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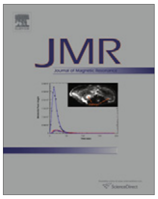

Historical Perspective

Addendum to the paper "Dead-time free measurement of dipole–dipole interactions between electron spins" by M. Pannier, S. Veit, A. Godt, G. Jeschke, and H.W. Spiess [J. Magn. Reson. 142 (2000) 331–340]

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ABSTRACT

The development of four-pulse DEER as described, which has been published in the Journal of Magnetic Resonance more than 10 years ago. The corresponding paper is an example where a slight advance, such as adding a refocusing pulse, which in retrospect looks so simple, can have a remarkable impact on an entire field of science. In our case it offered a simple way to exact measurements of distances between defined species in the nanometer range. The current applications are mainly in determining structures of proteins and nucleic acids.

Interview with the author(s).

A video interview with the author(s) associated with this Historical Perspective and the original article can be found in the online version, at doi:10.1016/j.jmr.2011.08.014.

1. Introduction

This paper is primarily an example of cross-fertilization between NMR and EPR. It describes four-pulse double electron–electron resonance (DEER) based on the ingenious approach introduced in Novosibirsk in the early 1980s by Milov, Salikhov and Tsvetkov. It can measure dipole–dipole couplings between electron spins [1,2] and was nicely demonstrated on a model system by Larsen and Singel in the 1990s [3]. It was clear that combined with site-directed spin labeling [4] this approach had potential for measuring distances in the nanometer range, which is of high importance in materials and life sciences alike. Yet it was not used for determining such distances in previously unknown structures. When Michael Hubrich set out to establish this technique in our lab, I did not worry about details and, therefore in our first paper on the subject we used the 'conventional' three-pulse approach, two for excitation and detection and one for inversion of the second spin [5].

Later, I realized that the conventional sequence, although based on the ingenious idea of a Hahn-echo, ignored the dead-time following the first excitation pulse. A simple refocusing pulse following this excitation, see Fig. 1 in our paper, generates a 'dead-time free' echo which can then be used even at negative evolution times to invert the second spin. For an NMR person like me this sounded very simple, as such refocusing is used throughout NMR these days 'to clean up the mess' generated by a simple excitation pulse. In fact, in our first paper using the four-pulse DEER sequence [6], we only stated that the limitations due to the dead-time can be overcome by our 'new' four-pulse sequence, but we didn't bother discussing details. In addition to that paper, we of course presented our work at conferences. Much to our surprise, the reaction of our EPR colleagues was not at all enthusiastic. In fact some of them claimed that the fourth pulse didn't make any difference. This encouraged us to submit a detailed description of our approach to JMR. In this paper we thoroughly discussed the sequence and described the analysis in both the time- and the frequency-domain, performed by Gunnar Jeschke, who had in the meantime joined my group and led the EPR-activities. In addition to demonstrating the technique on a model compound with an end-to-end distance of 2.8 nm, we determined both the mean cluster size and the mean distance between the clusters in disordered ionomers. Here the signals from spins in the same cluster are completely invisible in conventional DEER, see Fig. 8 in the paper.

The manuscript was handled by the late Arthur Schweiger as Associate Editor and accepted without problems, but also without special 'praise' by the reviewers. Why did nobody apparently propose this simple extension of the pulse sequence before? I think

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this was partly due to the technical difficulties associated with pulse EPR. At that time everyone tried to keep the pulse sequences as simple as possible, as additional pulses often generated problems. Moreover, it was virtually impossible, at least with our commercial set, to generate $\pi/2$ and π pulses covering sufficient spectral widths as 'required' in the four-pulse DEER sequence. The situation reminded me of the early days of Fourier transform deuteron NMR, where pulses covering the full spectral width of the ^{2}H-spectra were not available. Therefore, in our 1979 JMR paper [7] showing the first undistorted ^{2}H NMR spectrum covering the full width recorded by FT methods we applied $\pi/4$ rather than $\pi/2$ pulses as required by standard considerations. In our DEER paper we used pulses of 32 ns throughout and didn't even bother commenting on the fact that these pulses were not $\pi/2$ and π-pulses plotted in the figures and used in the discussion. In spite of this, the results were convincing.

When we wrote that paper we did not anticipate the remarkable impact it had on the EPR field. The four-pulse DEER sequence proved to be remarkably robust and the technical advances in pulsed EPR, including high-field applications, make this sequence nowadays easy to use, even for newcomers in the field. Moreover, Gunnar Jeschke's detailed recipes for extracting distances and distance distributions [8,9] were essential in promoting this technique to the current state. Today, four-pulse DEER (or PELDOR) can be considered a 'standard technique' of EPR spectroscopy.

In particular, DEER spectroscopy in combination with site-directed spin labeling [4,10] is extensively used for the study of structure and function of proteins [11,12] including their function as carriers of small molecules [13], and nucleic acids [14]. Moreover it is used to probe large, complex biomacromolecules and their assemblies [15] and protein folding [16]. Combining DEER and paramagnetic relaxation enhancement in high resolution NMR seems especially promising as it provides simultaneous access to intermediate and long-range distances in protein complexes [17]. Even the first in-cell measurements have recently been reported, which may open up a way to study processes *in vivo* [18,19].

Dead-time free DEER spectroscopy has also made an impact in the field of new materials and synthetic nanostructures, as it delivers valuable information in the very important distance range between 1.5 nm and ~8 nm [20]. In solution, distances in this range were not quantitatively accessible before DEER became available. So far, DEER has mainly been used to study the size and/or shape of synthetic nanostructures and supramolecular systems [20–22]. Furthermore, DEER has been employed to understand the complex self-assembly of counterions surrounding polyions in strongly charged polyelectrolyte systems [23,24].

Furthermore, developments starting from the four-pulse DEER sequence made it possible to measure distances not only between nitroxide radicals, but also between nitroxides and paramagnetic transition metal ions like Cu^{2+} [25,26] or recently Gd^{3+} [27,28] and even between transition metal ions [29–31]. DEER can now also be measured and analyzed at higher fields (Q- and W-band), with higher sensitivity and stronger orientation selection [32,33]. Furthermore, quantitative 'spin counting' is now a valuable tool to judge efficiencies of self-assembly [34,35] and the effect of multispin effects on the DEER data has been characterized [35,36]. In particular, these recent advances prove that beyond the use of DEER in biophysics and materials science, there is continuing interest of the magnetic resonance community to further develop this method.

To conclude, four-pulse DEER as described in our paper is an example where a slight advance, such as adding a refocusing pulse, which in retrospect looks so simple, can have a remarkable impact on an entire field of science. In our case it offered a simple way to exact measurements of distances between defined species in the nanometer range.

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References

[1] A.D. Milov, K.M. Salikhov, M.D. Shirov, Application of endor in electron-spin echo for paramagnetic center space distribution in solids, Fiz. Tverd. Tela 23 (1981) 975–982.

[2] A.D. Milov, A.B. Ponomarev, Y.D. Tsvetkov, Electron–electron double resonance in electron spin echo: model biradical systems and the sensitized photolysis of decalin, Chem. Phys. Lett. 110 (1984) 67–72.

[3] 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.

[4] W. L Hubbell, A. Gross, R. Langen, M. A Lietzow, Recent advances in site-directed spin labeling of proteins, Curr. Opin. Struct. Biol. 8 (1998) 649–656.

[5] V. Pfannebecker, H. Klos, M. Hubrich, T. Volkmer, A. Heuer, U. Wiesner, H.W. Spiess, J. Phys. Chem. 100 (1996) 13428–13432.

[6] R.E. Martin, M. Pannier, F. Diederich, V. Gramlich, M. Hubrich, H.W. Spiess, Determination of end-to-end distances in a series of TEMPO diradicals with a new four-pulse double electron electron resonance experiment, Angew. Chem Int. Ed. 37 (1998) 2834–2837.

[7] R. Hentschel, H.W. Spiess, Deuterium Fourier transform NMR in solids and solid polymers, J. Magn. Reson. 35 (1979) 157–162.

[8] G. Jeschke, A. Koch, U. Jonas, A. Goth, Direct conversion of EPR dipolar time evolution data to distance distributions, J. Magn. Reson. 155 (2002) 72–82.

[9] G. Jeschke, Distance measurements in the nanometer range by pulse EPR, ChemPhysChem 3 (2002) 927–932.

[10] W.L. Hubbell, D.S. Cafiso, C. Altenbach, Identifying conformational changes with site-directed spin labeling, Nat. Struct. Biol. 7 (2000) 735–739.

[11] J.P. Klare, H.-J. Steinhoff, Site-directed spin labeling and pulse dipolar electron paramagnetic resonance, Encyclopedia Anal. Chem. (2010), doi:10.1002/9780470027318.a9024.

[12] O. Schiemann, T.F. Prisner, Long-range distance determinations in biomacromolecules by EPR spectroscopy, Quart. Rev. Biophys. 40 (2007) 1–53.

[13] M.J.N. Junk, H.W. Spiess, D. Hinderberger, The distribution of fatty acids reveals the functional structure of human serum albumin, Angew. Chem. Int. Ed. 49 (2010) 8755–8759.

[14] O. Schiemann, P. Cekan, D. Margraf, T.F. Prisner, S.T. Sigurdsson, Relative orientation of rigid nitroxides by PELDOR: beyond distance measurements in nucleic acid, Angew. Chem., Int. Ed. 48 (2009) 3292–3295.

[15] J.E. Banham, C.R. Timmel, R.J.M. Abbott, S.M. Lea, G. Jeschke, The characterization of weak protein–protein interactions: evidence from DEER for the trimerization of a von Willebrand factor A domain in solution, Angew. Chem. Int. Ed. 45 (2006) 1058–1061.

[16] C. Dockter, A. Volkov, C. Bauer, Y. Polyhach, Z. Joly-Lopez, G. Jeschke, H. Paulsen, Refolding of the integral membrane protein light-harvesting complex II monitored by pulse EPR, Proc. Natl. Acad. Sci. USA 106 (2009) 18485–18490.

[17] Y. Yang, T.A. Ramelot, R.M. McCarrick, S. Ni, E.A. Feldmann, J.R. Cort, H. Wang, C. Ciccosanti, M. Jiang, H. Janjua, T.B. Acton, R. Xiao, J.K. Everett, G.T. Montelione, M.A. Kennedy, Combining NMR and EPR methods for homodimer protein structure determination, J. Am. Chem. Soc. 132 (2010) 11910–11913.

[18] R. Igarashi, T. Sakai, H. Hara, T. Tenno, T. Tanaka, H. Tochio, M. Shirakawa, Distance determination in proteins inside *Xenopus laevis* oocytes by double electron–electron resonance experiments, J. Am. Chem. Soc. 132 (2010) 8228–8229.

[19] I. Krstić, R. Hänsel, O. Romainczyk, J.W. Engels, V. Dötsch, T.F. Prisner, Long-range distance measurements on nucleic acids in cells by pulsed EPR spectroscopy, Angew. Chem. Int. Ed. 50 (2011) 5070–5074.

[20] G. Jeschke, Determination of the nanostructure of polymer materials by electron paramagnetic resonance spectroscopy, Macromol. Rapid Commun. 23 (2002) 227–246.

[21] G. Jeschke, M. Sajid, M. Schulte, N. Ramezanian, A. Volkov, H. Zimmermann, A. Godt, Flexibility of shape-persistent molecular building blocks composed of p-phenylene and ethynylene units, J. Am. Chem. Soc. 132 (2010) 10107–10117.

[22] J.E. Lovett, M. Hoffmann, A. Cnossen, A.T.J. Shutter, H.J. Hogben, J.E. Warren, S.I. Pascu, C.W.M. Kay, C.R. Timmel, H.L. Anderson, Probing flexibility in porphyrin-based molecular wires using double electron–electron resonance, J. Am. Chem. Soc. 131 (2009) 13852–13859.

[23] D. Hinderberger, H.W. Spiess, G. Jeschke, Probing how counterion structure and dynamics determine polyelectrolyte solutions using EPR spectroscopy, Appl. Magn. Reson. 37 (2010) 657–683.

[24] D. Kurzbach, D.R. Kattnig, B. Zhang, A.D. Schlüter, D. Hinderberger, Assessing the solution shape and size of charged dendronized polymers using double electron–electron resonance, J. Phys. Chem. Lett. 2 (2011) 1583–1587.

[25] E. Narr, A. Godt, G. Jeschke, Selective measurements of a nitroxide–nitroxide distance of 5 nm and a nitroxide–copper distance of 2.5 nm in a terpyridine-based copper(II) complex by pulse EPR, Angew. Chem. Int. Ed. 41 (2002) 3907–3910.

[26] M.J.N. Junk, H.W. Spiess, D. Hinderberger, Characterization of the solution structure of human serum albumin loaded with a metal porphyrin and fatty acids, Biophys. J. 9 (2011) 2293–2301.
[27] P. Lueders, G. Jeschke, M. Yulikov, Double electron–electron resonance measured between Gd^{3+} ions and nitroxide radicals, J. Phys. Chem. Lett. 2 (2011) 604–609.
[28] Y. Song, T.J. Meade, A.V. Astashkin, E.L. Klein, J.H. Enemark, A. Raitsimring, Pulsed dipolar spectroscopy distance measurements in biomacromolecules labeled with Gd(III) markers, J. Magn. Reson. 210 (2011) 59–68.
[29] T. Bund, J.M. Boggs, G. Harauz, N. Hellmann, D. Hinderberger, Copper uptake induces self-assembly of 18.5 kDa myelin basic protein (MBP), Biophys. J. 99 (2010) 3020–3028.
[30] A. Potapov, Y. Song, T.J. Meade, D. Goldfarb, A.V. Astashkin, A. Raitsimring, Distance measurements in model bis-Gd(III) complexes with flexible "bridge". Emulation of biological molecules having flexible structure with Gd(III) labels attached, J. Magn. Reson. 205 (2010) 38–49.
[31] A. Potapov, H. Yagi, T. Huber, S. Jergic, N.E. Dixon, G. Otting, D. Goldfarb, Nanometer-scale distance measurements in proteins using Gd^{3+} spin labeling, J. Am. Chem. Soc. 132 (2010) 9040–9048.
[32] P. Zou, H.S. Mchaourab, Increased sensitivity and extended range of distance measurements in spin-labeled membrane proteins: Q-band double electron–electron resonance and nanoscale bilayers, Biophys. J. 98 (2010) L18–L20.
[33] Ye. Polyhach, A. Godt, C. Bauer, G. Jeschke, Spin pair geometry revealed by high-field DEER in the presence of conformational distribution, J. Magn. Reson. 185 (2007) 118–129.
[34] B.E. Bode, D. Margraf, J. Planckmeyer, G. Dürner, T.F. Prisner, O. Schiemann, Counting the monomers in nanometer-sized oligomers by pulsed electron–electron double resonance, J. Am. Chem. Soc. 129 (2007) 6736–6745.
[35] M.J.N. Junk, H.W. Spiess, D. Hinderberger, DEER in biological multispin-systems: a case study on the fatty acid binding to human serum albumin, J. Magn. Reson. 210 (2011) 210–217.
[36] G. Jeschke, M. Sajid, M. Schulte, A. Godt, Three-spin correlations in double electron–electron resonance, Phys. Chem. Chem. Phys. 11 (2009) 6580–6591.