Pulsed Electron-Electron Double Resonance Spectroscopy between a High-Spin Mn^{2+} Ion and a Nitroxide Spin Label

Supporting information

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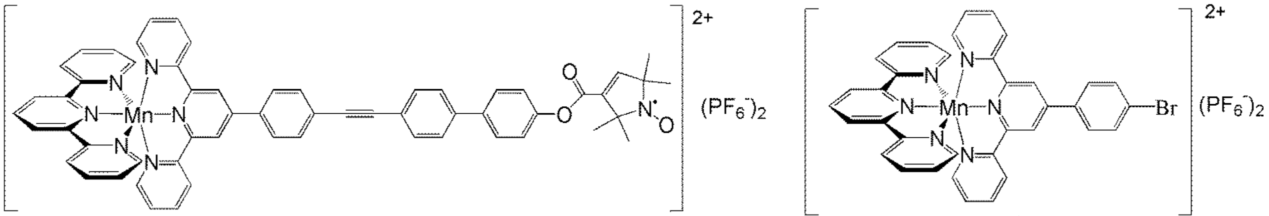
1. Chemical Structure of Compound 4 and 6

The synthesis of the compounds is described in the main text of the manuscript. The schematic representations of compounds **4** and **6** are given in Fig. S1.



4 **6**

Fig. S1. Heteroleptic Mn^{2+}-terpyridine complex (compound **4**) and a Reference Mn^{2+}-terpyridine complex (exact name is given in the main text) (compound **6**)

2. EPR spectra and Hahn-echo phase memory time measurements of compounds 4 and 6

Field-swept echo-detected EPR spectra on compounds **4** and **6** at Q-band and a temperature of 5 K are presented in Fig. S2. The spectra were normalized to the maximum of the Mn^{2+} ion spin system spectrum. The nitroxide line in the spectrum of compound **4** was artificially cut. The broad and featureless line, corresponding to the Mn^{2+} ion spin system practically does not change between compound **4** and **6**, however, to detect the change between ZFS parameters of these complexes is not possible in this experiment, because the Q-band frequencies are not high enough.

In Fig. S2 the phase memory time measurements obtained from the Hahn-echo decay is presented. The temperature used for Q-band measurements is 5 K. The G-band measurements on compound **4** were obtained at 10 K. The estimated phase memory times for both frequencies and both compounds are roughly 1 μs. Pronounced hyperfine coupling induced modulation of the echo evolution function (ESEEM modulation) could be observed at Q-band frequencies (probably due to the coupling to the close-by nitrogen nuclei of the terpyridine moieties), whereas at G-band frequencies this modulation is absent.

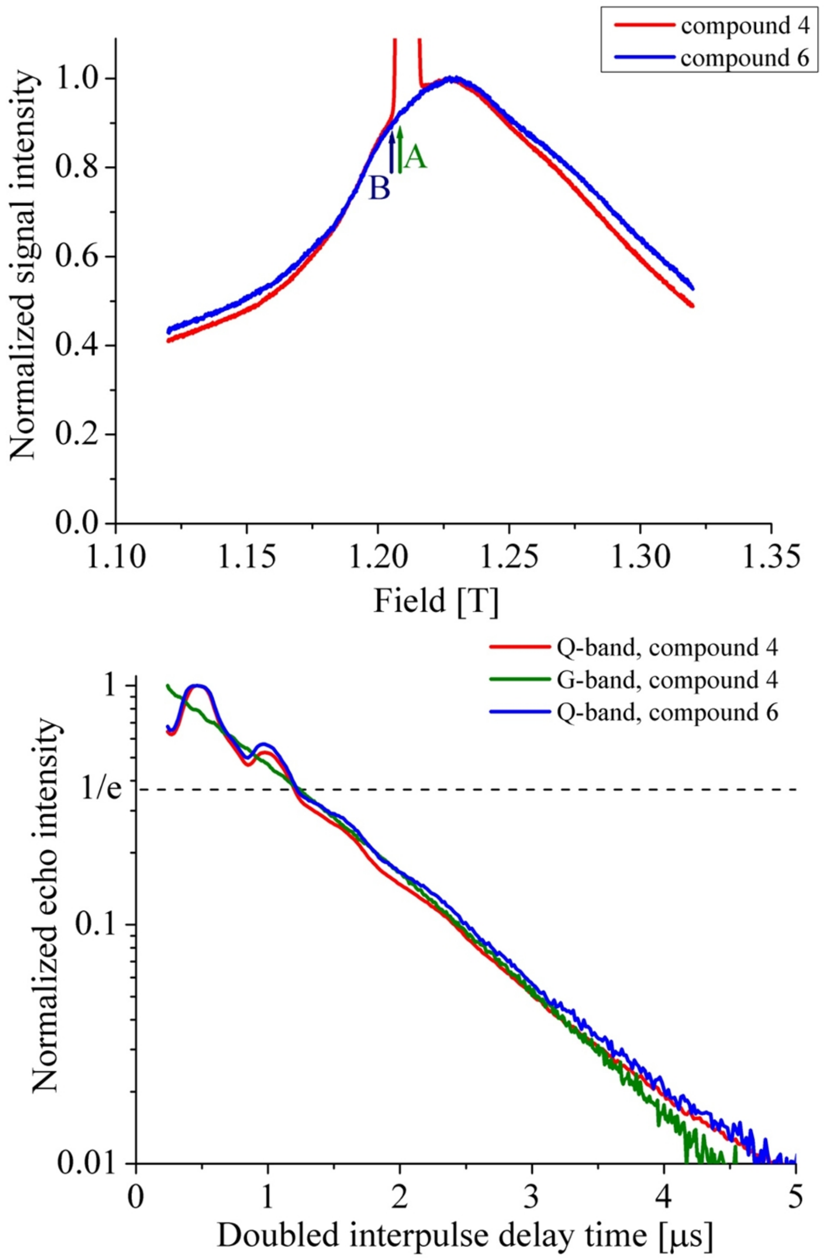


Fig. S2. Up - Field swept echo detected EPR spectra of compounds **4** and **6** at Q-band frequencies. The microwave frequency was 33.769 GHz. The pulse settings were optimized for Mn^{2+} ion spin system at the position B. The length of the pulses was set to 32 ns, the interpulse delay time was 120 ns; repetition time was 0.8 ms. The label A and B indicate the pump and probe positions, respectively, used for the experiment when pumping and detecting on the Mn^{2+} ion spin system of compound **6** (Fig. S6), control for testing nuclear modulation effects.

Down - Hahn-echo decay as a function of the double interpulse delay time. Compound **4**: the position in the spectrum was the position B; The pulse lengths were set to 32 ns, the initial interpulse delay time was 120 ns; repetition time was 0.8 ms. Compound **6**: the position on the spectrum was chosen roughly the same as for compound **4**, and all other parameters were identical. For the G-band experiment: the position in the spectrum was the position marked by up arrow at position 2 (Fig. 2 of the article - right upper spectrum); the temperature was 10 K; the pulses were 22.5 ns and 32.5 ns, the initial interpulse delay time was 300 ns; repetition time was 3 ms.

3. PELDOR experiments of compound 4 at Q-band frequencies

The 4-pulse PELDOR sequence was used. In Table S1 the experimental settings are given for pumping on the nitroxide (position A) and Mn^{2+} ion (position B), respectively.

Table S1. Experimental settings for the PELDOR experiments at Q-band

	Pump A detect B				Pump B detect A			
Pulses	$(\pi/2)_x$	$(\pi)_y$	$(\pi)_y$	π-pump	$(\pi/2)_x$	$(\pi)_y$	$(\pi)_y$	π- pump
Position of the pulses, ns	0	142	1784	190*	0	142	1784	190*
Length of the pulses, ns	32	32	32	20	32	32	32	20
Repetition time, μs	408				8160			
Shots per point	50				20			
Number of scans	1678				937			
Detection pulses frequency, GHz	33.85924				33.67894			
Pump pulse frequency, GHz	33.76910				33.76910			
Duration of the experiment	~ 4 hours				~ 16 hours			

*For the π-pump pulse the initial position of the pulse is given.

The power of the pulses was adjusted for the detection pulses by maximizing the echo intensity and for the pump pulse by obtaining the maximum inversion efficiency. A two-step phase cycling has been used: $(\pi/2)_x$ $(\pi)_y$ $(\pi)_y$ – $(\pi/2)_{-x}$ $(\pi)_y$ $(\pi)_y$.

Raw experimental PELDOR traces including the intermolecular background functions as well as the Pake patterns derived by Fourier transformation of the background divided time traces are depicted in Fig. S3.

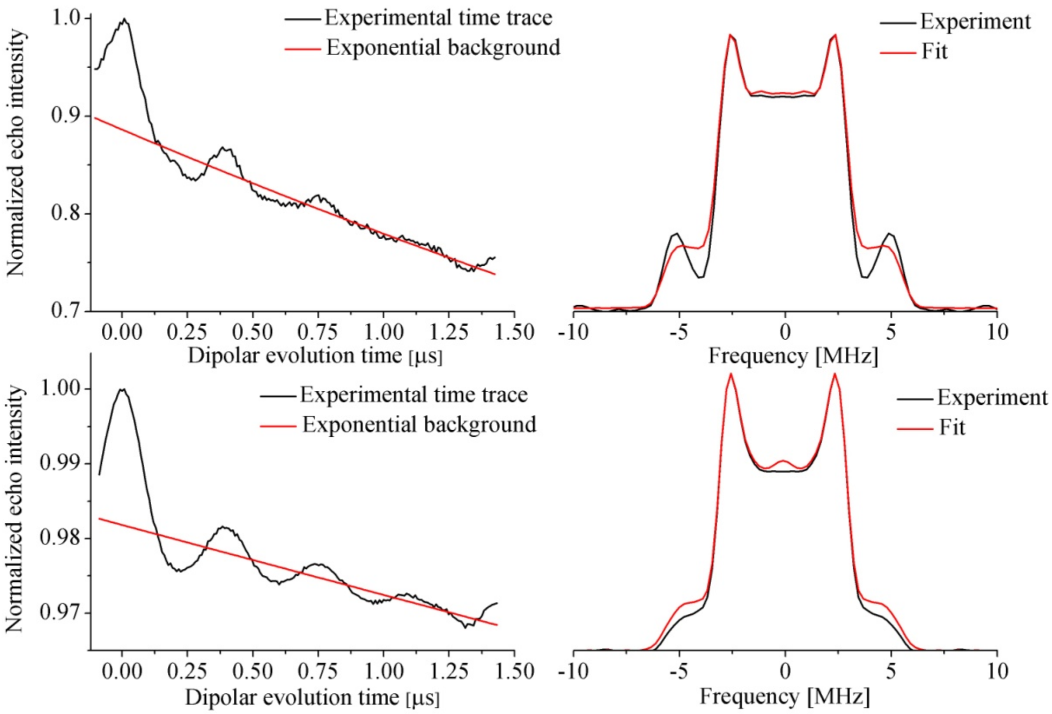


Fig. S3. Experimental PELDOR time traces including intermolecular background (red) and experimental Pake pattern obtained by Fourier transformation of the background divided time traces (black) and by Tikhonov regularization (red). Upper figures - pump position A, detect position B. Lower figures - pump position B, detect position A. The fits were obtained by DeerAnalysis 2013.

4. Control experiments with pump and detect frequency on the Mn^{2+} ion spin system

To check for artifacts by hyperfine coupling induced modulations or connected to the high-spin multiplicity of the Mn^{2+} ion two types of control experiments were performed:

1) Pump and detection was performed only on the Mn^{2+} ion spin system (positions C and D in Fig. S4, respectively) on compound **4**;

2) Pump and detection was performed only on the Mn^{2+} ion spin system approximately at the positions A and B (Fig. S2) on compound **6**.

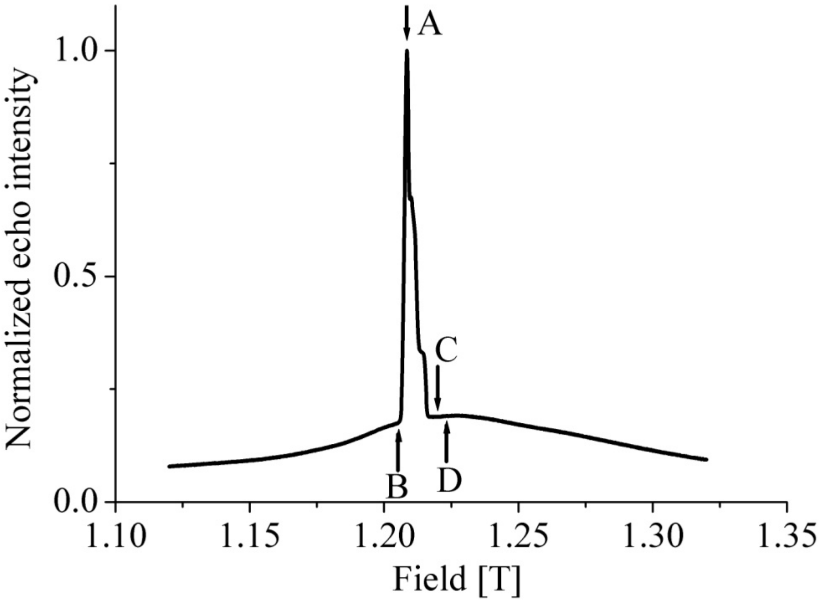


Fig. S4. Field swept echo detected EPR spectrum of compound 4. The spectral positions for probe and pump frequencies for the control experiments are shown.

Experiment 1

The experimental time trace upon pumping on position C and detection on the position D is depicted in Fig. S5. The parameters of the experiment are presented in Table S2. The power of the pulses was optimized with the strategy described in section 3.

Table S2. Experimental settings for the PELDOR-type experiment

	Pump C detect D			
Pulses	$(\pi/2)_x$	$(\pi)_y$	$(\pi)_y$	π- pump
Position of the pulses, ns	0	142	1784	190
Length of the pulses, ns	32	32	32	20
Repetition time, μs	816			
Shots per point	50			
Number of scans	1245			
Detection pulses frequency, GHz	33.67891			
Pump pulse frequency, GHz	33.76910			
Duration of the experiment	~ 5 hours			

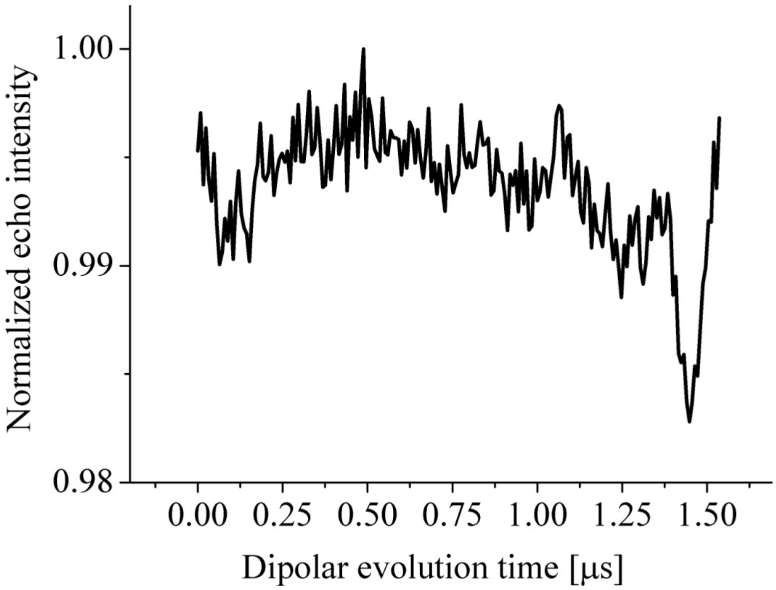


Fig. S5. Pumping and detecting on the Mn^{2+} ion spin system of compound **4** at Q-band frequencies and a temperature of 5K. The pump-detect frequency offset was set to 90MHz (positions C and D in Fig. S4).

Since none of the nitroxide spins are excited by the pump pulse at position C no dipolar oscillations are observed. The remaining weak oscillations could be caused by nuclear modulation effects.

Experiment 2

For this experiment pumping and detection was performed at positions A and B (Fig. S2) of compound **6**, containing no nitroxide. Pumping was performed on the Mn^{2+} ion spin system, however, the power of the pump pulse was the same as in the experiment with compound **4**, where pumping was performed on the nitroxide spin system. The power of the detection pulses was optimized for maximum echo intensity. All parameters of this experiment are given in Table S3.

Table S3. Experimental settings for the PELDOR-type experiment

	Pump A detect B			
Pulses	$(\pi/2)_x$	$(\pi)_y$	$(\pi)_y$	π- pump
Position of the pulses, ns	0	142	1784	190
Length of the pulses, ns	32	32	32	20
Repetition time, μs	816			
Shots per point	50			
Number of scans	2724			
Detection pulses frequency, GHz	33.85919			
Pump pulse frequency, GHz	33.76910			
Duration of the experiment	~ 12 hours			

The time trace for this experiment is shown in Fig. S6.

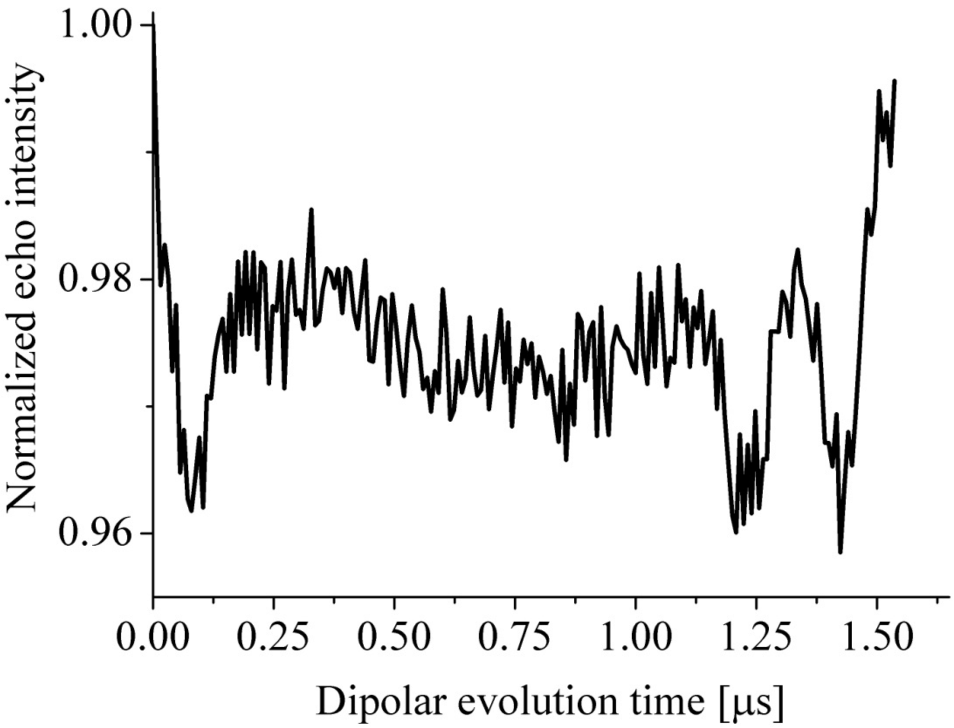


Fig. S6. Pumping and detecting on the Mn^{2+} ion spin system of compound **6** at Q-band frequencies and a temperature of 5 K. The pump-detect frequency offset was set to 90MHz.

More pronounced oscillations can be seen in the time trace under these experimental conditions. As it was discussed above, nuclear modulation effect cannot be ruled out from the consideration, assuming complexity of the high-spin Mn^{2+} ion spin system. These oscillations may be present in the PELDOR time trace when probing on Mn^{2+} spin system (Fig. S3 – upper trace). When probing on the nitroxide spin system this was not observed.

5. ESI mass spectrum of the heteroleptic Mn^{2+}-terpyridine complex (compound 4)

The ESI mass spectrum of compound **4** (Fig. S7) was obtained on a VG Platform II spectrometer, using an ionizing voltage of +3 kV. The solvent was a mixture of water:methanol 1:1, (v:v). Despite the fact, that solvent used for the EPR experiments was 2-methyltetrahydrofuran, the results obtained by mass spectroscopy give some qualitative information about the sample.

The peak with the m/z of 477.1 (double charged), corresponds to the expected mass of compound **4** without counter ions (m/z of 954.58±2.32 calculated 955.30). The peak with m/z of 1100.7 corresponds to the mass of compound **4** with only one counter ion (calculated m/z: 1100.27), so the complex appears as single charged.

The peak with the m/z of 666.0 most probably corresponds to a single charged Mn^{2+} bis-terpyridine complex $[(\mathrm{terpy})_2\mathrm{MnPF}_6]^+$ (calculated m/z of 666.09). However, it is worthwhile to mention that this is also close to the calculated m/z of 667.27 corresponding to ligand **3** (see article Fig. 1). The main peak with the m/z of 539 might be attributed to a single charged Mn^{2+} bis-terpyridine complex (calculated mass 521.13) solvated with one water molecule from the solvent: $[(\mathrm{terpy})_2\mathrm{Mn}(\mathrm{H_2O})]^+$. The double charged complex can attract an electron and become single charged, so that it also appears with actual m/1 in the spectrum.

In summary, from the mass spectrum the presence of another Mn^{2+} bis-terpyridine complex in solution besides of compound **4** cannot be excluded.

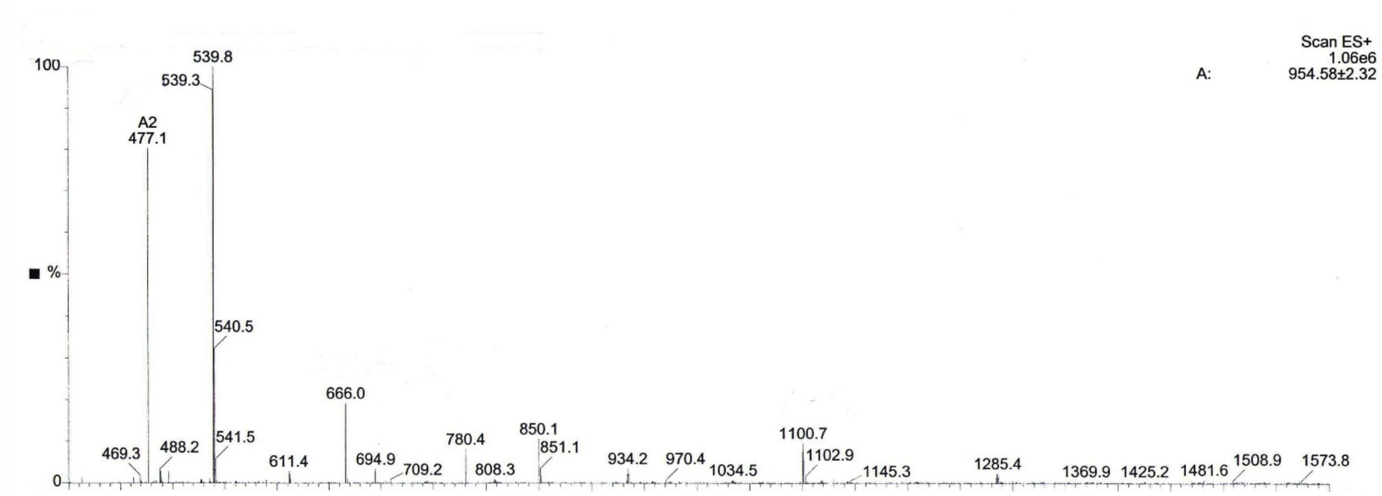


Fig S7. ESI mass spectrum of compound **4**.

6. Simulations of the G-band PELDOR time traces

The simulated dipolar evolution functions upon pumping on positions 1 and 2 (article Fig. 2 - right) are depicted in Fig S8.

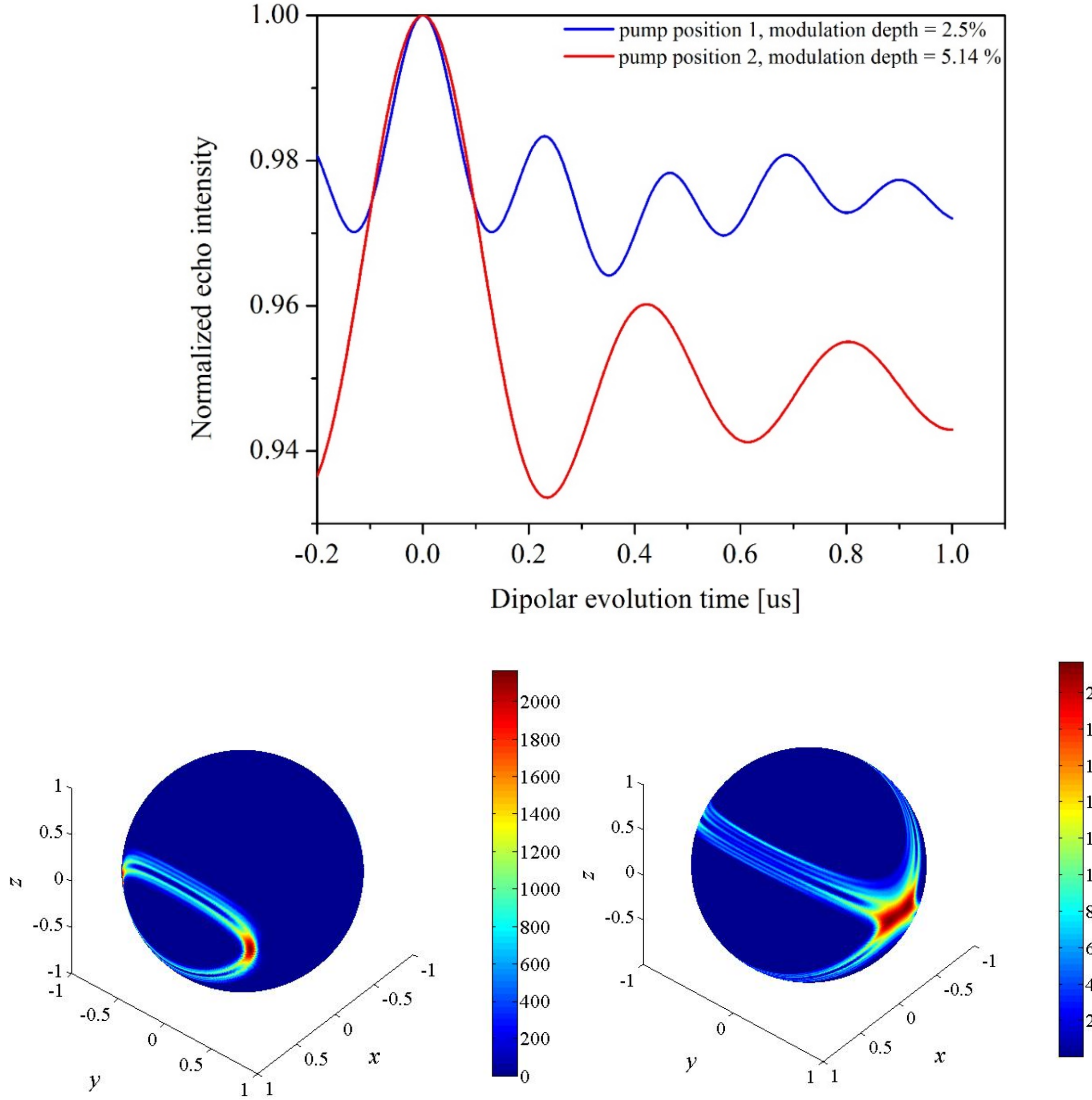


Fig. S8. Upper panel: simulated dipolar evolution functions upon pumping on the nitroxide spin system at positions 1 and 2 (Fig 2-left) with a fixed distance of 2.7 nm between the Mn^{2+} ion and the unpaired electron of the nitroxide; Lower panel - excited orientations of the nitroxide spin system upon pumping at position 1 (Left) and upon pumping at position 2 (Right). The *x*-, *y*-, *z*- axis represent the main axis of the g-tensor of the nitroxide spin system and the given colour bar represent the relative weight of the orientations excited by a pump pulse with pump pulse with 10 MHz bandwidth assuming an inhomogeneous linewidth of the nitroxide spin system of 10 MHz.

The excited orientations of the nitroxide spin system was obtained by the EasySpin function “orisel”.