Gd(III)-Gd(III) Relaxation-Induced Dipolar Modulation Enhancement for In-Cell Electron Paramagnetic Resonance Distance Determination

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Experimental details

Sample preparation.

The syntheses of Gd-ruler-2.1 and Gd-ruler-3.0 have been reported elsewhere.[1,2] Oocytes from *X. laevis* (stage V/VI) were purchased from EcoCyte Bioscience, Caustrop/Rauxel, Germany. The cell extract from the oocytes was prepared as described previously.[3]

All samples were prepared from aqueous solutions of Gd-rulers. Briefly, a 5 mM solution of Gd-ruler-2.1 in D_2O (pH ~ 8.0, containing ca. 37 mM NaCl) or a 5 mM solution of Gd-ruler-3.0 in D_2O (pH ~ 7.0, containing ca. 0.5 mM sodium trifluorocetate and ca. 30 mM NaCl) was lyophilized. The residual powder was dissolved in a predefined amount of H_2O (Milli Q).

For in-vitro samples, a 200 µM solution of the Gd-rulers was prepared in a mixture of H_2O (Milli Q) and glycerol (8:2, v/v).

For the in-extract sample, 1 µl of a 5 mM stock solution of Gd-ruler-3.0 in H_2O was mixed with 24 µl of cell extract to produce a final concentration of 200 µM. No glycerol was added. The samples were transferred into quartz capillaries 1.6/1.0 mm o.d./i.d. (Bruker Biospin), shock-frozen in liquid nitrogen and stored at -80 ^{o}C until further use.

For the in-cell sample, 5 mM stock solution of Gd-ruler-3.0 in H_2O was microinjected into oocytes (50 nl per oocyte) using a Nanojet II automatic nanoliter injector with fitting micromanipulator MM33 (DRUMMOND) to give a final in-cell concentration of 200 µM. After 2.5 h incubation at room temperature, 30 oocytes loaded with the Gd-ruler were transferred into a quartz tube 3.0/2.0 mm o.d./i.d., shock-frozen in liquid nitrogen and stored at -80 ^{o}C until further use.

EPR spectroscopy

All EPR measurements were performed at Q band on an Elexsys E580 spectrometer equipped with an arbitrary waveform generator (Bruker Biospin) and a 150 W TWT amplifier (Applied Systems Engineering). Temperature control was realized with the cryogen-free system consisting of a helium compressor F-70H (Sumitomo Cryogenics of America), a cryocooler ColdEdge (CE-FLEX-4K-0100, Bruker), and a MercuryITC (Oxford Instruments). A Q-band probehead with access for 3 mm tubes (ER5106QT-2, Bruker) was used for both 1.6 mm and 3 mm (o.d.) sample tubes. All the measurements were performed in the dip of the overcoupled resonator at 34 GHz. Rectangular pulses were used with the lengths fixed at 8 and 16 ns for $\pi/2$ and π, respectively.

The primary echo decay ($\pi/2 - \pi - echo$) was recorded by increasing the distance between the two microwave pulses with an 8 ns step. The length of the primary echo decay was 15 µs. The inversion recovery ($\pi - \pi/2 - \pi - echo$) was recorded by increasing the distance between the first and the second pulse with a 100 ns step. The length of the inversion recovery was 200 ms, In both experiments, the magnetic field was set to 12207 G, which corresponded to the maximum of the absorption curve.

In the RIDME measurement, a 5-pulse scheme was used and an 8-step phase cycle was applied during the acquisition of the refocused virtual echo.[4] The magnetic field was set to the maximum of the absorption spectrum of Gd-PyMTA label. A time delay between the first and the second pulse was 300 ns, a mixing time was 8 μs. The total length of the RIDME dipolar evolution was 2 μs, which was acquired with an 8 ns step.

The RIDME time traces were processed with the OvertoneAnalysis software package for Matlab, which includes a modified kernel function to account for overtone harmonics.[5] The background contributions to every RIDME time trace were approximated by a stretched exponential and eliminated by division. The distance distribution was extracted from the resulting form factor by Tikhonov regularization, where the fractions of overtones were fixed at $P_1 = 0.4$, $P_2 = 0.3$, and $P_3 = 0.3$ (for Gd-ruler-3.0) or at $P_1 = 0.69$, $P_2 = 0.21$, and $P_3 = 0.10$ (for Gd-ruler-2.1). Subsequently, the distance distribution at the optimum alpha-value (ranging from 7 to 39) was subjected to the validation. The validation by varying the background start, the modulation depth, and adding white noise at the level 1.2 to give the uncertainty regions for the distance distribution, which were plotted as grey area on top of the distance distribution.[5]

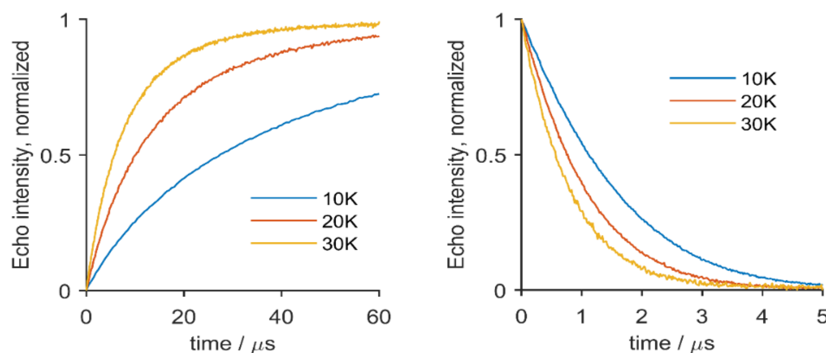

Figure S1. Inversion recovery (left) and primary echo decay (right) for Gd-ruler-3.0 in aqueous solution at different temperatures.

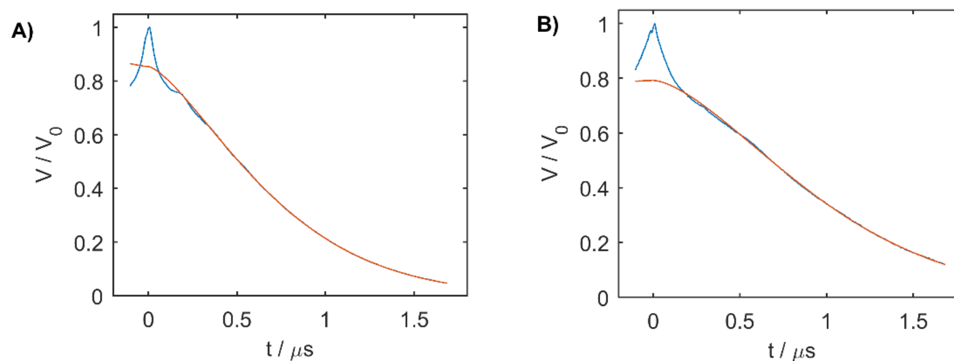

Figure S2. RIDME time traces (blue) and the background function (red) for Gd-ruler-2.1 (A) and Gd-ruler-3.0 (B) in frozen aqueous solutions.

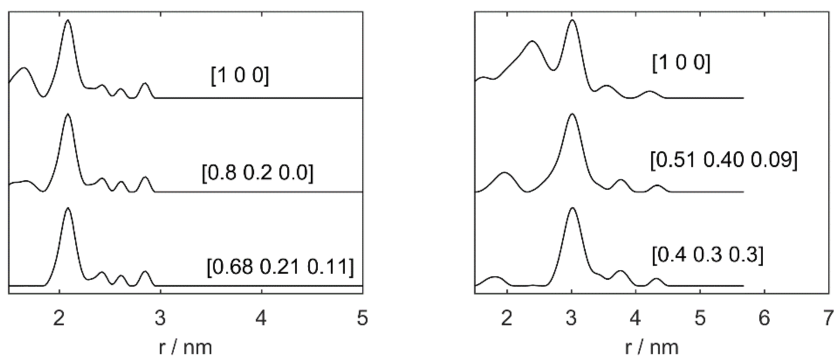

Figure S3. Distances Gd-ruler-2.1 (left) and Gd-ruler 3.0 (right) determined for aqueous protonated solutions with different overtone coefficients. From top to bottom: no overtones, overtone coefficients determined in a deuterated aqueous solution, overtone coefficients determined in a protonated aqueous solution.

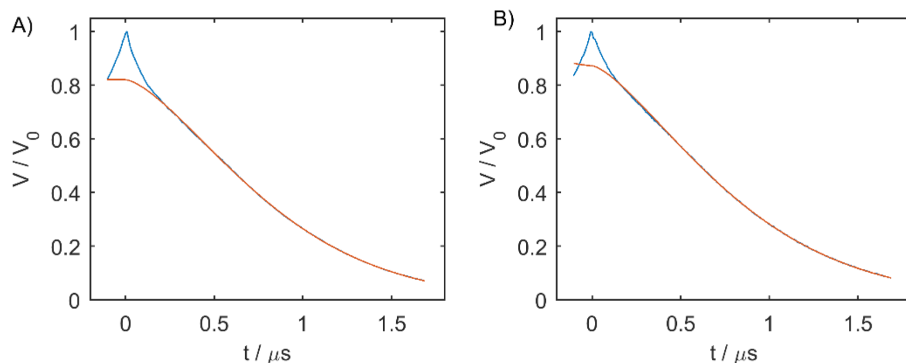

Figure S4. RIDME time traces (blue) and the background function (red) for Gd-ruler-3.0 in cell extract (A) and in oocytes (B).

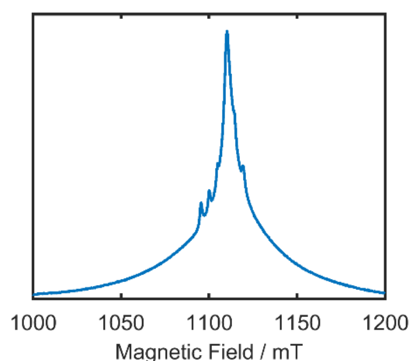

Figure S5. Echo-detected field sweep of Gd-ruler-3.0 in oocytes, recorded at 10K.

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