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Analytical distance distributions in systems of spherical symmetry with applications to double electron–electron resonance

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ABSTRACT

Based on a simple geometrical approach, we derive analytical expression of the probability density functions (pdfs) of distance of probe molecules distributed homogeneously in *spherical aggregates with shell structure*. These distance distributions can be utilized in the investigation of double electron–electron resonance (DEER) data of disordered nanometer-sized spin clusters. Structural insights and geometrical parameters of the aggregates can be extracted by modeling the DEER time traces based on the analytical pdfs. This approach is efficient and avoids difficulties of the model-free solution of the inverse problem that are related to multi-spin effects, limited excitation bandwidth, bias introduced by the regularization scheme, or ambiguity resulting from broad distance distributions. The derived pdfs can serve as building blocks, from which the distance distributions in arbitrary spherically symmetric objects can be assembled. The scenario of the pumped species being chemically distinct from the observed species is covered as well as that of a single type of probe molecules. We demonstrate the merits of analytical distance distributions by studying the distribution of three different spin probes in SDS micelles. By simultaneously analyzing DEER data corresponding to different spin probe concentrations, the distribution of the spin probes over the micelle can be determined. Employing Bayesian inference it is found that for all probes studied, a spherical shell model is most appropriate among the studied models and by orders of magnitude more likely than a homogeneous distribution in a ball. This statement also applies to probes that are deemed nonpolar. We envisage that the spin probe distributions in disordered soft and hard matter systems can now be quantified using DEER spectroscopy with greater precision and reduced ambiguity.

1. Introduction

Double electron–electron resonance (DEER) utilizes the characteristic r^{-3}-dependence of the electron–electron dipolar coupling for measuring distances, r, between paramagnetic centers [1–6]. The method has now evolved to a standard tool for assessing distances on the nanometer scale (1.8 nm < r < 6 nm; in exceptional cases up to 8 nm) with numerous applications in the fields of structural biology or soft matter research [7–11]. The majority of studies is devoted to (bio-)macromolecules carrying two covalently attached spin labels. In dilute solutions, these *bi-labeled* substrates can be treated as well-separated spin pairs and standard procedures can reveal the underlying distance distributions from experimental data. By variation of the labeling sites, information about the three dimensional conformation of the system can then be inferred by triangulation [12,13]. The extraction of the distance distribution usually involves the numerical inversion of a Fredholm integral equation of the first kind. This is an ill-posed problem implying that the inverse is not unique because more than one distance distribution is mapped to the same data, or because a small change in the data can cause a large change in the distance distribution. These issues can be addressed by Tikhonov regularization or the maximum entropy method, which are most straightforward if the underlying distance distribution is narrow [14–16].

Besides bilabeled substrates a wealth of structural information can be obtained from spin clusters. Spin clusters can result from the self-aggregation of mono-labeled compounds allowing the study of a variety of objects that do not lend themselves to the bilabeling strategy. Often, the milieu-directed accumulation of simple spin probes in micro-heterogeneous samples can be utilized to form spin clusters without covalently attaching radicals to the systems under study (spin probing). In this way, micelles [17–19], mesoporous materials resulting from templating with pluronics micelles [20], dendronized polymers [21,22], gold nano-particles [23], and even albumin [24–27] have been studied by DEER. Yet, the extraction of structural parameters from "statistically labeled" substrates is often more involved and more subject to uncertainty than the determination of solitary distances from well-defined bilabeled substrates. This is a consequence of inherently broad distance distributions often occurring in spin clusters due to the

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mutual mobility of the probe molecules and structural flexibility of the host systems. Furthermore, due to experimental constraints, multi-spins contributions resulting from clusters with more than two spins cannot always be excluded [28]. In these cases, the structural information can still be extracted by direct simulation of the DEER time trace and comparison with experimental data (e.g. in the least-squares sense). In general, this requires well-defined models of the underlying distance distributions. While complex geometrical models can easily be handled by Monte Carlo sampling, analytic models are in general more desirable, since they allow for fast evaluation of the dipolar evolution signal and the use of efficient, i.e. gradient/Hessian based, numerical algorithms for least-squares fitting.

Here, the focus is on spherically symmetric, statistically labeled systems, which e.g. occur (as idealized models) in the study of micelles [17–19], nanoparticles [23], or the globular, collapsed state of thermoresponsive polymers [29–32]. Several earlier works have relied on model distance distributions to investigate geometrical parameters of approximately spherical assemblies. Ruthstein et al. have investigated statistically labeled micelles from pluronics (poloxamers, i.e. block copolymers of poly(ethylene oxide) and poly(propylene oxide)) [17,18]. The micelle size and the aggregation number have been determined based on a model distance distribution proportional to the distance squared. In [17] a complementary model-free approach of determining the average number of spin-probes from the long-time limit of the dipolar evolution function was suggested. This approach, however, does not yield geometric insights and the required long acquisition times cannot always be realized due to fast phase relaxation. The authors also employ Monte Carlo simulations for generating distance distributions of different spherical shell models [17].

More recently, Bode et al. have studied several types of micelles by doping with spin-labeled fatty acids [19]. The DEER time traces were analyzed in terms of two analytically known distance distributions: the probability function for distance of points randomly picked from the interior, $p_B(r|R)$ [33,34], or the surface, $p_A(r|R)$ [35,36], of a sphere:

$$p_B(r|R) = \begin{cases} \frac{3r^5}{16R^6} - \frac{9r^3}{4R^4} + \frac{3r^2}{R^3} & 0 < r \leqslant 2R \\ 0 & \text{otherwise} \end{cases}, \tag{1}$$

and

$$p_A(r|R) = \begin{cases} \frac{r}{2R^2} & 0 < r \leqslant 2R \\ 0 & \text{otherwise} \end{cases}. \tag{2}$$

Here, the $p_i(r|R)$s denote the distance distribution functions, $r \geqslant 0$ the distance, and R the radius of the model sphere. Henceforth we will write $p(r)$ when we refer to a generic distance distribution and label specific distributions by superscripts (e.g. $p_A(r|R)$ and $p_B(r|R)$ with A and B hinting that the points are randomly chosen from the surface area and the volume of the sphere, i.e. the ball, respectively). A general method of evaluating probability density functions (pdfs) of distance in n-dimensional spherical objects has been proposed by Tu and Fischbach [34]. These authors address distance distributions for a single type of particle (employing nomenclature of this paper, cf. below) and a radially varying density. In [34], an expression for a two-shell model is given for the special case that the shells are of equal width. Homogeneous ellipsoids and spheroids have been treated in [37]. To the best of the authors' knowledge, Eqs. (1) and (2) are the only analytical distance distributions that have found application in DEER spectroscopy of confined spherical systems.[1] In the work of Bode et al. the following two points were, among other things, developed: Firstly, the extracted geometrical parameters critically depend on the choice of the model. E.g. the micelle diameter determined from the same dataset but the two models of $p(r)$ given above differ by as much as 1 nm. Secondly, it was argued that the model for $p(r)$ cannot be inferred from a single DEER time trace with significance, i.e. both models gave comparable fits, yet with significantly different parameter values [19].

Ionita et al. have addressed the lateral diffusion of spin-labeled thiols on spherical gold nano-particles [23]. The distance distribution of diffusively separated pairs of probe-molecules was modeled by Eq. (2). In a very recent publication, the study was extended by employing Gd^{3+} and nitroxide spin probes [38]. The distance distribution of Gd^{3+} and nitroxide centers was modeled by a broadened version of the distance distribution of points placed homogeneously over the surface of two concentric spheres. Monte Carlo simulations were employed to determine the distance distributions.

This publication has several aims: Firstly, we introduce a variety of analytic distance distributions of spherically symmetric, shell/onion-like models. These expressions can serve as building blocks from which more complex spherical models can easily be derived. We address the scenario of two different (spatially and spectrally separated) spin probes as well as distributions originating from a single type of probe molecules. The two probes scenario can e.g. be realized in practice by using structurally different ^{14}N- and ^{15}N-nitroxides (MISS-DEER, Mixed Isotopologues for Spectral Selectivity) or by using nitroxide type spin probes in combination with metal centers (Cu^{2+} or Gd^{3+}) [21,25,38]. The first approach has been recently applied to dendronized polymers, which were probed by ^{15}N-Fremy's salt and DOXYL-stearic acids [21]. Secondly, using these analytical models, we address the question of whether different models of the distance distribution can, under favorable conditions, be distinguished on the basis of experimental data alone. This would lay the basis of reliable size/geometry determination free of ad hoc assumptions for $p(r)$ motivated solely by chemical intuition. We will employ Bayesian inference to address this question [39,40]. As a model system, we employ SDS micelles doped with 5-DOXYL stearic acid (5DSA), 5-DOXYL stearic acid methyl ester (5DSM), and 4-hydroxy-TEMPO benzoate (TOBz) at different concentration of the spin probes. The properties of SDS micelles are known in detail. Critical micelle concentrations, the dependence of the aggregation number on concentration, and hydrodynamic radii are well established [41]. The group of Bales has employed the micro-polarity sensed by the probe (via the Mukherjee hydrophilicity index [42]) to locate spin probes within the micelle [43,44]. Many probes turned out to be confined to the shell-like Stern layer or a shell comprising the majority of the Stern layer plus a small fraction (widths less than 2 Å) of the micelle core or the aqueous surrounding.

This manuscript is structured as follows: We will initially develop analytical distance distributions for a variety of spherical scenarios. We suggest a simple geometrical approach that is applicable to two different types of spin probes as well as a single type. Then, the simulation of DEER time traces is briefly reviewed and two minor modifications from the common approach are introduced. Thereafter, we will discuss DEER results of spin-probed SDS micelles in the view of the analytical distance distributions derived in the theory section. We expect that the distance distributions derived here will aid the characterization of spherical, partly or fully disordered systems by DEER. Note that the lack of robust distance distributions has been mentioned at several places in the literature [17,19].

[1] $p(r) \propto r^2$ applies to the homogeneous distribution of radical centers in an extended, isotropic medium (however not to a homogeneous distribution of centers in a sphere, which is given by Eq. (1)). In spatially confined systems $p(r) \propto r^2$ may result if one of the radicals is always situated at the center.