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Observer-selective double electron–electron-spin resonance, a pulse sequence to improve orientation selection

Sergey Milikisyants, Edgar J.J. Groenen, Martina Huber *

Department of Molecular Physics, Huygens Laboratory, Leiden University, Niels Bohrweg 2, P.O. Box 9504, 2300 RA Leiden, The Netherlands

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ABSTRACT

By pulsed double electron–electron resonance (DEER), distances between spin labels in disordered systems up to 8 nm can be measured. In addition, the relative orientation of the interacting radicals can be determined, provided that the bandwidth of the pulses is sufficiently small. On the other hand, the bandwidth has to exceed the dipolar interaction considerably, because otherwise the DEER modulations become distorted and the modulation depth decreases, making distance determination impossible. Therefore, small bandwidths, i.e. long pulses, place a lower limit on the distance that can be determined. Two new pulse sequences, observer-selective DEER (os-DEER) and dead-time free os-DEER, are introduced that make it possible to use long observer pulses with bandwidths that are smaller than the dipolar interaction. The new pulse sequences do not suffer from the distortions caused by the limited bandwidth of the observer pulses, as demonstrated by measurements on a nitroxide biradical. With observer pulses of 140 ns, i.e., significantly longer than the 32 ns used in the conventional DEER sequence, a dipolar interaction of 7.8 MHz has been measured.

1. Introduction

In the last decade, structure determination in disordered systems has profited from the possibility to measure distances between two paramagnetic centers by DEER (double electron–electron resonance), a two-frequency, pulsed EPR method (for recent reviews, see [1–3]). It is selective for the dipolar and exchange interaction between the unpaired electron spins, and sensitive to distances up to 8 nm [4].

The basis of DEER, as initially proposed in Milov et al. [5], and further developed in Larsen and Singel [6], Pannier et al. [7], and Jeschke et al. [8], involves the use of two different microwave frequencies, the observer ω_{obs} and the pump ω_{pump} frequencies, which separately excite the two spins coupled by the dipolar interaction. The currently used sequences require that the spectral width of the pump pulse and the observer pulses must be larger than the spin–spin interaction to be measured. This makes it difficult to measure large dipolar interactions, i.e., short distances. To give an example for the instrumental limits of the presently available commercial equipment, for a pump pulse of 12 ns and observer pulses of 32 ns, distances below 1.55 nm become difficult to measure [9]. In addition, a large excitation bandwidth reduces the spectral selectivity of the pulses and thereby limits the possibility to determine the orientation of the paramagnetic centers with respect to each other. Here, often a compromise has to be sought between the desire to have narrow bandwidths for orientation selection and the need to have broad bandwidths to detect shorter distances. The importance to determine the relative orientation is increasingly recognized in the field [3,6,10–14].

The effect of pulses that are too narrow in bandwidth has been analyzed theoretically in reference [15,16]. It has been shown that the response curves become severely distorted [16]. Also, in reference [9], the effect of limited excitation bandwidth on the distance distribution is analyzed.

In the present account, we introduce a new pulse sequence that eliminates the restriction on the observer pulses. To demonstrate the pulse sequence, a biphenyl-based biradical [17] (Fig. 1) is used, in which the distance between the two nitroxide moieties is approximately 1.9 nm [8]. We show that distortion-free time traces can be obtained by observer pulses that are significantly longer than the currently used ones, i.e., that have a bandwidth of the order of the dipolar interaction. The time traces thus obtained are identical in shape and only somewhat reduced in modulation amplitude compared to those resulting from conventional, large excitation bandwidth, four-pulse DEER.

2. Experimental

All experiments were done using a Bruker (Bruker Biospin Rheinstetten) E680 ElexSys spectrometer equipped with an ER 4118X-MS3 split-ring resonator of the FlexLine series and an

* Corresponding author. Fax: +31 715275819.
E-mail address: mhuber@molphys.leidenuniv.nl (M. Huber).

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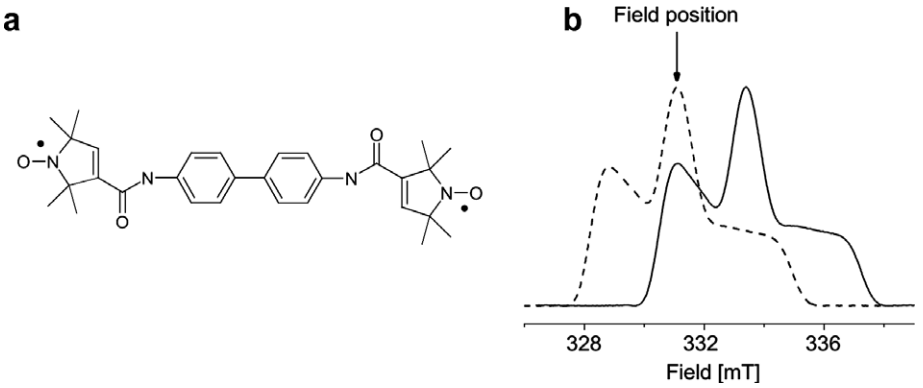

Fig. 1. (a) Chemical structure of biradical PH; (b) two pulse ($\pi/2$-τ – π, $\pi/2$ = π = 32 ns τ = 200 ns) echo detected EPR spectra of 0.5 mM PH in MTHF recorded at the pump frequency (dashed line) and the observer frequency (solid line), the arrow indicates the magnetic field at which the DEER experiments were performed.

Oxford cryostat. The resonator coupling was adjusted to obtain sufficient signal intensity and not too long ringing times. All measurements were done at a magnetic field value of $\mathbf{B}_0$ = 331.1 mT and at a temperature of 40 K. A bandwidth filter setting of 100 MHz was used throughout. A 0.5 mM solution of the biradical PH in MTHF was used for all experiments.

Fourier transforms are in the amplitude mode and were obtained from the DEER time traces by subtracting an exponential background, using a Gaussian-window function and zero-filling to 4096 points. Three-point averaging (4 ns step between points) was used to filter modulations at 65 MHz.

For the os-DEER sequence with observer pulses of 32 ns length, positions of the observer pulses were at 0 and 1200 ns, positions of the pump pulses at 100 and 140 ns. The position of the second pump pulse was incremented in steps of 4 ns. For the os-DEER sequence with observer pulses of 140 ns length, the position of the observer pulses was as before, but the pump pulses were at 200 and 240 ns, with the same increment of the second pump pulse.

For the dead-time free version of os-DEER, the initial positions of the pulses relative to the time of the first pulse were as follows: 300 and 340 ns for the first pair of pump pulses, 1200 ns for the second observer pulse, 1300 and 1500 ns for the last two pump pulses. The initial position of the pulses was chosen such that any temporal superposition of the echo observed with echos generated by the pump pulses is avoided for times $T > 0$. This is desirable since such a superposition causes modulations of the observed echo with the frequency $\Delta\nu$, i.e. 65 MHz in our case. Residues of such a modulation are seen in Fig. 5b at times around $T = -150$ ns. The position of the second pump pulse (starting at 340 ns) was increased in steps of 4 ns.

3. Results and discussion

3.1. Background of DEER and the new pulse sequence

The rotating-frame Hamiltonian of two interacting spins $S^1 = 1/2$ and $S^2 = 1/2$ can be written as [18]

$$\widehat{H}_{12} = \Omega_1 S_z^1 + \Omega_2 S_z^2 + \omega_{ee} S_z^1 S_z^2 \quad (1)$$

Here Ω_1 and Ω_2 are the resonance frequencies of spin 1 and 2, respectively, and ω_{ee} the frequency that represents the coupling between the spins. Non-secular terms of the spin–spin interaction have been omitted in Eq. (1). Neglecting the exchange interaction between the electrons and using a point-dipole approximation, ω_{ee} can be expressed as:

$$\omega_{ee} = \frac{\mu_0 g_1 g_2 \beta_e^2}{4\pi\hbar} \frac{1}{\mathbf{r}_{12}^3} (3\cos^2\theta_{12} - 1) \quad (2)$$

where g_1, and g_2 are the g values of the two interacting spins, $\mathbf{r}_{12}$ is the distance between them, and θ_{12} is the angle between the external magnetic field and the inter-radical vector $\mathbf{r_{12}}$. Assuming a random relative orientation of $\mathbf{r_{12}}$ with respect to the external magnetic field $\mathbf{B}_0$ the distribution of dipolar frequencies ω_{ee} can be described as a Pake pattern [19] with two sharp peaks at

$$\omega_{ee}^{\pm} = \pm \frac{\mu_0 g_1 g_2 \beta_e^2}{4\pi\hbar} \frac{1}{\mathbf{r}_{12}^3} \quad (3)$$

In the DEER experiment, two microwave frequencies are used that selectively excite two groups of spins. Those excited by the first frequency, the observer frequency ω_{obs}, are referred to as A spins, those excited by the pump frequency ω_{pump} as B spins [6,20]. The sequences for conventional three- and four-pulse DEER are shown in Fig. 2a and b. During the evolution time of the A spins, the B spins are inverted by the pump pulse. Without the pump pulse, the A spin evolves in an effective external magnetic field with a resonance frequency of $\Omega_A \pm \frac{\omega_{ee}}{2}$, where the sign depends on the quantum state of the B spin. A flip of the B spin changes the resonance frequency of the A spin by ω_{ee}, and the time at which that occurs determines how the A spins are refocused. This causes the modulation of the echo as a function of the time T at which the pump pulse is applied.

For this scheme to work, the excitation bandwidth of (i) the pump and (ii) the observer pulses has to be much larger than the dipolar interaction. The effect of a too small excitation bandwidth of the pump pulse (i) is a strong distortion of the DEER trace. This was analyzed theoretically for three-pulse DEER with different combinations of excitation bandwidths and values for ω_{ee} in [9,15,16]. To our mind, the distortions of the DEER traces are largely caused by the uncertainty of the time point at which the flip of the B spins occurs with respect to the characteristic time of the dipolar interaction. This results in a superposition of DEER traces with different time origins. The broadening of the modulations caused by the superposition is such that it becomes difficult to extract distances.

A different mechanism is responsible for the effect of the limited bandwidth of the observer pulses (ii). The inversion of the B spin changes the resonance frequency of the A spin by ω_{ee}. If this value becomes comparable with the bandwidth of the last observer pulse, these A spins cannot be properly refocused. This, in turn, strongly affects the DEER trace, suppressing the amplitude of the echo modulations.

This problem can be solved if, as we illustrate here, two consecutive π pulses are used as pump pulses instead of one. In this case, the dipolar modulations originate from the phase gained during the time between the pump pulses. By the second pump pulse, the resonance frequency of the A spins, shifted by the effect

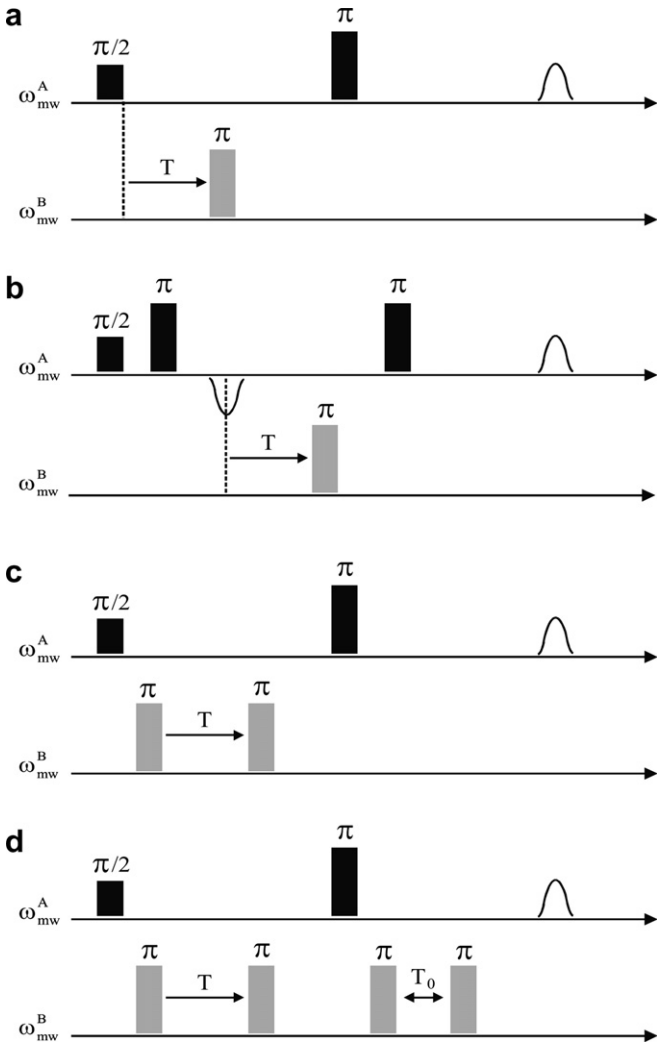

Fig. 2. Pulse sequences for the DEER experiments: (a) Three-pulse DEER; (b) Four-pulse DEER; (c) Four-pulse observer-selective (os) DEER; (d) Six-pulse, dead-time free os-DEER. The time T is varied, time T_0 is fixed.

of the first pump pulse, is reversed and no problem due to the limited bandwidth of the focusing (or refocusing) observer pulse should occur. The simplest sequence containing two pump pulses is shown in Fig. 2c. It is a variant of the three-pulse DEER sequence (Fig. 2a). The primary echo is modulated with the function $\cos(\omega_{ee}T)$, where T now is the time between the two pump pulses. The time at which the second pump pulse is applied is incremented. This sequence, however, suffers from a dead time because the two pump pulses may not overlap in time.

The simplest possibility to remove that dead time is to introduce two additional pump π-pulses with a fixed inter-pulse separation T_0 after the second observer pulse (Fig. 2d). In this case, the echo-envelope-modulation function becomes $\cos\{\omega_{ee}(T - T_0)\}$ and the zero point in the T-domain corresponds to $T = T_0$. To demonstrate that this approach works we performed conventional DEER experiments and experiments with the new sequences on the model compound PH (see Fig. 1).

3.2. Comparison of conventional DEER and observer-selective DEER for the model biradical

Fig. 3 shows the result of the application of the conventional four-pulse DEER sequence for PH. The pump frequency (9.327 GHz) was taken as the resonant frequency for spins at the spectral maximum, the observer frequency was by 65 MHz larger (see Fig. 1b). All observer pulses were of equal length and the amplitude was adjusted to obtain the $\pi/2$ and π pulses needed. The pulse lengths were 32 ns for the observer and 28 ns for the pump pulse. These pulses are much shorter than $2\pi/\omega_{ee}^{\perp} \approx 132$ ns, the pulse length at which the excitation bandwidth would be comparable to the dipolar interaction $\omega_{ee}^{\perp}$. The frequency $\omega_{ee}^{\perp}$ is estimated from the expected distance of 1.9 nm. The time trace shown in Fig. 3a (left) reveals pronounced modulations. The Fourier transform (Fig. 3a (right)) exhibits a clear peak at 7.7 ± 0.1 MHz, corresponding to $\omega_{ee}^{\perp}/2\pi$, which, according to Eq. (3). results in $r_{12} = 1.89$ nm. This distance is in good agreement with previous DEER investigations where a value of 1.92 ± 0.05 nm [8] was found.

In order to demonstrate the effect of a too small excitation bandwidth of the observer pulses, we performed the same experiment as shown in Fig. 3a, but with the length of the observer pulses set to 140 ns. In this case, the length of the observer pulses corresponds to a bandwidth of $\Delta\omega \sim \omega_{ee}^{\perp}$. The resulting trace (see Fig. 3b) is strongly distorted with respect to the correctly measured one, and any determination of the inter-spin distance becomes ambiguous.

Consider the new pulse sequence reproduced in Fig. 2c. To prove that it gives the proper DEER response under the usually applied conditions, first, it is applied with pulse lengths of 28 ns for the pump pulses and 32 ns for the observer pulses. The result of this measurement is shown in Fig. 4a. The trace obtained has the same period of modulation as the one of the four-pulse DEER experiment. The traces differ around the time $t = 0$ because of the dead time of 40 ns involved in the new sequence. The Fourier transform has a maximum at 7.8 ± 0.1 MHz, as in the four-pulse DEER experiment. Increasing the length of the observer pulses to 140 ns, the time trace shown in Fig. 4b is obtained. It is almost identical to the one obtained with pulses of 32 ns length except for a slightly reduced modulation amplitude. None of the distortions that were observed with the conventional DEER sequence (Fig. 3) are present in this case, demonstrating that the method works.

3.3. Dead-time free selective DEER

To remove the dead time, the sequence shown in Fig. 2d is employed. In Fig. 5 the traces resulting from this pulse sequence using 28 ns pump and 32 ns observer pulses are compared to those obtained with the longer observer pulses (140 ns). The traces are dead-time free and are similar to the four-pulse DEER trace (Fig. 3a). The longer observer pulses in Fig. 5b compared to Fig. 5a result in a smaller signal amplitude but the shape of the time traces remains unaffected. Fourier transformation yields a frequency spectrum that is identical to four-pulse DEER and has a $\omega_{ee}^{\perp}/2\pi \approx 7.7$ MHz. These results show that the limitation towards small excitation bandwidths of the observer pulses in DEER can be completely eliminated with the newly proposed sequence.

4. Summary and outlook

Two new pulse sequences are described, which effectively remove the restrictions caused by the lower limit on the bandwidth of the observer pulses in DEER experiments. By a second pump pulse, the shift of the resonance frequency of the observed spins caused by the spin flip of the pumped spin, is reversed, and the modulations are restored. Long observer pulses can be used, which, in the conventional DEER sequence, would result in severely distorted traces that cannot be analyzed anymore.

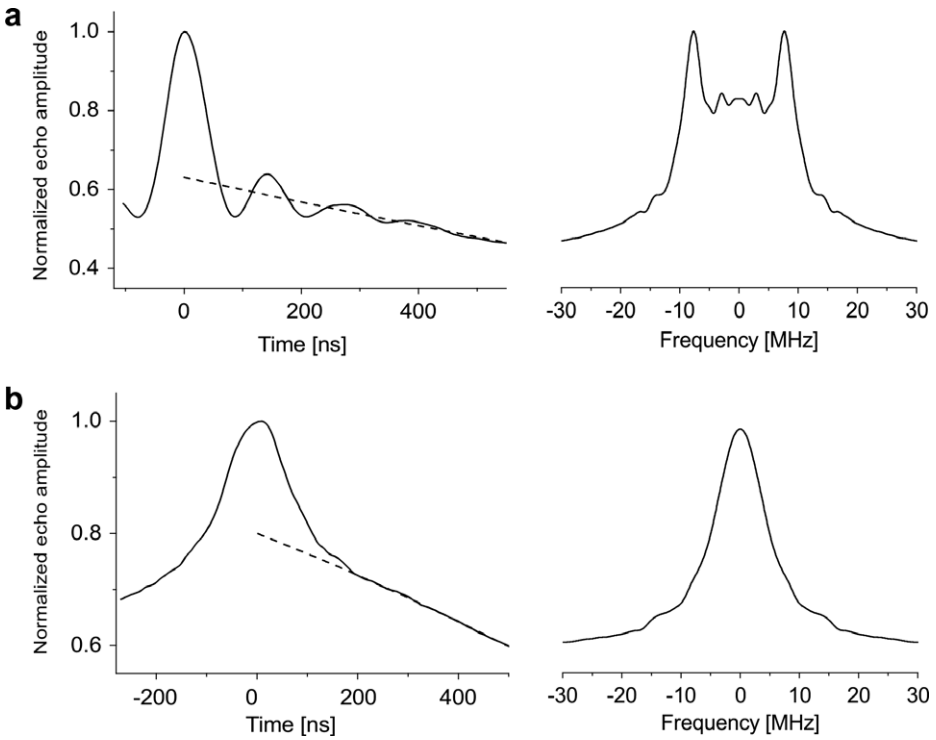

Fig. 3. Four-pulse DEER on biradical PH. Time trace (solid line, left panel) and simulated background curve (dashed line, left panel), Fourier transform of the trace after background subtraction (right panel): (a) Observer pulse: 32 ns, pump pulse: 28 ns; (b) Observer pulse: 140 ns, pump pulse: 28 ns.

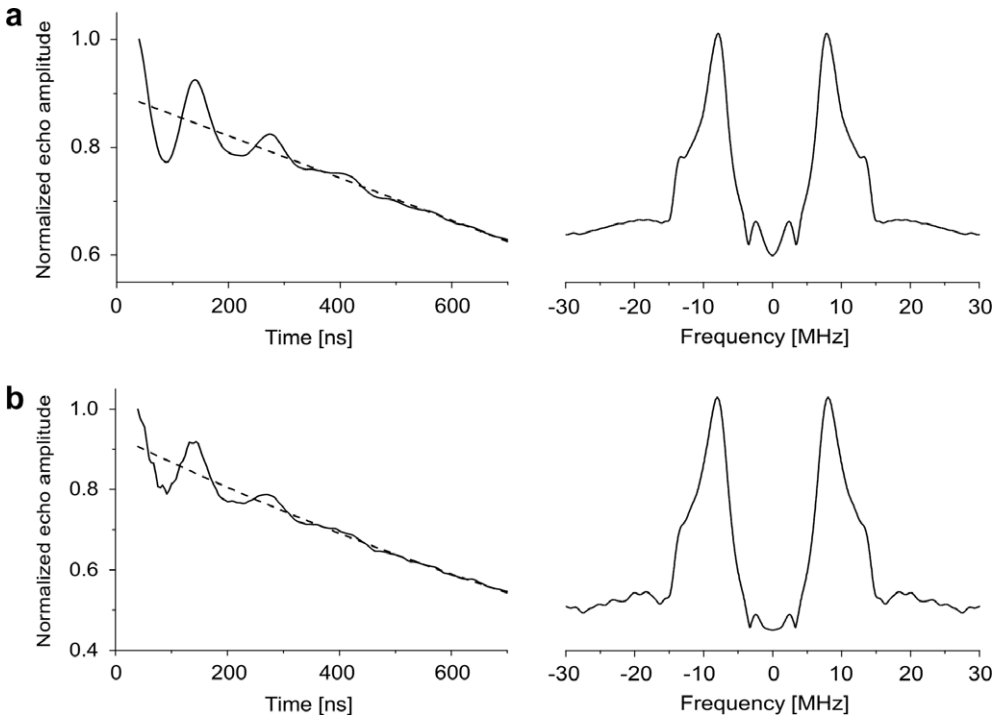

Fig. 4. Four-pulse os-DEER on biradical PH. Time trace (solid line, left panel) and simulated background curve (dashed line, left panel), Fourier transform of the trace after background subtraction (right panel): (a) Observer pulse: 32 ns, pump pulse: 28 ns; (b) Observer pulse: 140 ns, pump pulse: 28 ns.

An interesting, albeit in practice difficult to realize extension of the approach is that, if one could overcome the bandwidth limitations of the pump pulse, the os-DEER sequences would make it possible to measure shorter distances. It should be easier to increase the power of the pump pulses than that of the observer pulses, because the pump pulse could be amplified and applied in a separate pulse channel. Additional caveats would then be the necessary decoupling of pump and observer pulses in the resonator structure by e.g. double mode cavities.

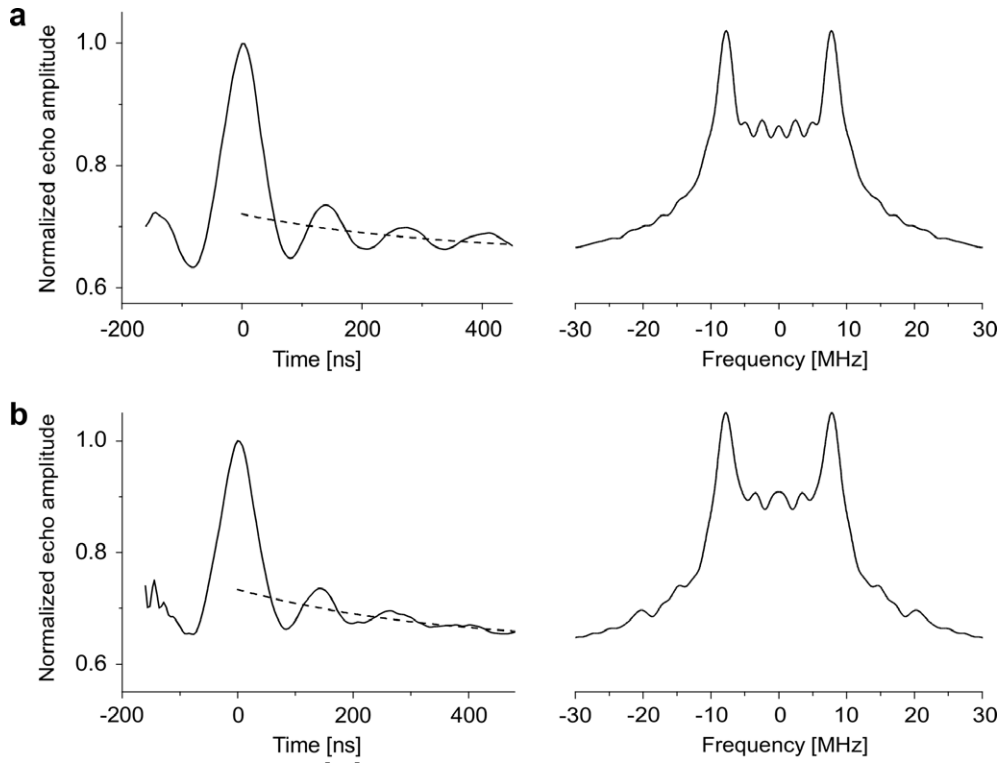

Fig. 5. Six-pulse, dead-time free os-DEER on biradical PH. Time trace (solid line, left panel) and simulated background curve (dashed line, left panel), Fourier transform of the trace after background subtraction (right panel): (a) Observer pulse: 32 ns, pump pulse: 28 ns; (b) Observer pulse: 140 ns, pump pulse: 28 ns.

The os-DEER sequence allows the use of smaller bandwidth observer pulses that improve spectral selectivity. This makes it possible to do orientation-selective experiments using a smaller frequency separation of observer and pump pulses. It could also be crucial in cases where short distances have previously required such large bandwidths of the observer pulses that the desired orientation selectivity could not be achieved.

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References

[1] P.P. Borbat, J.H. Freed, Measuring distances by pulsed dipolar ESR spectroscopy: spin-labeled histidine kinases, Methods Enzymol. 423 (2007) 52–116.
[2] O. Schiemann, T.F. Prisner, Long-range distance determinations in biomacromolecules by EPR spectroscopy, Q. Rev. Biophys. 40 (2007) 1–53.
[3] G. Jeschke, Y. Polyhach, Distance measurements on spin-labelled biomacromolecules by pulsed electron paramagnetic resonance, Phys. Chem. Chem. Phys. 9 (2007) 1895–1910.
[4] G. Jeschke, M. Pannier, H.W. Spiess, in: L.J. Berliner, S.S. Eaton, G.R. Eaton (Eds.), Double Electron–Electron Resonance, Distance Measurements in Biological Systems by EPR, Kluver Academic/Plenum Publishers, New York, 2000, pp. 493–512.
[5] A.D. Milov, A.G. Maryasov, Y.D. Tsvetkov, Pulsed electron double resonance (PELDOR) and its applications in free-radicals research, Appl. Magn. Reson. 15 (1998) 107–143.
[6] R.G. Larsen, D.J. Singel, Double electron–electron resonance spin-echo modulation—spectroscopic measurement of electron-spin pair separations in orientationally disordered solids, J. Chem. Phys. 98 (1993) 5134–5146.
[7] M. Pannier, S. Veit, A. Godt, G. Jeschke, H.W. Spiess, Dead-time free measurement of dipole–dipole interactions between electron spins, J. Magn. Reson. 142 (2000) 331–340.
[8] G. Jeschke, A. Koch, U. Jonas, A. Godt, Direct conversion of EPR dipolar time evolution data to distance distributions, J. Magn. Reson. 155 (2002) 72–82.
[9] G. Jeschke, V. Chechik, P. Ionita, A. Godt, H. Zimmermann, J. Bahman, C.R. Timmel, D. Hilger, H. Jung, Deer Analysis 2006—a comprehensive software package for analyzing pulsed ELDOR data, Appl. Magn. Reson. 30 (2006) 473–498.
[10] A. Weber, O. Schiemann, B. Bode, T.F. Prisner, PELDOR at S- and X-band frequencies and the separation of exchange coupling from dipolar coupling, J. Magn. Reson. 157 (2002) 277–285.
[11] A. Savitsky, A.A. Dubinskii, M. Flores, W. Lubitz, K. Möbius, Orientation-resolving pulsed electron dipolar high-field EPR spectroscopy on disordered solids: I. Structure of spin-correlated radical pairs in Bacterial Photosynthetic Reaction Centers, J. Phys. Chem. B 111 (2007) 6245–6262.
[12] V.P. Denysenkov, T.F. Prisner, J. Stubbe, M. Bennati, High-field pulsed electron–electron double resonance spectroscopy to determine the orientation of the tyrosyl radicals in ribonucleotide reductase, Proc. Natl. Acad. Sci. USA 103 (2006) 13386–13390.
[13] Y. Polyhach, A. Godt, C. Bauer, G. Jeschke, Spin pair geometry revealed by high-field DEER in the presence of conformational distributions, J. Magn. Reson. 185 (2007) 118–129.
[14] A. Godt, M. Schulte, H. Zimmermann, G. Jeschke, How flexible are poly(para-phenyleneethynylene)s?, Angew Chem. Int. Ed. 45 (2006) 7560–7564.
[15] A.G. Maryasov, Y.D. Tsvetkov, Formation of the pulsed electron–electron double resonance signal in the case of a finite amplitude of microwave fields, Appl. Magn. Reson. 18 (2000) 583–605.
[16] A.D. Milov, B.D. Naumov, Y.D. Tsvetkov, The effect of microwave pulse duration on the distance distribution function between spin labels obtained by PELDOR data analysis, Appl. Magn. Reson. 26 (2004) 587–599.
[17] G.A.A. Saracino, A. Tedeschi, G. D'Errico, R. Improta, L. Franco, M. Ruzzi, C. Corvaja, V. Barone, Solvent polarity and pH effects on the magnetic properties of ionizable nitroxide radicals: a combined computational and experimental study of 2,2,5,5-tetramethyl-3-carboxypyrrolidine and 2,2,6,6-tetramethyl-4-carboxypiperidine nitroxides, J. Phys. Chem. A 106 (2002) 10700–10706.
[18] A. Schweiger, G. Jeschke, Principles of Pulse Electron Paramagnetic Resonance, Oxford University Press, Oxford, 2001. p. 414.
[19] G.E. Pake, Nuclear resonance absorption in hydrated crystals—fine structure of the proton line, J. Chem. Phys. 16 (1948) 327–336.
[20] A.D. Milov, K.M. Salikhov, M.D. Shchirov, Application of the double resonance method to electron spin echo in a study of the spatial distribution of paramagnetic centers in solids, Sov. Phys. Solid State (Fizika Tverdogo Tela) 23 (1981) 565–569.