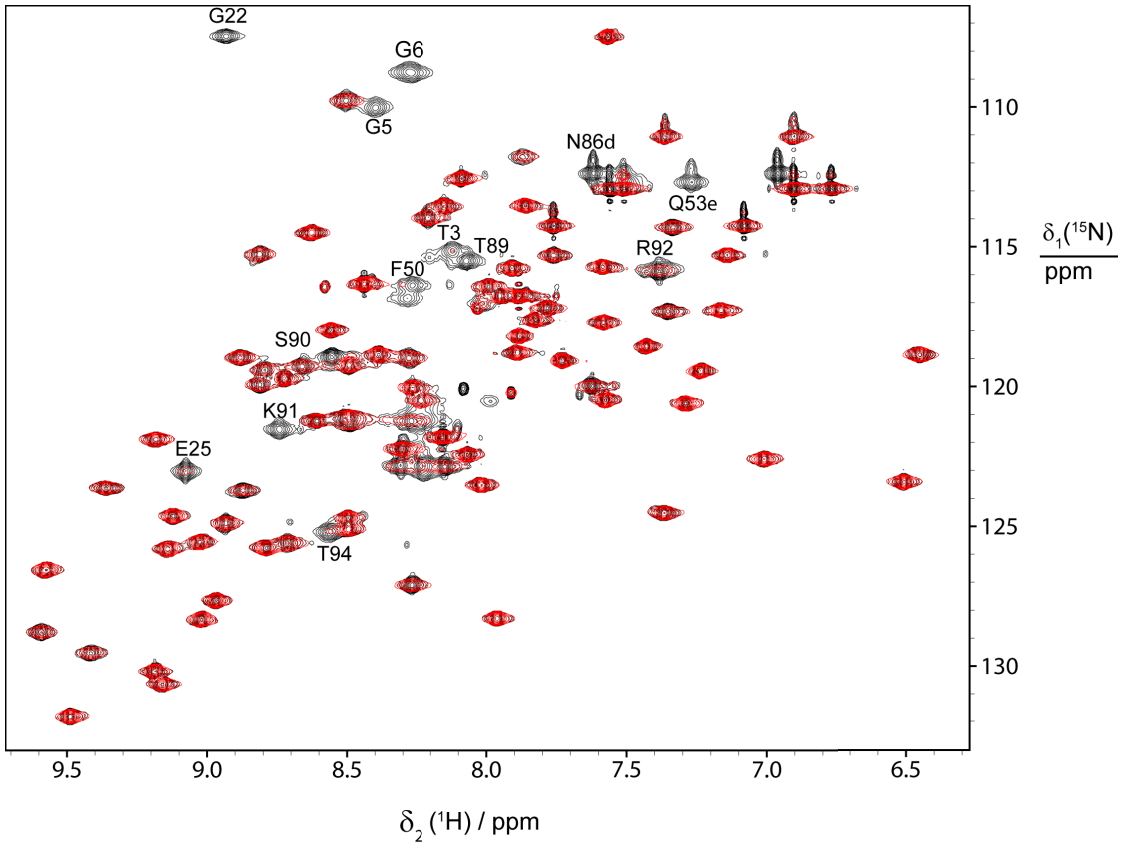
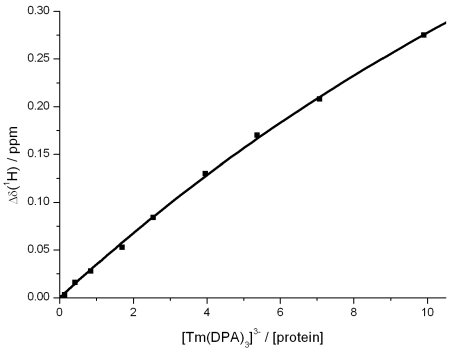
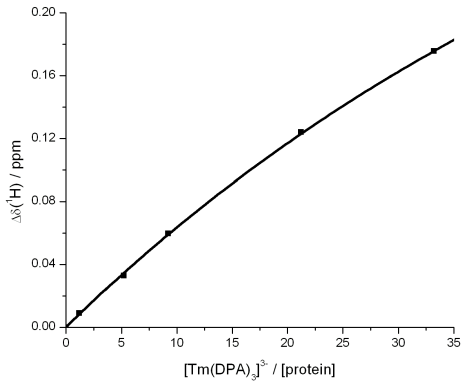



**Fig. S2** PREs induced by $[Gd(DPA)_3]^{3-}$ in the wild-type hnRNPLL RRM1 domain. The figure shows an overlay of $^{15}$N-HSQC spectra of the wild-type RRM1 domain without (black) and with (red) $[Gd(DPA)_3]^{3-}$. The concentrations of protein and $[Gd(DPA)_3]^{3-}$ complex were 0.47 and 0.235 mM, respectively, in standard NMR buffer (Table 1). Cross-peaks that broadened or disappeared due to the presence of $[Gd(DPA)_3]^{3-}$ are labelled with residue type and sequence number. The cross-peak of Arg92 is probably broadened as large PREs were observed for Lys91 and Thr94, but it is overlapped with the cross-peak of Lys51.

(a)




(b)




(c)

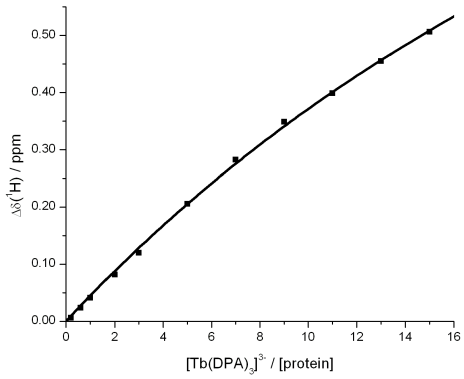



**Fig. S3** Binding affinities of wild-type and mutant RRM1 domains for $[Ln(DPA)_3]^{3-}$ measured in standard NMR buffer (Table 1). The proteins were titrated with $[Tm(DPA)_3]^{3-}$ or $[Tb(DPA)_3]^{3-}$ to magnify the chemical shift changes by pseudocontact shifts. 1:1 binding was assumed to fit the data. (a) Titration curve of a 0.25 mM solution of the wild-type RRM1 construct with $[Tm(DPA)_3]^{3-}$, monitoring the $^1$H chemical shift of the $^{15}$N-HSQC cross-peak of Thr89. The fitted curve corresponds to $K_d$ = 8±1 mM. (b) Titration curve of 0.1 mM solution of the RRM1(D62R) mutant with $[Tm(DPA)_3]^{3-}$. The $^1$H chemical shift of the amide of Ser15 was monitored. The fitted curve corresponds to $K_d$ = 10±1 mM. (c) Titration curve of a 0.1 mM solution of the RRM1(D62R/E66Q) mutant with $[Tb(DPA)_3]^{3-}$. The $^1$H chemical shift of Ser15 was monitored. The fitted curve corresponds to $K_d$ = 4.1±0.4 mM.

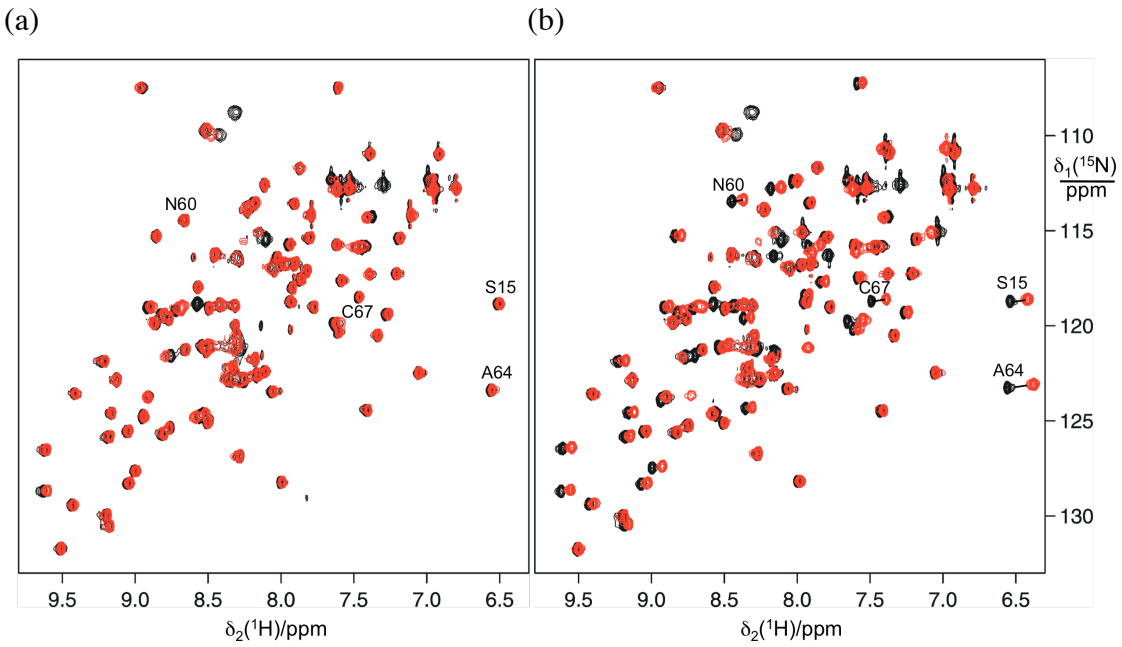



**Fig. S4** Superimposition of $^{15}$N-HSQC spectra recorded of 0.15 mM solutions of $^{15}$N-labeled wild-type and mutant RRM1 domains in standard NMR buffer and in the presence of diamagnetic or paramagnetic metal-DPA complexes to measure pseudocontact shifts. (a) Wild-type RRM1 in the presence of 1 mM $[Y(DPA)_3]^{3-}$ (black) or $[Tm(DPA)_3]^{3-}$ (red). (b) Same as (a), but for the mutant (D62R/E66Q). Some of the PCSs are identified by arrows connecting diamagnetic and paramagnetic peaks.

**Table S1** $\Delta\chi$ tensors generated in the RRM1(D62R/E66Q) mutant by different $[Ln(DPA)_3]^{3-}$ complexes[a]

| Metal ion | $\Delta\chi_{ax}/10^{-32}$ m$^3$ | $\Delta\chi_{rh}/10^{-32}$ m$^3$ |
|---|---|---|
| $Tb^{3+}$ | -0.63 | -0.39 |
| $Tm^{3+}$ | -0.46 | -0.20 |
| $Yb^{3+}$ | -0.32 | -0.08 |

[a] The PCSs induced by $[Tb(DPA)_3]^{3-}$, $[Yb(DPA)_3]^{3-}$, and $[Tm(DPA)_3]^{3-}$ were measured in standard NMR buffer at a protein concentration of 0.15 mM using a 7-fold excess of $[Ln(DPA)_3]^{3-}$ complex. The PCSs were corrected by subtracting very small paramagnetic shifts observed in the wild-type protein. The $\Delta\chi$ tensor parameters were fitted with the program Numbat (Schmitz C, Stanton-Cook MJ, Su XC, Otting G, Huber T (2008) J Biomol NMR 41:179-189) using PCSs of amide protons, the published protein structure (PDB ID 1WEX, model 12) and the metal coordinates determined from the PREs generated by $[Gd(DPA)_3]^{3-}$ (Figure 2). Model 12 was used because it fitted the PREs best.

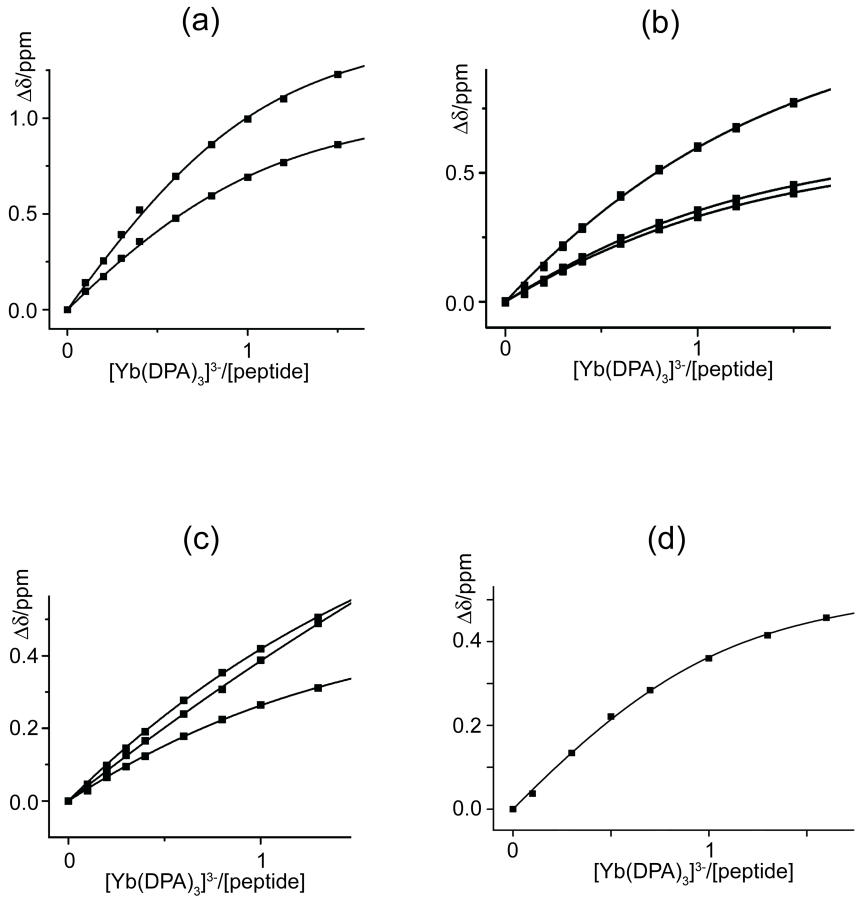



**Fig. S5** Titration curves of different peptides with $[Yb(DPA)_3]^{3-}$ at 25 $^{o}$C. Peptides were used at 1 mM concentration. The chemical shift changes observed for different NMR resonances of the peptides are plotted against the ratio of lanthanide complex to peptide. Only peptide resonances were used that could be resolved in 1D $^{1}$H NMR spectra. No specific resonance assignment was attempted. (a) Titration of $His_6$-$NH_2$ in 20 mM MES buffer at pH 7.2. The average fitted $K_d$ value was 0.25±0.08 mM. (b) Titration of N-acetyl-$His_6$ in 20 mM MES buffer at pH 6.5. The average fitted $K_d$ value was 0.6±0.2 mM. (c) Same as (b), except in 20 mM Tris buffer at pH 7.2 and in the presence of 100 mM NaCl. The average fitted $K_d$ value was 4±4 mM. (d) Titration of the hexa-peptide MRAAAR in 20 mM MES buffer at pH 6.5. The fitted $K_d$ value was 0.22±0.06 mM.