Rotational Correlation Functions of Single Molecules

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(Dated: August 16, 2016)

Single molecule rotational correlation functions are analyzed for several reorientation geometries. Even for the simplest model of isotropic rotational diffusion our findings predict non-exponential correlation functions to be observed by polarization sensitive single molecule fluorescence microscopy. This may have a deep impact on interpreting the results of molecular reorientation measurements in heterogeneous environments.

PACS numbers: 33.15.Vb, 87.64.Ni, 61.43.FS, 67.40.Fd

Reorientation of small molecules or segments of macromolecules undergoing conformational changes are elementary processes and often of crucial importance for the properties of materials. In most of the experimental techniques probing rotational dynamics, ensembles of molecules have been monitored. Actually, many chemical and biological systems are heterogeneous, a fact that is reflected in their dynamical properties. In the context of the non-exponential decay of bulk correlations in supercooled liquids this has been termed dynamic heterogeneity. Proteins, polymers, colloids and coarsening systems share this feature of non-exponential relaxation. In the past, bulk techniques have been developed that allow a characterization of heterogeneous rotational dynamics in complex systems in terms of a distribution of reorientational rates fluctuating in time. The orthogonal polarization resolved emission signals collected in fluorescence correlation spectroscopy have been analyzed in detail. A customary measure of dipole orientation can be obtained by calculating the reduced linear dichroism $d$ from $I_p$ and $I_s$ via

$$d = \frac{I_p - I_s}{I_p + I_s}$$

This frequently used quantity fluctuates in the course of time due to molecular rotational dynamics. The normalization ensures that $d$ depends solely on the orientation of the emission dipole and not on the absorption efficiency. Hence we represent the transition dipoles not by vectors but by their orientation using an-tric field vectors are aligned parallel to the transition dipoles of the molecule. Fluorophores preferentially absorb photons whose electric field vectors are aligned parallel to the transition dipoles of the molecule. These dipoles have a well defined orientation with respect to the molecular axes. Similarly, emission also occurs with light polarized along a fixed axis. The experimental situation we will analyze in the remainder of this paper is depicted in Fig. 1 together with the definition of the axes system and the angles used. The orthogonal polarization resolved emission signals $I_p$ and $I_s$ depend on both, absorption and emission efficiency. The contributions of $I_p$ and $I_s$ to the signals collected in fluorescence correlation spectroscopy have been analyzed in detail.

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of the respective interaction tensors distinct RCFs
\[ C_l(t) = \langle P_l(\cos(\theta(t))) P_l(\cos(\theta(0))) \rangle \] (3)
of the relevant Legendre polynomial are obtained. Here, \( \theta \) is defined in the same way as in Fig.1. For instance, the interaction of a permanent electric dipole moment with an applied electric field transforms in the same way as the first order Legendre polynomial \( P_1(\cos\theta) = \cos\theta \) and therefore \( C_1(t) \) is observed in dielectric spectroscopy. NMR and light scattering are \( l = 2 \) techniques and yield \( C_2(t) \). Below it will be shown that \( C_2(t) \), apart from being a local correlation function, cannot be related to one of the \( C_l(t) \) in a simple manner.

The theoretical description of reorientational dynamics in molecular liquids is a complicated many particle problem. For a qualitative understanding of the behavior of rotational correlation functions, however, a treatment in terms of rotational Brownian motion is usually sufficient. The rotational correlation functions, however, a treatment in terms of rotational Brownian motion is usually sufficient. The treatment general, in the following we treat auto-correlation functions of the form
\[ C_X(t) = \langle X(\Omega_{PL}(t)) X(\Omega_{PL}(0)) \rangle. \] (5)
Here, \( X \) denotes some arbitrary function of the molecular orientation \( \Omega_{PL} \) of the principal axis system (P) of the relevant interaction tensor in the laboratory fixed frame (L). In the present context we are primarily interested in the case of fluorescence, \( X = d \), in which case the z-axis of the P-system coincides with the direction of the transition dipole. However, other choices like e.g. \( X = e^{i\mathbf{q}\mathbf{r}} \) relevant for incoherent neutron scattering can be analyzed in an identical way. If the molecular orientation \( \Omega(t) \), given in terms of Eulerian angles \( \mathbf{P} \), is modelled as a stochastic process, \( C_X(t) \) can be written in the form (29):
\[ C_X(t) = \frac{1}{8\pi^2} \int d\Omega \int d\Omega_0 X(\Omega) X(\Omega_0) P(\Omega, t|\Omega_0) \] (6)
where \( P(\Omega, t|\Omega_0) \) denotes the conditional probability to find \( \Omega \) at time \( t \) given \( \Omega_0 \) at time \( t = 0 \). Throughout this letter we restrict ourselves to the model of anisotropic rotational diffusion of symmetric top molecules, in which case one has
\[ P(\Omega, t|\Omega_0) = \sum_{l,m,n} \left( \frac{2l+1}{8\pi^2} \right) D_{lmn}(\Omega) D_{lmn}(\Omega_0) e^{-[(l+1)D_z + m^2(D_x - D_y)]t} \] (7)
Here, the \( D_{lmn}(\Omega) \) are Wigner rotation matrix elements. The rotational diffusion coefficients \( D_y \) and \( D_x \) are equal, but different from \( D_z \). The limit of isotropic rotational diffusion is recovered for \( D_z = D_x \). It is important to point out that \( \Omega \) in eq. (7) denotes the orientation of the diffusion tensor (D) in the L-system, \( \Omega \equiv \Omega_{DL} \).

In order to proceed in the calculation of \( C_X(t) \), we expand the quantity \( X(\Omega_{PL}) \) in the expression for the correlation function, eq. (6), in terms of \( D_{nm}^{(l)}(\Omega) \), \( X(\Omega_{PL}(t)) = \sum_{l,m,n} X_{l,m,n} D_{lmn}^{(l)}(\Omega_{PL}(t)) \) with \( X_{l,m,n} = \frac{2l+1}{8\pi^2} \int d\Omega D_{lmn}^{(l)*}(\Omega) X(\Omega) \). Next, \( D_{lmn}^{(l)}(\Omega_{PL}(t)) \) is expressed in terms of the relevant \( \Omega_{DL}(t) \) via \( \Omega_{PL}(t) = \Omega_{PD} + \Omega_{DL}(t) \) using \( D_{lmn}^{(l)}(\Omega_{PL}(t)) = \sum_{\mu} D_{lmn}^{(l)}(\Omega_{PD}) D_{mn\mu}^{(l)}(\Omega_{DL}(t)) \). Here, \( \Omega_{PD} = (\alpha, \theta_{PD}, \pi - \phi_{PD}) \) is a shape and symmetry dependent molecular quantity. For most relevant cases \( X(\Omega_{PL}) \) has axial symmetry, which allows to replace the \( D_{lmn}^{(l)}(\Omega_{PL}(t)) \) by spherical harmonics \( Y_{lm} \). Performing the calculation using eqs. (6) and (7) yields:
\[ C_X(t) = \sum_l A_l C_l(t). \] (8)
With \( A_l = \frac{1}{8\pi^2} \sum_{\mu} \int_0^{2\pi} d\phi \int_0^\pi d\theta \sin \theta X(\theta, \phi) Y_{lm}(\theta, \phi) \) and
\[ C_l(t) = \sum_m \left| D_{lmn}^{(l)}(\Omega_{PD}) \right|^2 e^{-[(l+1)D_z + m^2(D_x - D_y)]t}, \] (9)
the RCFs eqs. (8) are calculated numerically. Typically, it is sufficient to use \( l \) values up to \( l = 20 \). Thus, \( C_l(t) \) is given by a weighted sum of a large number of RCFs.
fact has not been noticed in the literature before to the best of our knowledge. As already mentioned above, for the optical anisotropy one has \( \langle r(t)r(0) \rangle = C_2(t) \) which decays as a superposition of at most three exponentials.

For the case of isotropic rotational diffusion, i.e. \( D = D_x = D_y = D_z \), eq. (5) simplifies to

\[
C_X(t) = \sum_l A_l e^{-l(l+1)D t} \tag{10}
\]

For symmetry reasons, the odd components vanish. A similar expression has been reported recently \cite{24}. In table I the pre-factors are listed up to \( l = 20 \). The resulting correlation function significantly deviates from an exponential decay as shown in Fig.2. From fitting the computed data with a stretched exponential function \( f(t) = e^{-(t/\tau)^\beta} \) we obtain \( \tau_c = 0.87/6D \) and \( \beta = 0.871 \). Therefore, even for this simple model we find intrinsic non-exponential relaxation. The optical anisotropy decays exponentially, \( \langle r(t)r(0) \rangle = e^{-6D t} \).

The model of stochastic isotropic reorientations presents a drastic approximation as most molecules do not exhibit a spherical shape. Thus, in many cases of practical interest one is confronted with asymmetric top molecules. As noted above, however, in the present letter we restrict ourselves to the case of symmetric tops for simplicity. This means that we consider an ellipsoidal diffusion tensor with three components \( D_x = D_y \) and \( D_z \), see Fig. 3a. For standard \( l = 1,2 \) methods the RCFs resulting for this model consist of two and three exponentials, respectively, with time constants depending on the degree of anisotropy \( \delta = D_x/D_z \) \( (D_x = D_y) \). \( \tau_{PD} \) denotes the angle between the \( z \)-axis of the diffusion tensor and the transition dipole \( \vec{\mu} \).

\[
\tau / \tau_0 = \frac{\tau_{PD}}{\tau_0} = \frac{D_x}{D_x}
\]

The influence of the jump angle \( \gamma \) on the correlation function \( C_2(t) \) defined by eqns. [1] and [4] is plotted. With increasing \( \gamma \) the correlation decays become more exponential, which essentially originates from an convergence of the different \( l \) contributions to the RCF.

An entirely different approach to the calculation of RCFs via rotational random walk simulations has proved to be successful in the context of supercooled liquids\cite{27}. Here, molecular reorientation has been modelled by rotational jump processes. Starting from an arbitrary orientation, after a certain waiting time drawn from Poisson statistics the next orientation is chosen at random with the only restriction that starting and end position differ by an angle \( \gamma \). In the limit of \( \gamma \to 0 \) isotropic rotational diffusion is obtained with results identical to those obtained with the analytic approach. In Fig. 3b the influence of the jump angle \( \gamma \) on the correlation function \( C_2(t) \) is plotted. With increasing \( \gamma \) the correlation decays become more exponential, which essentially originates from an convergence of the different \( l \) contributions to the RCF.

So far, we have assumed that the principal axes sys-
FIG. 4: Influence of the reorientation mechanism on the stretching (○) and the time scale (●) of the correlation function, calculated for rotational jump processes with varying jump angles γ. For comparison the correlation times were normalized by the pure l = 2 correlation time τ2.

tem (P) relevant for experimental observables has a fixed orientation ΩPD relative to the 'diffusion tensor' system (D), and only the orientation of the latter with respect to the laboratory system (L) is time dependent: Ω(t) = ΩDL(t). In principle, one can treat the case of internal rotations by assuming a composite Markov process \{ΩPD(t), ΩDL(t)\} and using an appropriate master equation for the composite process. If the internal motion is independent of the tumbling motion of the whole molecule one can factorize the corresponding probability functions and averages. This case is of particular relevance for the conformational motions of polymers or proteins in solution and has been treated extensively in the literature to which we refer [28]. However, it was pointed out that even for NA = 1.25 the dichroism signal is only slightly influenced by this effect [12]. Additionally, if one were to measure rotational dynamics over an extended temperature range, it is experimentally more convenient to use low NA (< 1) objectives. Here still an influence of the NA is expected, which, however, becomes smaller the smaller the NA is. Nevertheless, the accurate incorporation of the NA into our theoretical description has still to be worked out. We also would like to mention that methods have been developed which utilize the longitudinal field component of high NA objectives to extract the full three dimensional orientation of transition dipoles [29, 30]. Although quite powerful, such measurements require a very good S/N ratio for subsequent data analysis and are limited with regard to time resolution.

While in the past only bulk experimental techniques were available for studying molecular dynamics, recent experimental progress has enabled to study dynamical processes at the single molecule level. In particular, fluorescence microscopy has allowed to track molecular orientation in time. Here the merits of single molecule microscopy emerge in probing rotational dynamics in heterogeneous environments in a direct way. We found that even for the model of isotropic rotational diffusion the obtained correlation function decays non-exponentially. Motional anisotropy increases the deviation from exponentiality. If the RCFs calculated for the simple model of anisotropic rotational diffusion are parametrized by a stretched exponential, we find stretching parameters β ≥ 0.85 for physically reasonable values of the anisotropy δ between 0.3 and 3. From this we conclude that only β values smaller than roughly 0.85 can be taken as an indication for intrinsically non-exponential dynamics. Our findings suggest a careful interpretation of single molecule rotational correlation functions as obtained by polarization resolved microscopy.

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