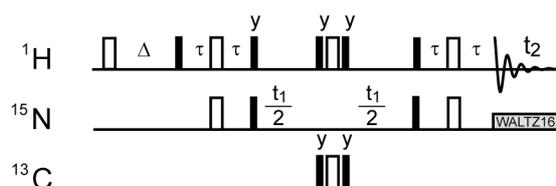


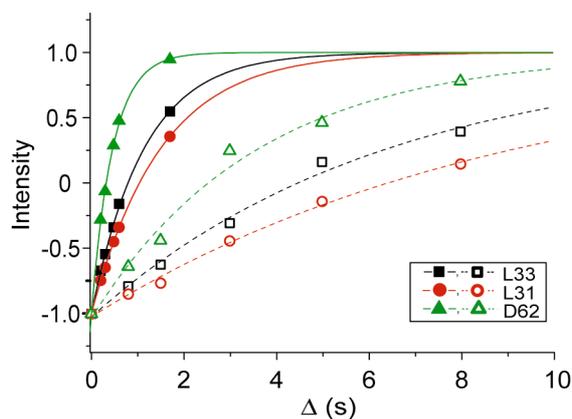
## Probing Surface Accessibility of Proteins using Paramagnetic Relaxation in Solid State NMR Spectroscopy

by

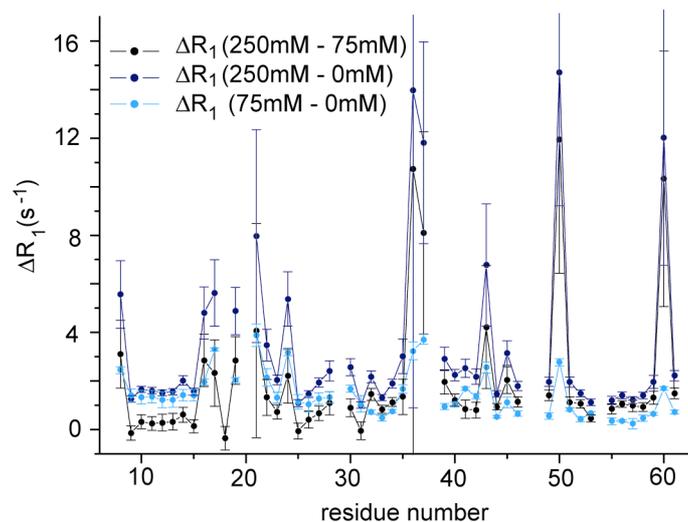
Rasmus Linser, Uwe Fink, and Bernd Reif



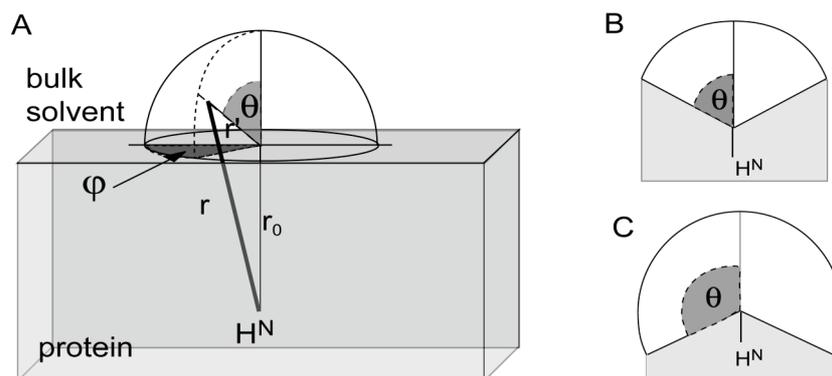
**Supporting Figure 1.** Pulse sequence for the 2D inversion recovery experiments. Open bars refer to  $180^\circ$  pulses, closed ones to  $90^\circ$  pulses. The first  $^{15}\text{N}$   $90^\circ$  pulse was phase shifted by  $90^\circ$  steps according to TPPI.  $\tau$  was set to 2.4 ms, the recovery delay is depicted as  $\Delta$ . Decoupling from  $^{15}\text{N}$ - $^1\text{H}$ - $^1J$ -coupling during acquisition was performed using 2 kHz decoupling.



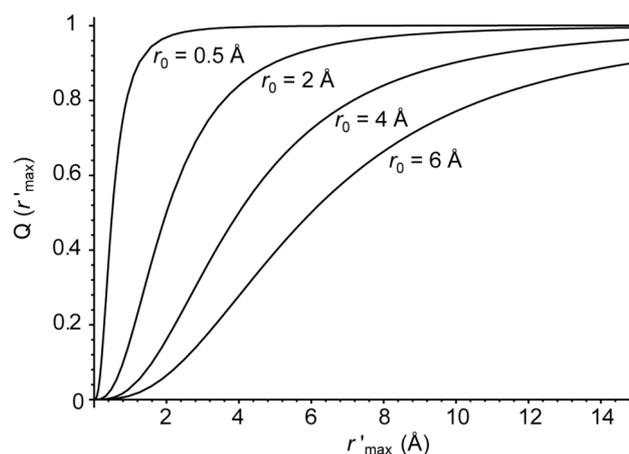
**Supporting Figure 2.** Representative  $^1\text{H}$ - $T_1$  curves for selected residues. The figure displays the experimental data for the amide resonances of L33 (black), L31 (red), and D62 (green). Solid and dashed lines refer to crystals containing 75 mM  $\text{Cu}^{\text{II}}$  and 0 mM  $\text{Cu}^{\text{II}}$ .



**Supporting Figure 3.** Difference relaxation rates extracted from 3 different protein preparations containing 250 mM, 75 mM, and 0 mM  $\text{Cu}^{\text{II}}$ . We find similar trends for the difference  $\text{H}^{\text{N}}$   $R_1$  relaxation rates  $\Delta R_1$  for all three combinations of the three data sets.



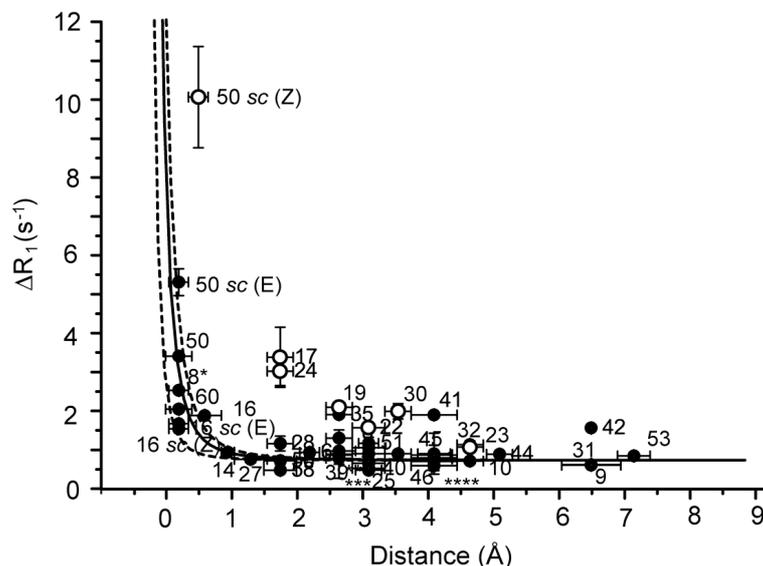
**Supporting Figure 4.** A) Integration procedure for the calculation of the distance dependent difference rate  $\Delta R_1$ . For any  $\text{H}^{\text{N}}$  proton, an effective distance to the dissolved chelate is obtained by calculation of the weighted sum, with  $r \geq r_0$ . Assuming a half-sphere for the solvent accessible volume, the integration was performed for  $0 \leq \theta \leq 90^\circ$  and  $0 \leq \varphi \leq 360^\circ$ . For  $0 \leq r' \leq r'_{\text{max}}$ , a cut-off  $r'_{\text{max}}$  of  $8 \text{ \AA}$  was used.  $r$  is calculated as  $r = \sqrt{r_0^2 + r'^2 - 2r_0r' \cos(\pi - \theta)} = \sqrt{r_0^2 + r'^2 + 2r_0r' \cos \theta}$ . B,C) In order to approximate concave and convex surfaces, the maximum value of  $\theta$  was chosen to be smaller or larger than  $\pi/2$ , respectively.



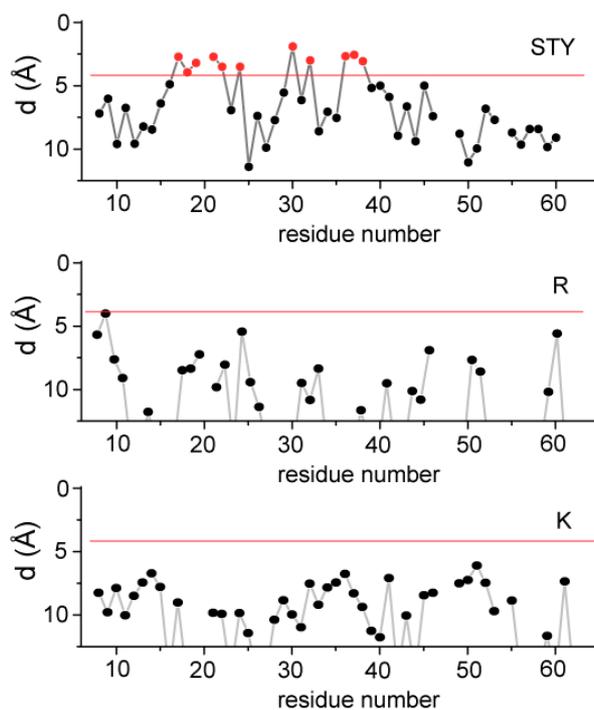
**Supporting Figure 5.** Convergence of the spherical integral for different  $r_0$

$$Q(r'_{\max}) = \frac{1}{r'_{\max}{}^6} \cdot \left[ \lim_{r'_{\max} \rightarrow \infty} \left( \frac{1}{r'_{\max}{}^6} \right) \right]^{-1}$$

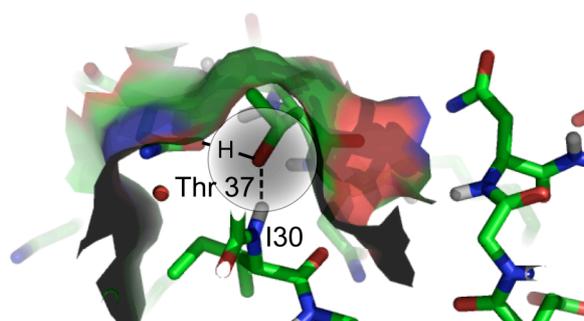
To take contributions from remote chelates properly into account, the spherical integral has to be carried out for  $r'_{\max} \gg r_0$ . For amide protons located directly beneath the surface, small cut-off values  $r'_{\max}$  are sufficient, while an effective distance for amide protons further away can only be calculated employing sufficiently large spheres. However, the absolute contribution to the difference rate  $\Delta R_1$  for amide protons with large  $r_0$  is rather small. The poor convergence for those protons does therefore not compromise the analysis.



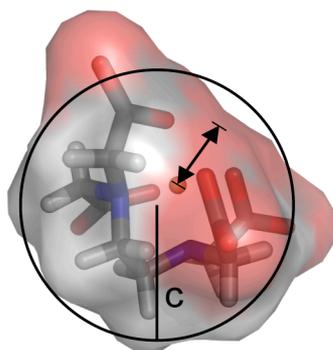
**Supporting Figure 6.** Difference relaxation rates  $\Delta R_1$  versus distance to the chelate accessible surface for all residues as in Figure 3 of the main manuscript. The figure includes amide protons which are close to sidechain hydroxyl groups (depicted as white circles). All other residues are represented in black. Dotted lines refer to convex and concave surfaces, respectively.



**Supporting Figure 7.** Distances between  $H^N$  and hydroxyl groups (top), arginine side chains (center), and lysine side chain  $NH_3^+$  (bottom), respectively (for distances  $< 15 \text{ \AA}$ ). The red line marks the employed cut-off distance of  $3.5 \text{ \AA}$ .



**Supporting Figure 8.** SH3 structure around residue T37. Due to its localisation in the interface between two symmetry related molecules and its involvement in an H-bond with N35, the OH group of T37 is supposedly not accessible for chemical exchange with water. Consequently, no strong PRE is observable for I30, although the intermolecular distance of  $I30-H^N$  to  $T37-OH$  is exceptionally short ( $1.89 \text{ \AA}$ ).



**Supporting Figure 9.** For the exploration of the chelate accessible volume in the crystal structure, Cu-edta was assumed to be a perfect sphere with radius “ $c$ ” = 4 Å. Although this simplification is reasonable for the calculation of the Connolly surface, the precise geometry of the molecule is obviously important for the quantitative analysis of the chelate induced relaxation. In [Figure 3](#) of the main manuscript, we find a horizontal offset  $B = 0.5$  Å, which is due to the distance between the paramagnetic center and the surface of the chelate. Alternatively, the PRE might be induced indirectly via water molecules. In this case, a very small radius “ $c$ ” ([Figure 2A](#)) can be assumed for the relaxation inducing agent.