THE JOURNAL OF PHYSICAL CHEMISTRY B

Cite This: J. Phys. Chem. B 2019, 123, 2454−2456

Comment

pubs.acs.org/JPCB

Comment on "Distinct Populations in Spin-Label EPR Spectra from Nitroxides"

Eva Meirovitch,*[,†] Boris Dzikovski,[‡] and Jack H. Freed*[,‡]

[†]The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 5290002, Israel

[‡]Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853-1301, United States

J. Phys. Chem. B **2018**, *122* (23), 6129−6133. DOI: 10.1021/acs.jpcb.7b11294

J. Phys. Chem. B **2019**, *123*. DOI: 10.1021/acs.jpcb.8b11960

Marsh, in his recent article,[1] criticizes a 34 year old article of ours.[2] He also points out that he cannot reproduce the simulated electron spin resonance (ESR) spectra in figure 8 of ref 2. We found a convergence issue with this figure, which we corrected, as described below. But Marsh goes on to imply that the rest of the paper must not be sound, and this is not justified. His aim is to disprove the finding in ref 2 that a single-component model is a possible alternative to the rather frequent two-component interpretation of ESR spectra from protein−lipid mixtures. In this Comment, we refute such claims by Marsh. We also point out that the issue of whether an ESR spectrum is composed of one or two components, which had led to ambiguity in the past, has been moot for more than 22 years since an objective least-squares method to address this was introduced[3] based on ref 2 and successfully applied to distinguish between one and two components (for example, refs 4 and 5).

Meirovitch, Nayeem, and Freed (MNF)[2] introduced the model of Microscopic Order and Macroscopic Disorder (MOMD) for interpreting restricted (i.e., locally ordered) slow-motional ESR spectra in dispersed morphologies. Of particular interest were lipid dispersions and lipid/protein mixtures. Prior to this development, ESR spectroscopists had to settle for much simpler models that are easier to calculate but often not physically sound because the key element of local ordering is not accounted for. Typical examples are the "fluid" model (rotational diffusion), the "immobilized" model (effective time-independent Hamiltonian), and the two-component model (superposed "fluid" and "immobilized" components).[2] The main achievement of MNF was the correct calculation of restricted slow-motional ESR in randomly dispersed media. It requires summing the locally ordered slow-motional spectra from all angles between the local director and the magnetic field.[2,6]

MNF contains 11 figures comprising simulations of ESR spectra; eight are for MOMD, the main focus of that article. Marsh disregards all of the MOMD spectra and the MOMD model and addresses only figure 8, which is for the Very Anisotropic Rotation (VAR) model proposed 10 years earlier.[7] VAR is a very simple model assuming much faster parallel than perpendicular motion about a diffusion axis tilted in the magnetic-tensor frame; it does not include local ordering. Marsh could not reproduce figure 8A,B using the EasySpin package.[8] Without further justification or other examples to support his case, he concludes that the results of MNF are wrong, and hence that article is "misleading and unhelpful". This statement has been contradicted by many studies since MNF, such as those of refs 4 and 5.

The slow-motional software in EasySpin[8] is directly based on the NLSL slow-motional software we developed,[3,6,9] and hence corresponding results should be virtually identical. Thus Marsh is using software that derives from the same source as the one he claims leads to flawed results; however, he only uses it for the VAR model, mainly to interpret ESR spectra from dispersions of lipid bilayers.[1] Because "lipid bilayers" invariably implies local ordering, one should, in general, use MOMD.

Another objective of MNF was to show that ESR lineshapes from lipid/protein mixtures, which appear to be interpretable as superimposed spectra from "fluid" bulk lipids and "immobilized" boundary lipids, could, at least in some cases, be interpretable as single-component MOMD spectra; this is due to the properties of derivative ESR spectra from slow-motional microscopically ordered systems, as delineated in detail in section IV.A of ref 2. MNF cites a number of articles offering such a pre-MOMD interpretation and shows a room-temperature (RT) spectrum from *Halobacterium halobium* doped with stearic acid I(1,14) (cf. figure 11A (iii)[2]) and an RT spectrum from dipalmitoylphosphatidylcholine (DPPC)−gramicidin A dispersion doped with phospholipid spin probe (figure 11B (i)[2]). A demonstration that MOMD adequately reproduces these experimental lineshapes is shown in figure 11B (ii).[2] Marsh[1] ignores this, stating that "neither from simulation nor from experiment is there any basis to assert that single-component nitroxyl electron paramagnetic resonance (EPR) spectra resemble those containing two components."

Marsh also ignores illustrations that MOMD correctly reproduces ESR spectra from pure lipids. In his work, Marsh simulates such spectra with the VAR model using the tilt angle between the diffusion and magnetic axes, Ψ, as an *apparent* order parameter to derive flexibility gradients in membranes.[1] But Ψ is just a geometric feature associated with how the nitroxide moiety is attached to the labeled lipid molecule.[7] It does not report on the actual ordering (i.e., alignment) of the lipid within the membrane. The VAR model lacks the physical feature of ordering in the membrane, whereas the MOMD model does include this, thereby enabling one to extract the extent of ordering of the lipid in the membrane.

Received: August 8, 2018
Revised: November 15, 2018
Published: February 15, 2019

We checked the simulated spectra in MNF with the NLSL program,[3,6,9] the successor of that used in MNF, as well as with EasySpin version 5.2.9. The MOMD spectra of MNF are properly reproduced by both NLSL and EasySpin, which give identical results (see Figure 1a as well as www.acert.cornell.edu); so are the VAR spectra in figure 13c of MNF (taken from ref 7). There is, however, a flaw noted above in the VAR spectra of figures 7 and 8 in MNF, but *only* in those two figures. It arose because these simulations in MNF were calculated with the Mmax parameter needed for the MOMD program to simulate VAR likely chosen too small (Mmax = 2), resulting in unconverged spectra. We show in Figure 1b the correct results with Mmax = 6 obtained for a tilt of 35° of the diffusion axis with NLSL (black) and the perfect agreement with EasySpin (red). (The same results are also obtained with just the simple VAR program.) In the inset to Figure 1b, we show the unconverged result for Mmax = 2 for the case of $R_{\|}$ = 1.5 × 10^7 s^{-1} and 35° tilt (black), which is very similar to its counterpart in figure 8B of MNF (blue). This emphasizes the importance of checking simulated spectra for convergence with respect to convergence parameters lemx, lomx, kmx, Mmax, and ipnmx.

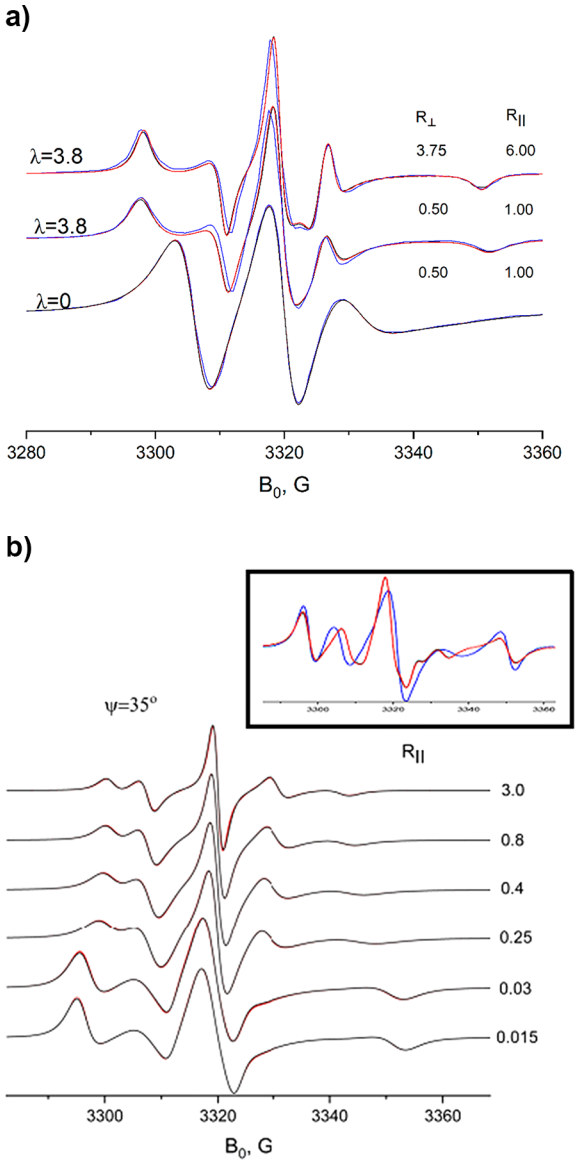

Figure 1. (a) MOMD spectra obtained with NLSL for Ψ = 0°, $(T_2^*)^{-1}$ = 1.5 G, $R_\perp$, $R_\|$, and λ as depicted in the figure in units of 10^8 s^{-1}. Magnetic parameters: set B of MNF. Convergence parameters: lemx, lomx, kmx, Mmax, ipnmx = 30, 23, 10, 6, 2, respectively (black); spectra scanned from MNF (blue); spectra from EasySpin (red). λ is the ordering potential in units of kT.[2,6] (b) VAR spectra obtained with NLSL (black) and EasySpin (red) for Ψ = 35°, $(T_2^*)^{-1}$ = 1.5 G, $R_\perp$ = 5.0 × 10^6 s^{-1}, and $R_\|$ as depicted, in units of 10^9 s^{-1}. lemx, lomx, kmx, Mmax, ipnmx = 30, 23, 10, 6, 2, respectively. Inset: Lowest spectrum redone with NLSL Mmax = 2 (black), EasySpin (red), and scanned from MNF (blue). Other convergence parameters: lemx, lomx, kmx, ipnmx = 30, 23, 10, 2, respectively.

Thus there is nothing wrong with the original MNF (except for a minor convergence issue in MNF figures 7 and 8 that has been repaired here, cf. Figure 1b, and is not repeated in later work) as well as existing software, contrary to Marsh's unjustified assertion. We urge colleagues to employ the MOMD model for microscopically ordered but macroscopically disordered media rather than the simpler VAR model. Furthermore, both NLSL[3] and EasySpin[8] versions of MOMD allow for the least-squares fitting of experimental spectra with two or more components, yielding the relative fractions and their respective dynamical and ordering parameters. This has been applied to extensive studies; the two noted above are for plasma membrane vesicles[4] and plasma membranes of live cells,[5] which enabled the determination of the fraction of liquid-ordered versus liquid-disordered regions and the corresponding dynamics and ordering in each region for an extensive range of spin-labeled lipids.

In summary, the MOMD approach introduced by MNF constitutes the current state-of-the-art method of analysis in spin-label ESR studies of lipid−protein interactions and other microscopically ordered systems. This statement is not impacted by an input error to a VAR calculation performed 34 years ago.

AUTHOR INFORMATION

Corresponding Authors

*E.M.: E-mail: eva.meirovitch@biu.ac.il. Tel: 972-3-531-8049.
*J.H.F.: E-mail: jhf3@cornell.edu. Tel: 607-255-3647.

ORCID

Eva Meirovitch: 0000-0001-5117-5079
Jack H. Freed: 0000-0003-4288-2585

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Stefan Stoll for useful advice on slow motional spectral simulations using EasySpin. This work was supported by NIH grant P41GM103521 to J.H.F., ISF grant 369/15 to E.M., and BSF grant 2016097 to E.M. and J.H.F.

REFERENCES

(1) Marsh, D. Distinct Populations in Spin-Label EPR Spectra from Nitroxides. *J. Phys. Chem. B* **2018**, *122*, 6129−6133.

(2) Meirovitch, E.; Nayeem, A.; Freed, J. H. Analysis of Protein-Lipid Interactions Based on Model Simulations of ESR Spectra. *J. Phys. Chem.* **1984**, *88*, 3454−3465.

(3) Budil, D. E.; Lee, S.; Saxena, S.; Freed, J. H. Nonlinear Least-Squares Analysis of Slow-Motion EPR Spectra in One and Two Dimensions Using a Modified Levenberg-Marquart Algorithm. *J. Magn. Reson., Ser. A* **1996**, *120*, 155−189.

DOI: 10.1021/acs.jpcb.8b07730
J. Phys. Chem. B 2019, 123, 2454−2456

(4) Ge, M.; Gidwani, A.; Brown, H. A.; Holowka, D.; Baird, B.; Freed, J. H. Ordered and Disordered Phases Coexist in Plasma Membrane Vesicles of RBL-2H3Mast Cells. An ESR study. *Biophys. J.* **2003**, *85*, 1278−1288.
(5) Swamy, M. J.; Ciani, L.; Ge, M.; Smith, A. K.; Holowka, D.; Baird, B.; Freed, J. H. Coexisting Domains in the Plasma Membranes of Live Cells Characterized by Spin-Label ESR Spectroscopy. *Biophys. J.* **2006**, *90*, 4452−4465.
(6) Meirovitch, E.; Igner, D.; Igner, E.; Moro, G.; Freed, J. H. Electron Spin Relaxation and Ordering in Smectic and Supercooled Nematic Liquid Crystals. *J. Chem. Phys.* **1982**, *77*, 3915−3938.
(7) Mason, R. P.; Polnaszek, C. F.; Freed, J. H. Interpretation of ESR Spectra of Spin Labels Undergoing Very Anisotropic Rotational Reorientation. *J. Phys. Chem.* **1974**, *78*, 1324−1329.
(8) Stoll, S.; Schweiger, A. EasySpin, a Comprehensive Software Package for Spectral Simulation and Analysis in EPR. *J. Magn. Reson.* **2006**, *178*, 42−55.
(9) Moro, G.; Freed, J. H. Calculation of ESR Spectra and Related Fokker-Planck Forms by the Use of Lanczos Algorithm. *J. Chem. Phys.* **1981**, *74*, 3757−3772.

DOI: 10.1021/acs.jpcb.8b07730
J. Phys. Chem. B 2019, 123, 2454−2456