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Marsh in his recent article\(^1\) has made several unjustified criticisms of a 34 year-old article of ours.\(^2\) In this comment we wish to set the record straight. 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-site 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.\(^2,3\)

MNF contains eleven 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 ten years earlier.\(^4\) 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 Figures 8A and 8B using the EasySpin package.\(^5\) Without further justification, or other examples to support his case, he concludes that all of the results of MNF are wrong, hence that article is “misleading and unhelpful.”

The slow motional software in EasySpin\(^5\) is directly based on the NLSL slow-motional software we developed;\(^3,6,7\) 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 MOMD spectra. MNF cite a number of articles offering such a pre-MOMD interpretation and show a room-temperature (RT) spectrum from Halobacterium Halobium doped with stearic acid I(1,14) (cf. Figure 11A (iii)²), and a 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. Marsh1 completely ignores this, calling the MOMD spectra “artefactual.” He also ignores illustrations that MOMD correctly reproduces ESR spectra from pure lipids. Marsh simulates such spectra with the VAR model which he misconstrues by recasting the tilt angle between the diffusion and magnetic axes into an apparent order parameter, used in an unjustified fashion4 to derive flexibility gradients in membranes.¹

We checked the simulated spectra in MNF with the NLSL program,³,⁶,⁷ 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, www.acert.cornell.edu, and biorxiv.org; so are the VAR spectra in Figure 13c of MNF (taken from ref 4). There is, however, a flaw 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 in the MOMD program 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 obtained whether using the MOMD program for VAR with Mmax = 6, or just the simple VAR program. In the inset we show the unconverged result for Mmax = 2 for the case of $R_\parallel = 1.5 \times 10^7\, s^{-1}$ 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, max, and ipnmx.

**Figure 1A.** MOMD spectra obtained with NLSL for $\Psi = 0^\circ$, $(T_2^*)^{-1} = 1.5\, G$, $R_\perp$ and $R_\parallel$ as depicted on the Figure in units of $10^8\, s^{-1}$, magnetic parameters – set B of MNF, truncation parameters – lemx, lomx, kmx, Mmax, ipnmx = 30, 23, 10, 6, 2 (black); spectra scanned from MNF (blue); spectra from EasySpin (red).

**Figure 1B.** VAR spectra obtained with NLSL (black) and EasySpin (red) for $\Psi = 35^\circ$, $(T_2^*)^{-1} = 1.5\, G$, $R_\perp = 5.0 \times 10^6\, s^{-1}$ and $R_\parallel$ as depicted, in units of $10^9\, s^{-1}$. **Inset:** Lowest spectrum redone with NLSL Mmax = 2 (black), EasySpin (red), and scanned from MNF (blue).
In summary, there is nothing wrong with the original MNF and 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. Both NLSL and EasySpin versions of MOMD allow for the fitting of experimental spectra with two or more components, when appropriate.

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References


