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Rotational diffusion coefficients of a small, spherical subunit flexibly tethered to a larger sphere

A. Iniesta and J. García de la Torre*

Departamento de Química Física, Facultad de Ciencias Químicas y Matemáticas, Universidad de Murcia, E-30001 Murcia, Spain

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Abstract. The rotational diffusion coefficients of a small spherical particle, which is flexibly anchored to the surface of a much larger sphere, are calculated using the hydrodynamic theory of segmentally flexible particles. The model is intended for representing the rotational mobility of a small residue or chromophore in the surface of a globular macromolecule. The coefficients are found to be essentially independent, or to vary slowly with the relative dispositions of the spheres. They are also insensitive to the size ratio when this ratio is high enough. These findings support the use of an approximative treatment proposed by Wegener in which the small conformation dependence is averaged out. The resulting averages are tentatively used in the Lipari-Szabo model for restricted rotational diffusion in a cone. It is concluded that the rotational relaxation of the small sphere has three components: *(i)* a torsional rotation with the same diffusion coefficient as the free sphere; *(ii)* a perpendicular wobbling with a diffusion coefficient several (five in a typical case) times smaller; and *(iii)* an overall rotation of the whole macromolecule, that will appear in a much longer time scale if the two spheres have quite distinct sizes.

Key words: Rotational diffusion, tethered sphere, anisotropy decay, segmental flexibility

Introduction

The dynamics in very dilute solution of segmentally flexible macromolecules is a problem that has recently attracted much theoretical work (Harvey 1979; Harvey and Cheung 1980; Wegener 1980, 1982a, b; Wegener et al. 1980; García de la Torre et al. 1985). Although the relationship between rotational diffusion coefficients and the time decay of spectroscopic or electro-optical properties is not completely established, it is at least possible to calculate very reliable values, including hydrodynamic-interaction and volume effects (García de la Torre and Rodes 1983), of the rotational diffusion coefficients. From these coefficients, the time dependence of various properties like fluorescence anisotropy decay, can be estimated using the approximations of Wegener et al. (1980).

A particularly interesting situation arises when the experimentally detected property cames from a small, external part of the macromolecule that is flexibly anchored to the rest of it. For instance, the chromophore causing the fluorescence of a globular protein could be attached to the surface by means of a flexible connector which allows for independent rotations. In this case the anisotropy decay would reflect not only the global motions of the protein, but also the independent motions of the chromophore. If the size difference between the fluorescing group and the remainder is large enough, the two kinds of motion can be separated in the decay. The model studied in this paper, in which a small sphere is flexibly tethered to a larger one, is intended for representing such situations.

The hydrodynamic theory of rigid particles composed of spherical elements (for reviews see García de la Torre 1981; García de la Torre and Bloomfield 1981; Bloomfield 1985) was extended for segmentally flexible particles by including in the treatment the additional degrees of freedom corresponding to internal motions. There are two options for assigning the rotational degrees of freedom, proposed respectively by Wegener et al. (1980) and Harvey et al. (1983). In a recent work (García de la Torre et al. 1985) the treatment has been applied to a model, the segmentally flexible dumbbell, in which two spherical subunits are joined to a swivel by means of frictionless spacers. The model studied in this paper is a

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* To whom offprint requests should be sent

particular case of the swivel-jointed dumbbell: for one of the spheres the length of the spacer is zero and therefore the joint is on its surface.

In our previous paper the rotational coefficients were obtained in Harvey coordinates, to be next transformed to the representation of Wegener. The latter seems more suitable for the present model, since Wegener coordinates correspond to individual motions of the subunits. In the Theory section of this paper we show how the various Wegener coefficients can be obtained rather directly using a strategy recently proposed by Goldstein (1985). The results should obviously be the same as those obtained with the indirect procedure, but the formalism and the computational procedure are simpler. Application is made to cases with varying size ratios, and the tentative use of configuration-dependent rotational coefficients to predict decay curves is discussed.

Theory

For a system of two independent, free spheres, the description of its dynamics requires 12 degrees of freedom, which are the components of the linear velocities, $\boldsymbol{v}^{(1)}$ and $\boldsymbol{v}^{(2)}$, and angular velocities $\boldsymbol{\omega}^{(1)}$ and $\boldsymbol{\omega}^{(2)}$. Thus, the diffusion matrix, $\underset{\sim}{\boldsymbol{D}}'$, is of dimension 12×12 and depends on the vector joining the centers of the spheres,

$$\boldsymbol{r} = \boldsymbol{r}_1 - \boldsymbol{r}_2 \,, \tag{1}$$

where $\boldsymbol{r}_1$ and $\boldsymbol{r}_2$ are the position vectors of the spheres. $\underset{\sim}{\boldsymbol{D}}'$ depends also on the radii of the spheres, σ_1 and σ_2. The generalized velocity vector for two independent spheres has 12 components and can be written as

$$\boldsymbol{U}' = (\boldsymbol{v}^{(1)}, \boldsymbol{v}^{(2)}, \boldsymbol{\omega}^{(1)}, \boldsymbol{\omega}^{(2)})^T \,. \tag{2}$$

In Eq. (2) all the velocities are expressed in a common system of reference, and the superscript T indicates transposition of a row vector.

The hydrodynamic interaction between two spheres is a classical problem in hydrodynamics, and many approaches to $\underset{\sim}{\boldsymbol{D}}'$ are available. In the present study, the hydrodynamic interaction effect is strong since $\sigma_1 + \sigma_2$ is close to the distance between centers, r. Consequently, we have chosen for $\underset{\sim}{\boldsymbol{D}}'$ the series-expansion formulas of Reuland et al. (1978) that have been shown to give accurate results in such situations (García de la Torre and Rodes 1983).

Now, we turn to the particular case of the segmentally flexible dumbbell, depicted in Fig. 1 a, in which two spheres of radii σ_1 and σ_2 are joined, by means of frictionless connectors of length d_1 and d_2, to a point O that acts as a universal joint. The model has three translational degrees of freedom less than the system of two independent spheres. Then, the generalized velocity vector for the flexible dumbbell has nine components that can be chosen in a number of ways (Harvey et al. 1983). If $\boldsymbol{U}$ is the nine-dimensional velocity vector, the linear relationship between U and U' can be written as

$$\boldsymbol{U}' = \underset{\sim}{\boldsymbol{B}} \cdot \boldsymbol{U} \tag{3}$$

where $\underset{\sim}{\boldsymbol{B}}$ is a 12×9 transformation matrix which accounts for the constraints in the model. The diffusion tensor of the flexible dumbbell, $\underset{\sim}{\boldsymbol{D}}$, is 9×9. According to Goldstein (1985), it is obtained from $\underset{\sim}{\boldsymbol{D}}'$ as

$$\underset{\sim}{\boldsymbol{D}} = (\underset{\sim}{\boldsymbol{B}}^T \cdot \underset{\sim}{\boldsymbol{D}}'^{-1} \cdot \underset{\sim}{\boldsymbol{B}})^{-1} \,. \tag{4}$$

Three of the nine degrees of freedom correspond to translation of some point (the joint O, for instance) in the particle. We are interested in the other degrees of freedom which correspond to rotational motions. It is therefore useful to divide $\underset{\sim}{\boldsymbol{D}}$ into four blocks,

$$\underset{\sim}{\boldsymbol{D}} = \begin{pmatrix} \underset{\sim}{\boldsymbol{D}}_{tt} & \underset{\sim}{\boldsymbol{D}}_{tr} \\ \underset{\sim}{\boldsymbol{D}}_{rt} & \underset{\sim}{\boldsymbol{D}}_{rr} \end{pmatrix} . \tag{5}$$

The dimensions of $\underset{\sim}{\boldsymbol{D}}_{tt}$, $\underset{\sim}{\boldsymbol{D}}_{tr}$, $\underset{\sim}{\boldsymbol{D}}_{rt}$ and $\underset{\sim}{\boldsymbol{D}}_{rr}$ are 3×3, 3×6, 6×3 and 6×6, with $\underset{\sim}{\boldsymbol{D}}_{rt} = \underset{\sim}{\boldsymbol{D}}_{tr}^T$. $\underset{\sim}{\boldsymbol{D}}_{tt}$ and $\underset{\sim}{\boldsymbol{D}}_{rt}$ depend on the choice of the point to which translation is referred, but $\underset{\sim}{\boldsymbol{D}}_{rr}$ is independent.

Now we have to specify the components of $\boldsymbol{U}$. We have selected the coordinates of Wegener et al. (1980) who assigned independent Cartesian axes to each subunit as shown in Fig. 1 a. The translational velocity of point O, denoted as $\boldsymbol{v}$, is expressed, as well as $\boldsymbol{\omega}^{(1)}$, in the system of coordinates of subunit 1, while $\boldsymbol{\omega}^{(2)}$ is expressed in the system of coordinates of subunit 2. The nine components of U are

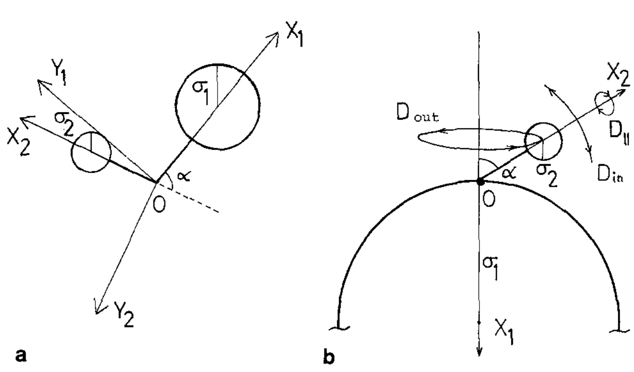

Fig. 1. **a** Segmentally flexible dumbbell, showing the two subunit fixed Cartesian systems of coordinates. Point O is the swivel-like universal joint. Axis x_i ($i = 1, 2$) is aligned with the connector; y_i lies in the instantaneous plane (z_1 and z_2 coincide). σ_i and d_i are, respectively, the radius of the subunit and the length of its connector. **b** Sphere tethered to a larger sphere as a particular case with $d_1 = 0$. The individual rotations of the small subunit corresponding to the Wegener coefficients $D_{\parallel}$, D_{out} and D_{in} are indicated

written explicitly as

$$U = (v_{x1}, v_{y1}, v_{z1}, \omega^{(1)}_{x1}, \omega^{(1)}_{y1}, \omega^{(1)}_{z1}, \omega^{(2)}_{x2}, \omega^{(2)}_{y2}, \omega^{(2)}_{z2})^T . \quad (6)$$

If the common system of reference needed to express the 12-dimensional generalized velocity vector $\boldsymbol{U}$ is the one attached to subunit 1, it is easy to show that the Goldstein transformation matrix is formed by the following 3×3 blocks:

$$\underset{\sim}{B} = \begin{pmatrix} \underset{\sim}{I} & -\underset{\sim}{U}_1 & \underset{\sim}{0} \\ \underset{\sim}{I} & \underset{\sim}{0} & -\underset{\sim}{A}\cdot\underset{\sim}{U}_1 \\ \underset{\sim}{0} & \underset{\sim}{I} & \underset{\sim}{0} \\ \underset{\sim}{0} & \underset{\sim}{0} & \underset{\sim}{A} \end{pmatrix} . \quad (7)$$

$\underset{\sim}{I}$ and $\underset{\sim}{0}$ are the identity and null 3×3 matrices, respectively. $\underset{\sim}{U}_i$ (not to be confused with the velocities $\boldsymbol{U}$ and $\boldsymbol{U}'$) is given by

$$\underset{\sim}{U}_i = \begin{pmatrix} 0 & -z_i & y_i \\ z_i & 0 & -x_i \\ -y_i & x_i & 0 \end{pmatrix} , \quad i = 1, 2 . \quad (8)$$

(x_i, y_i, z_i) are the coordinates of $\boldsymbol{r}_i$ in the system of coordinates of subunit i. Particularly for the present model, $x_i = \sigma_i + d_i$ and $y_i = z_i = 0$. $\underset{\sim}{A}$ is the matrix which transforms coordinates from system 2 to system 1,

$$\underset{\sim}{A} = \begin{pmatrix} -\cos\alpha & -\sin\alpha & 0 \\ \sin\alpha & \cos\alpha & 0 \\ 0 & 0 & 1 \end{pmatrix} . \quad (9)$$

The model considered in this paper consists of a spherical subunit tethered to a larger sphere, as indicated in Fig. 1 b. This is a particular case of the flexible dumbbell with $\sigma_2 < \sigma_1$ and $d_1 = 0$. We are interested in the diagonal components of D_{rr} belonging to subunit 2. In the notation of Wegener, they are

$$\begin{aligned} D_{\parallel} &= D^{4,4}_{rr} \\ D_{\text{in}} &= D^{5,5}_{rr} \\ D_{\text{out}} &= D^{6,6}_{rr} . \end{aligned} \quad (10)$$

$D_{\parallel}$ corresponds to rotation of subunit 2 around axis x_2. D_{in} is for rotation of subunit 2 around axis z, in the plane defined by axes x_1 and x_2. Finally, D_{out} corresponds to rotation out of that plane, with α fixed, around x_1. In these rotational motions, which are illustrated in Fig. 1 b, subunit 1 is at rest.

The computational procedure starts from the radii of the subunits and their coordinates (that depend on the value of α) expressed in the axes of subunit 1. Then $\underset{\sim}{D}'$ is evaluated using the series expansions of Reuland et al. (1978). Alternatively, hydrodynamic interaction can be neglected, and in such case $\underset{\sim}{D}'$ takes a very simple, diagonal form (see, for instance, García de la Torre et al. 1985). $\underset{\sim}{B}$ is constructed from Eqs. (7)–(9), and $\underset{\sim}{D}$ is calculated from Eq. (4). The lower diagonal block $\underset{\sim}{D}_{rr}$ is extracted according to Eq. (5) and its diagonal elements are identified with the rotational diffusion coefficients of Wegener (Eq. (10)). The procedure is repeated for various values of α.

Results and discussion

Assuming that in real cases the length of the connector may be of the same order as the size of the subunit, we have restricted the calculation to two cases with $\sigma_2 = d_2$ and $\sigma_2 = d_2/2$ respectively. The choice of σ_2 will be shown later to be quite irrelevant. For each case, the calculations were carried out with and without hydrodynamic interaction.

The size ratio σ_1/σ_2 was varied from 2 to 40. α was varied between 0 and the $\alpha_{\max}$ at which the two spheres touch each other in each case. It is convenient to give the values of $D_{\parallel}$, D_{in} and D_{out} normalized to the rotational diffusion coefficient of the smaller sphere if it were free,

$$D_{\text{free}} = \mathrm{kT}/(8\pi\eta_0\sigma_2^3) , \quad (11)$$

where kT is Boltzmann's factor and η_0 is the solvent viscosity.

Regarding $D_{\parallel}$, we found that in the whole ranges of σ_1/σ_2 and α, it was always very close to 1, going down to 0.98 at most for small σ_1/σ_2 and large α. Thus it does not seem necessary to present numerical values. This finding yields a first important conclusion of our study: the torsional diffusivity of the tethered sphere, represented by $D_{\parallel}$, is practically the same as the rotational diffusivity of the sphere free in solution. This conclusion can be helpful in the analysis of experimental data, although the other rotations do complicate such analysis, as we will describe later on.

In Tables 1 and 2 we present values for the normalized D_{in} and D_{out} coefficients for the $\sigma_2 = d_2$ case. The most remarkable trend shown by the results is that these coefficients depend rather weakly on conformation, i.e., on the value of α. This is particularly notable for D_{out} and, for both coefficients, when σ_1/σ_2 is large. Considering $\sigma_1/\sigma_2 = 10$ as a typical case, D_{in} and D_{out} for $\alpha \cong \alpha_{\max}$ are only 23% and 5%, respectively, lower than the value for $\alpha = 0$. In regard to the dependence with σ_1/σ_2 for α fixed, there is a shallow minimum at about $\sigma_1/\sigma_2 = 10$, and the values seem to converge to a constant for very high σ_1/σ_2. The results without hydrodynamic interaction show that for any α both D_{in} and D_{out} decrease monotonically with increasing σ_1/σ_2. The limiting value when $\sigma_1/\sigma_2 \to \infty$ of both coefficients coincide with the diffusion coefficient of sphere 2 rotating around point O fixed in space:

$$D_{\text{fixed}} = \mathrm{kT}/[8\pi\eta_0\sigma_2^3 + 6\pi\eta_0(d_2+\sigma_2)^2\sigma_2] . \quad (12)$$

Table 1. Values of D_{in}, normalized to D_{free} obtained for the small sphere in a model with $d_1 = 0$, $\sigma_2 = d_2$ using the series expansions of Reuland et al. (1978)

α	σ_1/σ_2				
	2	4	10	20	40
0.00	0.3112	0.2483	0.2288	0.2332	0.2395
5.00	0.3103	0.2477	0.2285	0.2328	0.2393
10.00	0.3073	0.2457	0.2273	0.2322	0.2390
15.00	0.3027	0.2425	0.2253	0.2310	0.2383
20.00	0.2962	0.2380	0.2227	0.2293	0.2375
25.00	0.2885	0.2323	0.2192	0.2273	0.2363
30.00	0.2795	0.2257	0.2150	0.2250	0.2350
35.00	0.2698	0.2178	0.2102	0.2222	0.2337
40.00	0.2593	0.2088	0.2047	0.2192	0.2320
45.00	0.2482	0.1988	0.1985	0.2158	0.2303
50.00	0.2367	0.1875	0.1918	0.2123	0.2287
55.00	0.2247	0.1748	0.1845	0.2087	0.2270
60.00	0.2120	0.1603	0.1765	0.2048	0.2252
65.00	0.1983	0.1435	–	–	–
70.00	0.1833	0.1237	–	–	–
75.00	0.1657	–	–	–	–
80.00	0.1440	–	–	–	–

Table 2. Values of D_{out}, normalized to D_{free} obtained for the small sphere in a model with $d_1 = 0$, $\sigma_2 = d_2$ using the series expansions of Reuland et al. (1978)

α	σ_1/σ_2				
	2	4	10	20	40
0.00	0.3113	0.2483	0.2288	0.2332	0.2395
5.00	0.3112	0.2482	0.2283	0.2330	0.2395
10.00	0.3108	0.2478	0.2285	0.2330	0.2393
15.00	0.3103	0.2473	0.2282	0.2328	0.2393
20.00	0.3097	0.2463	0.2278	0.2327	0.2393
25.00	0.3087	0.2452	0.2273	0.2323	0.2392
30.00	0.3075	0.2438	0.2265	0.2322	0.2392
35.00	0.3062	0.2420	0.2255	0.2317	0.2392
40.00	0.3043	0.2398	0.2243	0.2313	0.2390
45.00	0.3023	0.2372	0.2232	0.2308	0.2388
50.00	0.2998	0.2342	0.2217	0.2303	0.2372
55.00	0.2970	0.2305	0.2198	0.2297	0.2385
60.00	0.2935	0.2262	0.2180	0.2290	0.2383
65.00	0.2893	0.2210	–	–	–
70.00	0.2845	0.2147	–	–	–
75.00	0.2785	–	–	–	–
80.00	0.2710	–	–	–	–

Table 3. Values of $\bar{D}_{\text{in}}$, $\bar{D}_{\text{out}}$, $D_{\perp}$ and D_{fixed} in units of D_{free}

σ_2/d_2	σ_1/σ_2	With hydrodynamic interaction			Neglecting hydrodynamic interaction		
		$\bar{D}_{\text{in}}$	$\bar{D}_{\text{out}}$	$D_{\perp}$	$\bar{D}_{\text{in}}$	$\bar{D}_{\text{out}}$	$D_{\perp}$
1.	2	0.223	0.294	0.26	0.352	0.385	0.37
	4	0.186	0.233	0.21	0.308	0.332	0.32
	10	0.201	0.223	0.21	0.277	0.281	0.28
	20	0.217	0.231	0.22	0.266	0.263	0.26
	40	0.223	0.239	0.23	0.258	0.257	0.26
				$D_{\text{fixed}} = 0.25$			
2.	2	0.323	0.412	0.37	0.502	0.526	0.51
	4	0.296	0.343	0.32	0.449	0.460	0.45
	10	0.310	0.335	0.32	0.405	0.410	0.41
	20	0.338	0.347	0.34	0.390	0.392	0.39
	40	0.353	0.358	0.36	0.382	0.381	0.38
				$D_{\text{fixed}} = 0.37$			

The D_{in} and D_{out} coefficients must be considered as instantaneous values corresponding to a "frozen" conformation of the macromolecule. On the other hand, rotational dynamics are experimentally studied in the decay or relaxation of properties like fluorescence polarization or NMR. While the hydrodynamic theory used to calculate the coefficients for segmentally flexible macromolecules is well established, the theoretical link between the coefficients and the relaxation of properties has not been developed yet. Thus, one necessarily has recourse to approximations or reasonable assumptions, and therefore the following rationale is somewhat speculative.

We first employ a procedure proposed by Wegener et al. (1980) to reduce the α-dependent D_{out} and D_{in} to a single value. This dependence is first averaged out between $\alpha = 0$ and $\alpha = \alpha_{\max}$ with a $\sin\alpha$ weighting (see Eqs. (87) and (88) of García de la Torre et al. 1985). Such an approximation is acceptable for our results because of the weakness of that dependence. The resulting values $\bar{D}_{\text{out}}$ and $\bar{D}_{\text{in}}$ are quite close and Wegener et al. suggest taking their mean,

$$D_{\perp} = \tfrac{1}{2}\left(\bar{D}_{\text{out}} + \bar{D}_{\text{in}}\right) \tag{13}$$

as a measure of the rotational diffusivity in a perpendicular direction. Values of $\bar{D}_{\text{out}}$, $\bar{D}_{\text{in}}$ and $D_{\perp}$ are listed in Table 3 as functions of σ_1/σ_2 and for the two cases of σ_2/d_2. We see how the $D_{\perp}$ values with and without hydrodynamic interaction progressively approach each other and in the $\sigma_1/\sigma_2 \to \infty$ limit both coincide with D_{fixed}. In this limit, sphere 2 moves as if it were just anchored to point O, and the hydrodynamic interaction effect is unimportant. The values of $D_{\perp}$ with hydrodynamic interaction present a rather weak dependence on σ_1/σ_2, and in fact the approximation

$$D_{\perp} \cong D_{\text{fixed}} \tag{14}$$

works rather well: D_{fixed} overestimates $D_{\perp}$ by 20% at most, in the region of intermediate σ_1/σ_2. Indeed, Eq. (14) is a better approximation than using the σ_1/σ_2-dependent values of $D_{\perp}$ without hydrodynamic interaction. This conclusion can be very helpful in the interpretation of experimental results, since it allows the use of Eq. (12) for $D_\perp$, thus avoiding complex calculations of diffusion coefficients.

In the remainder of Wegener's procedure, if the perpendicular rotation of the subunit is unrestricted, the relaxations of properties are determined by at most three relaxation times, whose reciprocals are simple combinations of $D_\parallel$ and $D_\perp$ (Wegener et al. 1980; García de la Torre et al. 1985). In the system under study here, however, such rotation is clearly restricted sterically by the other bulky subunit. Then, the motion of the small subunit could be represented by the wobbling-in-a-cone model (Kinosita et al. 1977; Lipari and Szabo 1980, 1981; Szabo 1984) with $D_\perp$ playing the role of the wobbling coefficient (we implicitely assume that motion within the cone is completely free). If the dipole vectors pertinent to the observed property are along axis X_2, according to an oversimplified version of the cone theory the dynamics can be formulated in terms of two relaxation times, τ_{eff} and τ_M. τ_{eff} is the relaxation time for the wobbling motion, and can be approximated as (Lipari and Szabo 1980):

$$\tau_{\text{eff}} \cong 7\,\theta_0^2/24\,D_\perp\,. \tag{15}$$

θ_0 is the cone semiangle in radians. For our model θ_0 would be identified with $\alpha_{\max}$, which is about 60° for $\sigma_1/\sigma_2 > 4$, although somewhat smaller values can be expected in real cases. Taking, for instance $\sigma_1/\sigma_2 = 10$ and $\theta_0 \cong 45$, we obtain $\tau_{\text{eff}} \cong 5\,\tau_{\text{free}}$, where $\tau_{\text{free}} = 1/6\,D_{\text{free}}$. For other values of σ_2 and d_2, τ_{eff} can be estimated using Eqs. (12), (14) and (15).

If the subunit is appreciably smaller than the rest of the macromolecule, τ_M is the relaxation time for the overall rotation of the whole macromolecule,

$$\tau_M = 1/6\,D_M \tag{16}$$

where $D_M \cong \mathrm{kT}/(8\,\pi\,\eta_0\,\sigma_1^3)$, so that

$$D_M \cong (\sigma_2^3/\sigma_1^3)\,D_{\text{free}}\,. \tag{17}$$

For the case with $\sigma_1/\sigma_2 = 10$, $\tau_M = 10^3\,\tau_{\text{free}}$.

When the relevant dipoles are not coaxial with X_2, the theory is very complicated even in approximate versions (Lipari and Szabo 1980; Szabo 1984). An approximation for the anisotropy decay is (Gottlieb and Wahl 1963):

$$\begin{aligned} r(t) = {} & (A \exp(-t/\tau_\parallel^{(1)}) + B \exp(-t/\tau_\parallel^{(2)}) + C) \\ & \cdot (E \exp(-t/\tau_{\text{eff}}) + F) \exp(-t/\tau_M)\,, \end{aligned} \tag{18}$$

where A, B, C, E and F are constants related to the orientations of the dipoles, and

$$\begin{aligned} \tau_\parallel^{(1)} &= D_\parallel^{-1} \\ \tau_\parallel^{(2)} &= (4\,D_\parallel)^{-1}\,. \end{aligned} \tag{19}$$

As our results have shown, $D_\parallel \cong D_{\text{free}}$, and therefore

$$\begin{aligned} \tau_\parallel^{(1)} &\cong 6\,\tau_{\text{free}} \\ \tau_\parallel^{(2)} &\cong \tfrac{3}{2}\,\tau_{\text{free}}\,. \end{aligned} \tag{20}$$

Summarizing our tentative picture, the rotational diffusion of the small subunit tethered to a larger sphere is a superposition of relaxations with characteristic times $\tau^{(i)}$, $i = 1, 2$, τ_{eff} and τ_M such that

$$\tau_\parallel^{(i)} \lesssim \tau_{\text{eff}} \ll \tau_M \tag{21}$$

$\tau_\parallel{}^{(i)}$ and τ_{eff} are expected to be a few times greater than τ_{free} but of a close order of magnitude, and τ_M belong to a much longer time scale. Equation (18) can be used along with the results of this study (namely, Eqs. (14) and (20)) to predict anisotropy decays. Similar treatments can be given to other properties. This description seems to be simple enough to be compared with observations. From the theoretical side, we plan to check its validity by simulating the Brownian dynamics of the model using procedures similar to that of Harvey and Cheung (1980).

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