Supporting information

Nanometer range distance measurement in a protein using Mn^{2+} tags

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Contents

Protocol for the preparation of the p75DD sample with Mn^{2+}-EDTA tags

The p75 death domain gene coding for residues 334-425 of the intracellular domain of the neurotrophin low-affinity receptor p75 (p75DD) was cloned with an N-terminal MASMTGHHHHHH tag into the expression plasmid pETMCSI [1] for overproduction of the protein under control of the T7 promoter. The protein was expressed in *E. coli* Rosetta (λDE3)/pRARE cells and purified by a HisTrap HP column (GE Healthcare, 5 mL). The EDTA tag ([S-methanethiosulfonylcysteaminyl]ethylenediamine-N,N,N',N'-tetraacetic acid; purchased from TRC) was attached to the p75DD sample as follows. A 0.2 mM protein solution in reaction buffer (20 mM Tris-HCl, pH 7.6) was reduced with 5 equivalents of DTT. The DTT was washed out using Millipore Ultra-4 centrifugal filters (MWCO 3,000). 4 equivalents of EDTA tag were added to the solution and allowed to react for 5 hours at room temperature. Excess EDTA tag was washed out and the buffer was exchanged to 20 mM HEPES in D_2O, pD 7.0, using Millipore Ultra-4 centrifugal filters (MWCO 3,000). The ligation efficiency was almost 100% as indicated by ESI mass spectrometry. For EPR measurements, a 0.1 mM solution of EDTA-tagged protein was prepared in 80% D_2O/20% glycerol-d_8. Two equivalents of an aqueous $MnCl_2$ solution were added to form the di-Mn^{2+} complex.

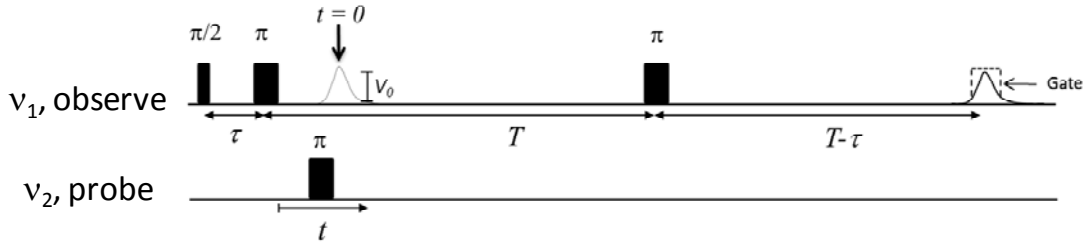

Figure S1. The four-pulse double electron-electron resonance (DEER) sequence used in the present work. Measurements were carried out on a home-built pulse EPR spectrometer.[2] A rectangular detection gate (duration ~90 ns) was placed on the refocused echo generated by the 2nd observe π pulse at time 2*T*. Parameters used: ν_1 = 94.975 GHz, ν_2 = 94.9 GHz, τ = 200 ns, T = 5.8 μs, 1 ms recovery delay between scans, probe (observe) π/2 pulse = 30 ns, probe (observe) π pulse = 60 ns, pump π pulse = 15 ns. The DEER traces were generated by systematically incrementing the delay *t* from -140 ns to 5.14 μs in increments of 20 ns. The time t=0 (zero time), marked with an arrow, is the time where the first observer echo with intensity V_0 is generated. Sample preparation involved loading of about 2-3 μL of protein solution into a quartz capillary (0.6 mm I.D.)

followed by rapid freezing and cooling to 10 K by insertion into a pre-cooled cryostat attached to the EPR magnet.

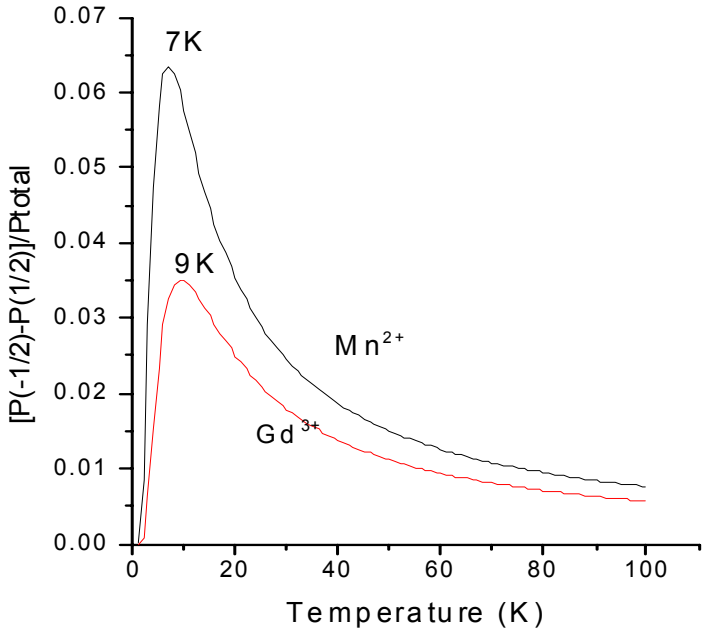

Figure S2. Calculated Boltzmann population difference of the central transition (|-1/2> →|1/2>) relative to the total population for Mn^{2+} and Gd^{3+} at 95 GHz as a function of temperature.

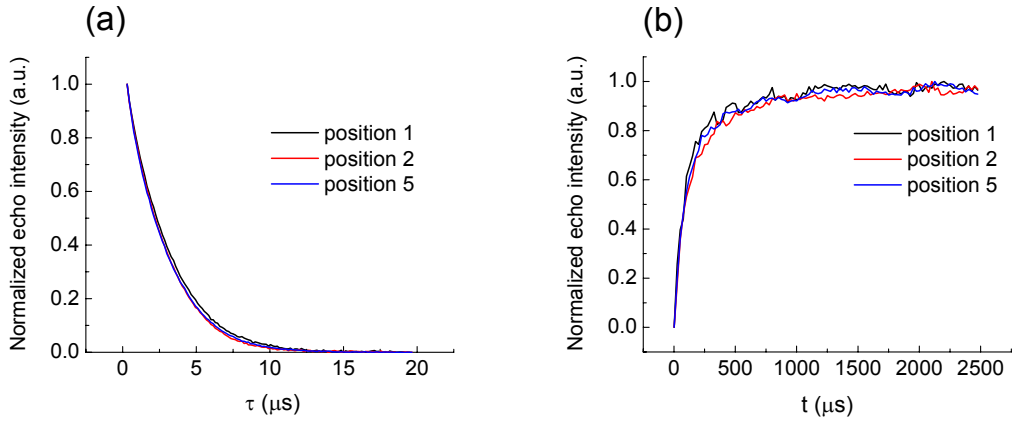

Figure S3. W-band two-pulse echo decays (a) and saturation recovery curves (b) at 10 K. The pulse lengths used were $t_{\pi/2}$ = 30 ns and t_{π} = 60 ns, with a saturation pulse of 5 ms, applied at the magnetic field positions indicated in Figure 1 of the main text.

References

1. Neylon, C.; Brown, S. E.; Kralicek, A. V.; Miles, C. S.; Love, C. A.; Dixon, N. E., Interaction of the Escherichia coli replication terminator protein (Tus) with DNA: A model derived from DNA-binding studies of mutant proteins by surface plasmon resonance. *Biochemistry* **2000,** 39, 11989-11999.
2. Goldfarb, D.; Lipkin, Y.; Potapov, A.; Gorodetsky, Y.; Epel, B.; Raitsimring, A. M.; Radoul, M.; Kaminker, I., HYSCORE and DEER with an upgraded 95 GHz pulse EPR spectrometer. *J. Magn. Reson.* **2008,** 194, 8-15.