SUPPLEMENTARY INFORMATION:

Pulsed EPR spectroscopy distance measurements of DNA internally labelled with Gd$^{3+}$-DOTA

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Experimental procedures

All reactions were performed in oven-dried glassware under nitrogen. Chemicals and solvents were purchased from Sigma. Solvents were of laboratory grade. Thin-layer chromatography (TLC) was performed on aluminium-backed plates pre-coated with silica gel 60 (0.2 mm, UV254, Merck, Germany). Flash column chromatography (FCC) was performed using silica gel 60 (Merck, Germany). ESI mass spectra were recorded on a Bruker Daltonics esquire 3000plus. NMR spectra were recorded using Avance 400 (400 MHz, Bruker, Germany). 31P NMR spectra was recorded at 161.8 MHz using 85% H3PO4 as an external standard.

DNA synthesis was performed on a 1.0 µmole scale on a Applied-Biosystem 392 DNA/RNA synthesizer. Chemicals and the solid support was purchased from AzcoBiotech (USA), J.T. Baker (Netherlands) and Link-Technologies (Scotland, UK). 5′-(ethyliothio)-1H-tetrazole (0.25 M in acetonitrile) was used as the activator and coupling times of 3 min were used for the modified building block 2.

DNA synthesis
Two resin-bound modified oligodeoxynucleotides were synthesized ODN 1 5'-GCCAAGTGACTGTATGATGATGCT-3' and ODN 2 5'-AGCATCATCATACAGXACACTGC-3' containing the modified building block X. The resin-bound DNA was placed in a 1.5 mL microcentrifuge tube with a screw cap. The resin-bound DNA was placed in a 1.5 mL microcentrifuge tube with a screw cap, anhydrous DMF (1.0 mL) was added followed by sodium azide (45 mg, 0.69 mmol). The vial was placed in an eppendorf thermomixer, and shaken for 24 hours at 55°C. The microcentrifuge tube was cooled to room temperature and spun down in an eppendorf centrifuge MiniSpin. The DMF was removed and the resin was washed with acetonitrile/water (1:1, 3 x 1 mL), then acetonitrile (3 x 1 mL).

To the resin-bound azide containing DNA was added DMF (0.5 mL), water (0.5 mL), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (1 mg, 1.88 μmol), CuSO4 (1.5 mg, 9.4 μmol), and sodium ascorbate (10 mg, 51 μmol) and 12 mg (20 μmol) of alkyne-(Gd³⁺-DOTA) was added. The vial was placed in an eppendorf thermomixer, and shaken for 24 hours at 50°C. The microcentrifuge tube was cooled to room temperature and spun down in an eppendorf centrifuge MiniSpin. The resin was washed with DMF (3 x 1 mL), then acetonitrile (3 x 1 mL).

Cleavage from the solid support and final deprotection was achieved with 30% NH₄OH solution (50°C, 12 h). HPLC purification was performed using a Prominence HPLC (Shimadzu, Japan) equipped with a Phenomenex Jupiter semi-preparative RP-HPLC column (5μm, C18, 300Å) using A = 0.1 M triethylammonium acetate in H₂O, pH 7.0; B = 0.1 M triethylammonium acetate in CH₃CN/H₂O 4:1, pH 7.0, detection wavelength at 260 nm. The oligonucleotides were desalted using Sephadex-G10.

The modified oligonucleotides were confirmed by ESI-MS; ODN 1 5'-GCCAAGTGACTGTATGATGATGCT-3' C₂₅H₂₅GdN₁₀O₁₅P₂₅ Exact Mass: 8162.4 found 8161.3 and ODN 2 5'-AGCATCATCATACAGXACACTGC-3' C₂₅H₂₅GdN₂₉O₁₄₇P₂₅ Exact Mass: 7940.4 found 7939.4.

**Thermal denaturation studies**

Thermal denaturation studies were recorded on a Varian Cary 100-Bio UV/VIS spectrophotometer (Varian Inc.), equipped with a Peltier element at 260 nm with a heating/cooling rate of 0.5°C/min. A heating cycle in the temperature range 20–90°C was applied. All measurements were carried out in a buffer consisting of 50 mM NaCl and 10 mM Tris (pH = 7.4) at 1.0 μM strand concentration.

**CD spectra**

CD spectra were measured on a JASCO J-715 spectropolarimeter using quartz cuvettes with a path length of 1 cm. All measurements were carried out in a buffer consisting of All measurements were carried out in a buffer consisting of 50 mM NaCl and 10 mM Tris (pH = 7.4) at 1.0 μM strand concentration.

**Microinjection of DNA samples**

Sample preparation of intracellular EPR samples were done as described by Qi et al. Briefly, the Xenopus laevis oocytes on stage V/VI (purchased from EcoCyte Bioscience, Castrop-Rauxel, Germany) were kept in MBS at 18°C. Three oocytes were prepared on a poly (tetrafluoroethylene) holder and microinjected with 50 nL of a 3.7 mM solution of the Gd(III)-labelled duplex DNA sample solution and washed with MBS buffer. The oocytes were collected in a Q-band tube (quartz glass, 1 mm inner diameter, Bruker Biospin) via slightly applied negative pressure and were visually inspected directly afterwards and after 15 min of incubation time using a Stemi 2000-C binocular microscope mounted with an AxiaCam ERC 5s camera (ZEISS). After removal of supernatant MBS buffer the oocytes were shock-frozen in liquid nitrogen and stored at -80°C until measuring them without de-freezing them.

**Investigating cytotoxic effects of injected samples**

Gd(III) is highly toxic. The toxicity can significantly be reduced by complexation. Consequently, Gd(III) chelates are routinely used as MRI contrast agents in medicine. However, even for injection of 50 nL 8 mM uncomplexed GdCl₃ per Xenopus laevis oocyte, we observe morphological stability for several hours, see Figure S1. In contrast, upon microinjection 50 nL 3.7 mM Gd(III)-DOTA labelled duplex DNA we found an increased cytotoxic effect, resulting in apoptosis after 1.5 h. This suggests that the duplex DNA contributes to the toxic effect.
\pi\text{ pulse} = 16 \text{ ns} \text{ and a sweep width of 4000 G with } B_0 \approx 12200 \text{ G.}

The four-pulse, dead-time free DEER was obtained using the following pulse sequence:

\[ \left( \frac{\pi}{2} \right)_\text{obs} \cdots \tau_1 \cdots (\pi)_{\text{obs}} \cdots t \cdots (\pi)_{\text{pump}} \cdots (\tau_1 + \tau_2 - t) \cdots (\pi)_{\text{obs}} \cdots \tau_2 \cdots \text{echo}. \]

The pump pulse (length 16 ns for \textit{in vitro}, resp. 18 ns for \textit{in cellulo} experiments) was set to the maximum of the Gd(III) spectrum and the observer pulses (lengths 14/14/28 ns) were shifted \( \Delta \nu = 125 \text{ MHz} \) to lower frequencies. The time separation \( \tau_1 \) was 400. Shot repetition time was set to 2 \( \mu \text{s} \).

The transverse relaxation time \( T_2 \) was determined by incremental elongation of the time separation \( \tau_1 \) between \( \pi/2 \) pulse and \( \pi \) pulse shown in Figure S2 \textit{in vitro} and \textit{in cellula}.

\textbf{Fig. S2} \( T_2 \) relaxation time experiment of doubly Gd(III)-DOTA labelled duplex DNA at 10 K in deuterated Tris-HCl buffer (pH = 7.4, DNA concentration = 100 \( \mu \text{M} \)) (black) and inside \textit{Xenopus laevis} oocytes after microinjection of 50 nL 3.7 mM labelled duplex DNA in Tris-HCl buffer (pH = 7.4) (15 min incubation time, absolute spin concentration = 180 \( \mu \text{M} \)) (orange); initial pulse separation time = 400 ns, mw-frequency = 34 GHz, pulse lengths: 14/28 ns. The observed ESEEM in the \textit{in vitro} experiment (black) is due to Gd(III) interaction with the deuterated solvent.

\textbf{References of Supporting Information:}