Supplement of

High sensitivity Gd^{3+}- Gd^{3+} EPR distance measurements that eliminate artefacts seen at short distances

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	D (mT)	FWHH (mT)-Q	FWHH (mT)-W
Gd-rulers with [Gd^{III}(PyMTA)] as the spin label	37.4[1]	14.2	4.38 (this work)
[Gd-DOTA]	22.8	-	1.30 (Raitsimring et al., 2014)
[Gd-4-iodo-PyMTA]	40.6	-	4.00 (Dalaloyan et al., 2015)
[Gd-595]	22.9	-	1.23 (Collauto et al., 2016)
[Gd-538]	44.2	-	3.81 (Collauto et al., 2016)
[Gd-4MMDPA]	63.6	-	15.00 (Gordon-Grossman et al., 2011; Potapov et al., 2010)
[Gd-DO3A-BrPSPy]	51.2	-	6.40 (Yang et al., 2018)
[Gd.sTPATCN]	-	2.54	1.14 (Shah et al., 2019)
[Gd-NO_3Pic]	18.3	-	- (Clayton et al., 2018)

Table S1: ZFS splitting parameter D and the full width half height estimated from the central lines of the ED-FS spectra recorded at T=10 K and at Q and W-band. It should be noted that FWHH depends on solvent and whether the complex is measured as such, or as attached to another molecule. The literature should be consulted for details.

[1] ZFS determined for Gd-ruler (2.1 nm) and Gd-ruler (6.0 nm) was found to be identical within the uncertainty intervals.

Compound	$\boldsymbol{T_m^a}$(µs)	$\boldsymbol{A_1}$(%)	$\boldsymbol{T_m^b}$(µs)	$\boldsymbol{A_2}$(%)	10% signal[1]
Gd-ruler (6.0 nm)	4.6±0.3	45±2	13.7±0.1	55±2	18.2
Gd-ruler (2.1 nm)	9.1±0.3	78±2	13.7±0.1	22±2	20.6

Table S2: Echo decay parameters derived from the fit of the experimental data. The data were well fitted by a sum of two stretched exponential functions with fixed exponents to 1 and 2. The associated parameters to the fit are shown in the fitting function.

$$y=A_1.\, e^{-\left(\frac{x}{T_m^a}\right)^1} + A_2.\, e^{-\left(\frac{x}{T_m^b}\right)^2}$$

[1] Corresponds to the T_m value determined at the time at which the echo decayed to 10% of its initial value. These values are more representative of the echo decay.

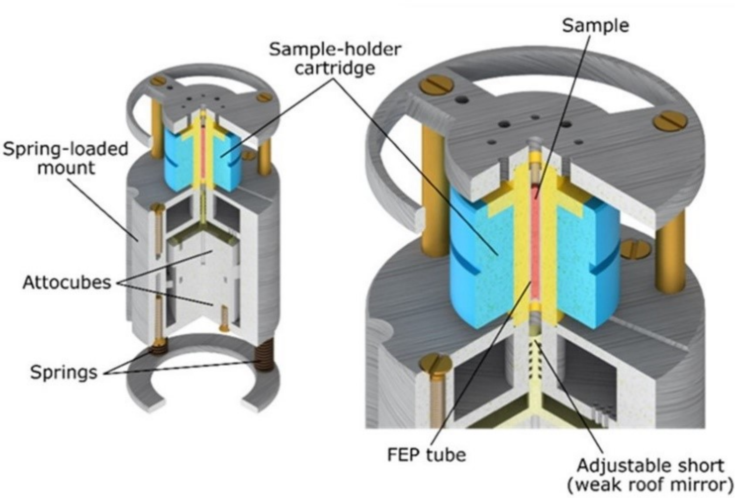
Compound	$\boldsymbol{T_1^a}$(μs)	$\boldsymbol{A_1}$(%)	$\boldsymbol{T_1^b}$(μs)	$\boldsymbol{A_2}$(%)	$\boldsymbol{T_1}$(μs)[1]
Gd-ruler (6.0 nm)	15.5±0.3	40±2	45.7±0.3	60±2	31.3±0.3
Gd-ruler (2.1 nm)	14.8±0.3	29±2	53.1±0.3	71±2	36.8±0.3

Table S3: Inversion recovery parameters derived from the fit of the experimental data performed for both samples. The data were well fitted by a sum of two exponential functions. The parameters to the fit are shown in the fitting function.

$$y=A_1.[1-2*e^{-\left(\frac{x}{T_1^a}\right)}]+A_2[1-2*e^{-\left(\frac{x}{T_1^b}\right)}]$$

[1] T_1^a and T_1^b refer to T_1 values that have been derived from a mono-exponential fitting function.

a)



b)

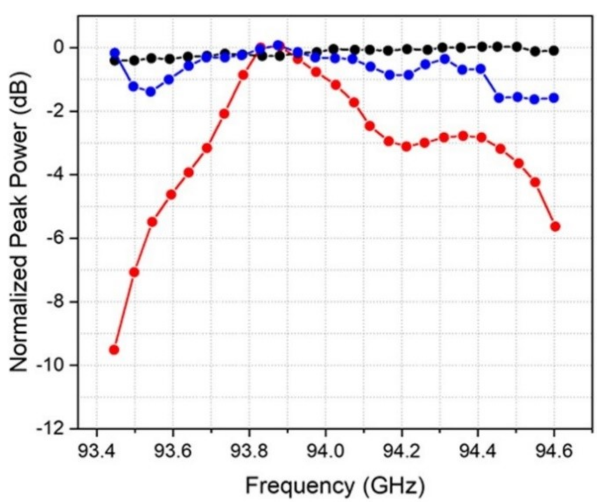
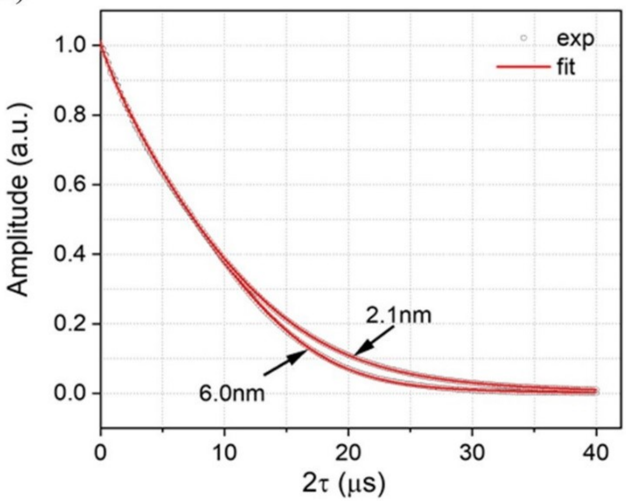


Fig. S1: a) CAD images of HiPER sample-holder showing the sample-holder cartridge mounted into the spring-loaded mount. b) Variation of the power level with respect to the frequency measured at different points in the transmit chain of HiPER. The measurements of the power output have been performed via couplers placed at the output of the multiplier (black), 1 W amplifier (blue) and 1 kW EIK amplifier (red). (Adapted from (Motion et al., 2017)).

a)



b)

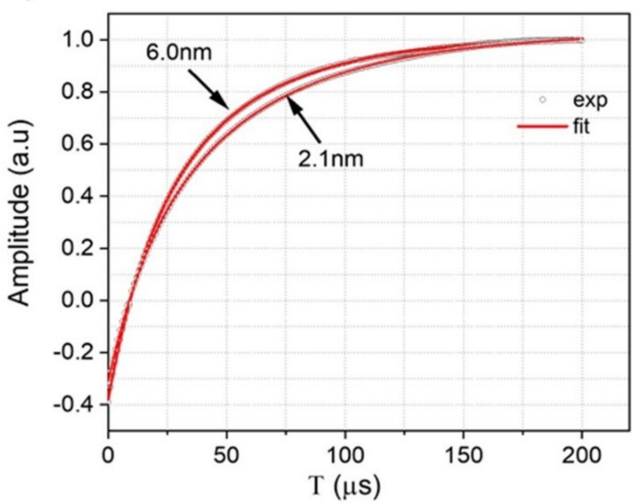


Fig. S2: Experimental and fitted a) echo decay and b) inversion recovery data recorded at 10 K at the maximum of the ED-FS spectra of Gd-ruler (2.1 nm) and Gd-ruler (6.0 nm). τ is the delay between the $\frac{\pi}{2}$ and π pulses in the echo decay pulse sequence and T is the delay between the inversion π pulse and $\frac{\pi}{2}$ --π observer sequence.

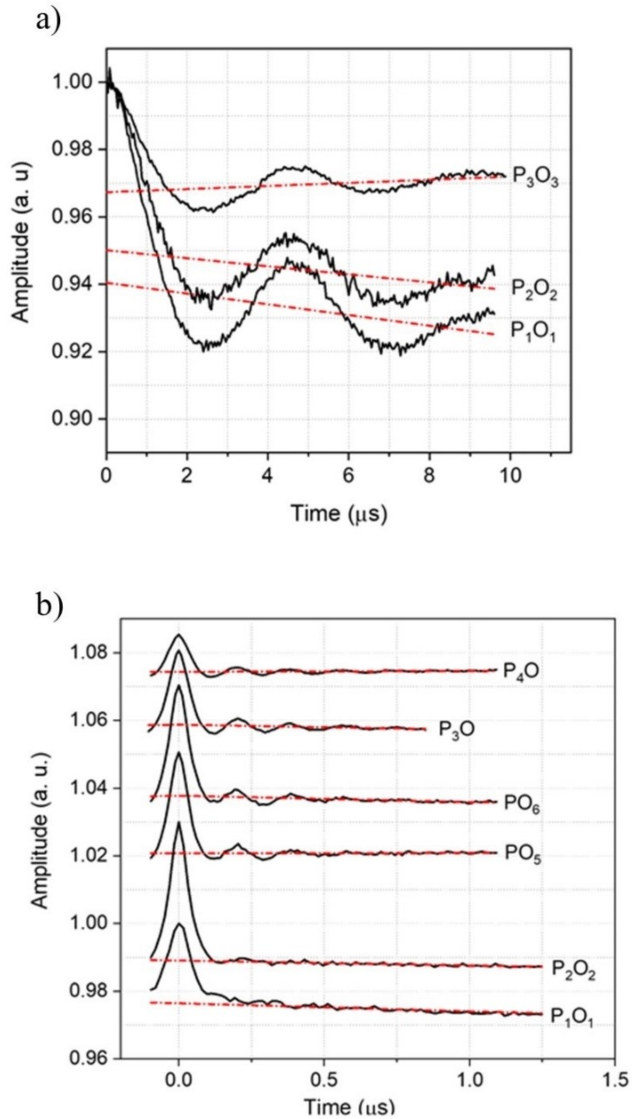
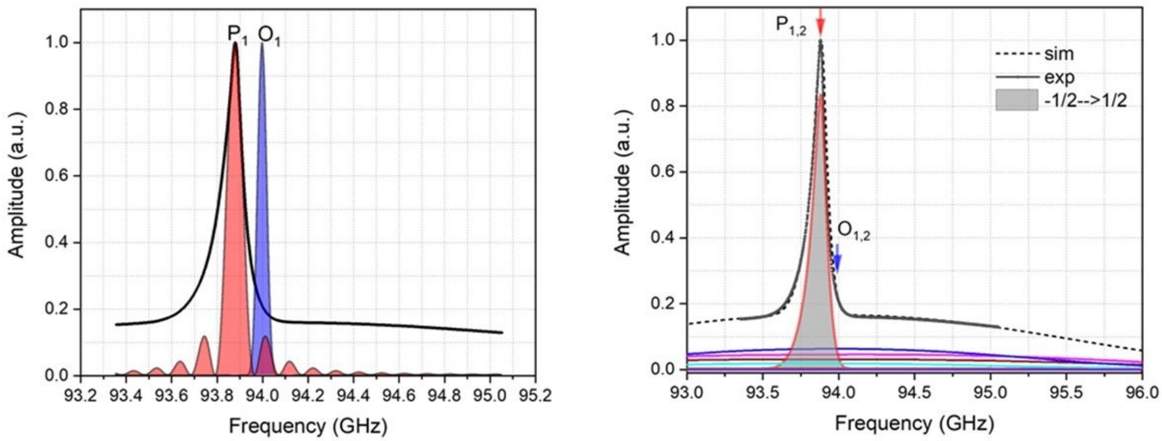


Fig. S3: Primary DEER data of a) Gd-ruler (6.0 nm), and b) Gd-ruler (2.1 nm) recorded at different PO offsets. The background fitting used in DEER data analysis is shown in red. The positions of the pump (P) and observer (O) pulses are reported in Figs. S5, S6 and S7. Note that in the case of P_3O_3 the functional form of the background deviates from the expected exponential decay. We do not yet have an explanation for this unphysical effect. However, for all the data shown, the background were chosen to maximise the fit to the expected Pake pattern and, without loss of argument in the rest of the paper, we have chosen to take this fitting approach for this experiment.

a)



b)

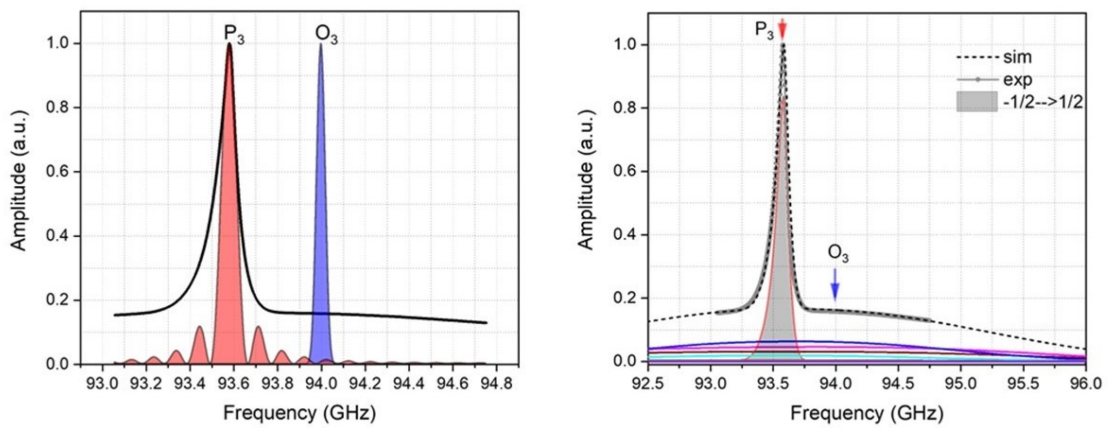


Fig. S4: ED-FS spectra of Gd-ruler (6.0 nm) sample with (left) simulated excitation profiles of the pump and observer pulses and (right) the associated field positions with respect to the central transition $\left|-\frac{1}{2}\right\rangle \rightarrow \left|\frac{1}{2}\right\rangle$. PO offsets: a) 120 MHz, b) 420 MHz. Note that pump frequencies are different although located at the central transition. The excitation plots in a) and b) are the same as those in the main text. They were added here for convenience.

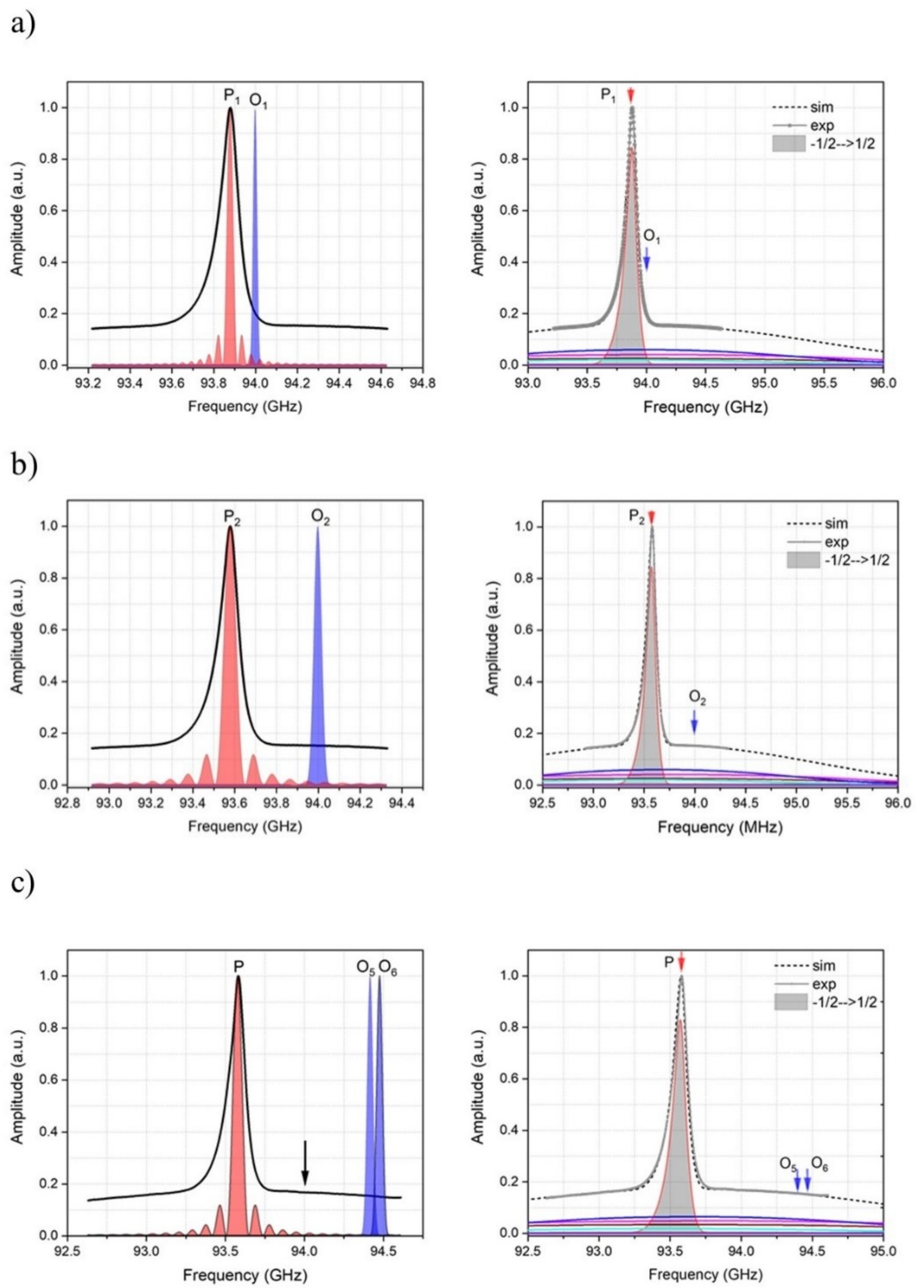


Fig. S5: ED-FS spectra of Gd-ruler (2.1 nm) sample with (left) simulated excitation profiles of the pump and observer pulses and (right) the associated field positions with respect to the central transition $\left|-\frac{1}{2}\right\rangle \rightarrow \left|\frac{1}{2}\right\rangle$. PO offsets: a) 120 MHz, b) 420 MHz and c) 840 and 900 MHz. Note that pump frequencies are different although located at the central transition. The excitation plots are the same as those in the main text. They were added here for convenience.

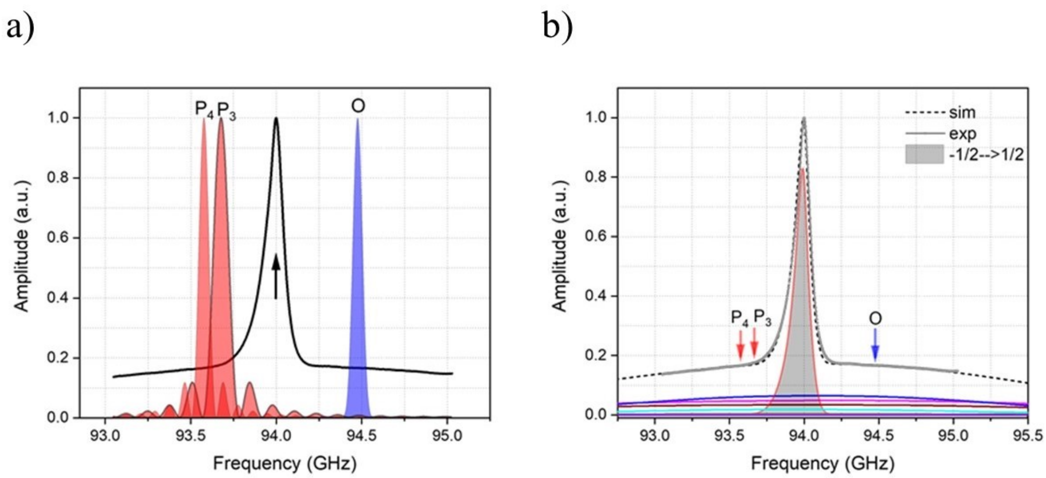


Fig. S6: ED-FS spectrum of the Gd-ruler (2.1 nm) with a) simulated excitation profiles of the pump and observer pulses separated by 800 and 900 MHz and b) the pump and observer pulses field positions with respect to the central transition $\left|-\frac{1}{2}\right\rangle \rightarrow \left|\frac{1}{2}\right\rangle$. The excitation plots are the same as those in the main text. They were added here for convenience.

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