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Dynamics of the Orientational Factor in Fluorescence Resonance Energy Transfer

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Abstract

A one-dimensional diffusion equation is derived for the time evolution of the orientational factor, κ^2 , in the Förster energy transfer rate. The κ^2 -dependent diffusion coefficient is obtained in three different ways: (1) by requiring the κ^2 autocorrelation function, calculated using the κ^2 -diffusion equation, to be single exponential with the exact characteristic time; (2) by projecting the multidimensional diffusion equation for the transition dipoles onto κ^2 using the local equilibrium approximation; and (3) by requiring exact and approximate κ^2 trajectories to be as close as possible using a Bayesian approach. Within the framework of this simple theory, the distance dependence of the fluorescence resonance energy transfer (FRET) efficiency can be calculated for all values of the ratio of the rotational correlation time of the transition dipoles to the lifetime of the donor excited state. The theoretical predictions are compared to the exact values obtained from Brownian dynamics simulations of the reorientation of the donor and acceptor transition dipoles.

Graphical Abstract

INTRODUCTION

The dramatic advances in single-molecule methods during the last two decades have made it possible to study a remarkable array of biomolecular processes. Single-

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molecule fluorescence spectroscopy has proved particularly powerful as a probe of both structural and dynamic properties of macromolecules in solution. Fluorescence resonance energy transfer (FRET) spectroscopy exploits the strong, $1/r^6$ distance dependence of the energy transfer rate between an initially excited donor dye and an acceptor dye. However, in Förster theory, the rate of energy transfer, k_{ET} , between donor and acceptor dyes depends not only on the distance between them but also on their relative orientation. Specifically, when the geometry is fixed,

$$k_{ET} = \frac{3}{2} \kappa^2 k_D (R_0/r)^6 \quad (1)$$

where r is the distance between the dyes, R_0 is the Förster radius (for isotropic dye orientations, $\langle \kappa^2 \rangle = 2/3$), $(k_D)^{-1}$ is the fluorescence lifetime of an isolated donor, and κ^2 is the orientational factor defined in terms of unit vectors pointing along the donor (μ) and acceptor (ν) transition dipoles as

$$\kappa^2(\mu, \nu) = (\mu \cdot \nu - 3(\mu \cdot \hat{r})(\nu \cdot \hat{r}))^2 \quad (2)$$

where $\hat{r}$ is a unit vector pointing along a line connecting the centers of the two dyes.

The orientational factor κ^2 can take on values between 0 and 4. If the rotational dynamics of the two transition dipoles is unrestricted, then the average of κ^2 is $\langle \kappa^2 \rangle = 2/3$. When the dyes reorient on a time scale that is much faster than the fluorescence lifetime of the donor, then κ^2 in eq 1 can be replaced by its average value and the resulting energy transfer rate, $k_{ET} = k_D (R_0/r)^6$, is determined solely by the interdy distance, r . Now this distance can also fluctuate and the amplitude of such fluctuations is expected to be large when the dyes are attached to the ends of a disordered polymer such as a protein in the unfolded state.

Dynamics in condensed phase can often be described accurately as diffusion on the potential of mean force (which is proportional to the logarithm of the probability density of the interdy distance at equilibrium) of a suitably chosen collective variable. In particular, the dynamics of the interdy distance is commonly approximated^{1,2} as diffusion on the potential of mean force between the dyes. This is arguably the simplest possible description of the dynamics that is consistent with the exact equilibrium distribution since it involves only a single additional parameter, namely the diffusion constant of the interdy distance.

The purpose of this paper is to propose an analogous approximate description of the dynamics of the orientational factor, κ^2 . Specifically, we are interested in finding a diffusion equation for the time evolution of the probability density of κ^2 , $p(\kappa^2, t)$, of the form:

$$\frac{\partial p(\kappa^2, t)}{\partial t} = \frac{\partial}{\partial \kappa^2} D(\kappa^2) p_{eq}(\kappa^2) \frac{\partial}{\partial \kappa^2} \frac{p(\kappa^2, t)}{p_{eq}(\kappa^2)} \quad (3)$$

where $p_{\text{eq}}(\kappa^2)$ is the exact equilibrium probability distribution of κ^2 and $D(\kappa^2)$ is an orientation-factor-dependent diffusion coefficient. We shall obtain expressions for $D(\kappa^2)$ in three different ways. The first and simplest is to require that the time dependence of the autocorrelation function of the deviation of κ^2 from its equilibrium average, $\delta\kappa^2 = \kappa^2 - \langle\kappa^2\rangle$, calculated using eq 3, to be a single exponential with the exact relaxation time. The second is to project the multidimensional rotational diffusion equation for the time-dependent probability density $p(\boldsymbol{\mu}, \boldsymbol{\nu}, t)$ onto κ^2 using the local equilibrium approximation. This approximation is rigorously valid only when the dynamics “perpendicular” to κ^2 is much faster than the dynamics of κ^2 , **including motions of the solvent and the linkers connecting the dyes to macromolecules**. The corresponding autocorrelation function of κ^2 is guaranteed to be exact only at short times. The third choice is to find the $D(\kappa^2)$ that makes the exact and approximate κ^2 trajectories resemble each other as closely as possible.³ We will then test these approximations by examining the interdyer dependence of the fluorescence energy transfer efficiency when the dynamics of κ^2 is faster or slower than the fluorescence lifetime of the donors, or when the two are comparable.

THEORY

Dynamics of κ^2

Let us assume that the donor ($\boldsymbol{\mu}$) and acceptor ($\boldsymbol{\nu}$) unit transition dipoles diffuse freely and independently with the same rotational diffusion coefficient, D . Then the joint probability density $p = p(\boldsymbol{\mu}, \boldsymbol{\nu}, t)$ satisfies

$$\frac{\partial p}{\partial t} = D \left(\boldsymbol{\mu} \times \frac{\partial}{\partial \boldsymbol{\mu}} \cdot \boldsymbol{\mu} \times \frac{\partial}{\partial \boldsymbol{\mu}} + \boldsymbol{\nu} \times \frac{\partial}{\partial \boldsymbol{\nu}} \cdot \boldsymbol{\nu} \times \frac{\partial}{\partial \boldsymbol{\nu}} \right) p \quad (4)$$

in the notation of Edwards and Doi.⁴ The corresponding stochastic difference equations are

$$\boldsymbol{\mu}_{n+1} = (1 - 2D\delta t)\boldsymbol{\mu}_n + (2D\delta t)^{1/2}(\mathbf{g} - (\boldsymbol{\mu}_n \cdot \mathbf{g})\boldsymbol{\mu}_n) \quad (5)$$

and an analogous equation for $\boldsymbol{\nu}_{n+1}$. Here $\boldsymbol{\mu}_n$ is the unit transition dipole vector at time $n\delta t$ where δt is the time step. $\mathbf{g}$ is a random vector, $\mathbf{g} = g_1\mathbf{i} + g_2\mathbf{j} + g_3\mathbf{k}$. The g_n ($n = 1, 2, 3$) are uncorrelated Gaussian random numbers with zero mean and unit variance, and $\mathbf{i}, \mathbf{j}, \mathbf{k}$ are the orthonormal unit vectors defining the Euclidian coordinate system. As in standard Brownian dynamics algorithms,⁵ the update combines deterministic and stochastic displacements. Note that the deterministic force points along $-\boldsymbol{\mu}_n$, and the random force is perpendicular to $\boldsymbol{\mu}_n$. If $\boldsymbol{\mu}_n$ is a unit vector, then on the average $\boldsymbol{\mu}_{n+1} \cdot \boldsymbol{\mu}_{n+1} = 1 + 4(D\delta t)^2$. To ensure that $\boldsymbol{\mu}_{n+1}$ is precisely a unit vector, it should be normalized after each time step.

If we define the volume element $d\boldsymbol{\mu}$ (and analogously $d\boldsymbol{\nu}$) as $d\boldsymbol{\mu} = \sin \theta d\theta d\phi/4\pi$, where θ and ϕ are the polar and azimuthal angles of $\boldsymbol{\mu}$, then $\int d\boldsymbol{\mu} = \int d\boldsymbol{\nu} = 1$ and the normalized equilibrium probability density is equal to one, $p_{\text{eq}}(\boldsymbol{\mu}, \boldsymbol{\nu}) = 1$. The equilibrium probability density of κ^2 is defined as

$$p_{\text{eq}}(\kappa^2) = \iint d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}^\dagger \boldsymbol{\nu})) \quad (6)$$

where $\delta(x)$ is the Dirac delta function and $\kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})$ is given in eq 2. Remarkably, $p_{\text{eq}}(\kappa^2)$ can be found analytically.⁶ We will now obtain $p_{\text{eq}}(\kappa^2)$ in a way that can immediately be generalized to solve a problem that we will face later.

To evaluate the integral in eq 6, it is convenient to use the Steinberg representation⁷ of κ^2

$$\kappa^2 = (\boldsymbol{\nu} \cdot \hat{\boldsymbol{e}})^2 (1 + 3(\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}})^2) \quad (7)$$

where $\hat{\boldsymbol{e}}$ is a unit vector pointing along the electric field produced by the donor transition dipole, $\hat{\boldsymbol{e}} = (3\hat{\boldsymbol{r}}(\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}}) - \boldsymbol{\mu}) / (1 + 3(\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}})^2)^{1/2}$. Consequently

$$\begin{aligned} p_{\text{eq}}(\kappa^2) &= \int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - (\boldsymbol{\nu} \cdot \hat{\boldsymbol{e}})^2 (1 + 3(\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}})^2)) \quad (8) \\ &= \frac{1}{4} \int_0^\pi \sin\theta d\theta \int_0^\pi \sin\psi d\psi \delta(\kappa^2 - \cos^2\psi (1 + 3\cos^2\theta)) \\ &= \int_0^1 \int_0^1 dx dy \delta(\kappa^2 - y^2 (1 + 3x^2)) \end{aligned}$$

where $\psi(\theta)$ is the polar angle between $\boldsymbol{\nu}$ and $\hat{\boldsymbol{e}}$ ($\boldsymbol{\mu}$ and $\hat{\boldsymbol{r}}$). The last line was obtained after making the substitution $x = \boldsymbol{\mu} \cdot \hat{\boldsymbol{r}} = \cos\theta$ and $y = \boldsymbol{\nu} \cdot \hat{\boldsymbol{e}} = \cos\psi$. Our strategy is to evaluate the integral over y first. Using the identity $\delta(a(x-b)) = \delta(x-b)/a$, we have $\delta(\kappa^2 - y^2 (1 + 3x^2)) = \delta((1 + 3x^2)(\kappa^2/(1 + 3x^2) - y^2)) = \delta(\kappa^2/(1 + 3x^2) - y^2)(1 + 3x^2)^{-1}$. Now using the identity $\delta(a^2 - y^2) = \delta((a-y)(a+y)) = \delta(2a(a-y)) = \delta(a-y)/2a$, valid for $a, y \geq 0$, $p_{\text{eq}}(\kappa^2)$ in eq 8 can be written as

$$p_{\text{eq}}(\kappa^2) = \frac{1}{2\sqrt{\kappa^2}} \int_0^1 \frac{dx}{\sqrt{1 + 3x^2}} \int_0^1 dy \delta\left(\sqrt{\frac{\kappa^2}{1 + 3x^2}} - y\right) \quad (9)$$

When $\kappa^2 < 1$ then $\sqrt{\kappa^2/(1 + 3x^2)}$ is less than 1 for all values x and so

$$p_{\text{eq}}(\kappa^2) = \frac{1}{2\sqrt{\kappa^2}} \int_0^1 \frac{dx}{\sqrt{1 + 3x^2}} = \frac{\ln(2 + \sqrt{3})}{\sqrt{12}\kappa^2}, \quad \kappa^2 < 1 \quad (10)$$

However, when $1 < \kappa^2 \leq 4$, then $\sqrt{\kappa^2/(1 + 3x^2)} < 1$ only when $x > \sqrt{(\kappa^2 - 1)/3}$ and thus

$$p_{\text{eq}}(\kappa^2) = \frac{1}{2\sqrt{\kappa^2}} \int_{\sqrt{(\kappa^2-1)/3}}^1 \frac{dx}{\sqrt{1+3x^2}} \quad (11)$$

$$= \frac{1}{\sqrt{12\kappa^2}} \ln \left(\frac{2+\sqrt{3}}{\sqrt{\kappa^2} + \sqrt{\kappa^2-1}} \right), \quad 1 < \kappa^2 \leq 4$$

Note that when $\kappa^2 = 1$, eqs 10 and 11 give the same value for $p_{\text{eq}}(1)$. For completeness, we also give the cumulative distribution

$$P_{\text{eq}}(\kappa^2 < k) = \begin{cases} \ln(2+\sqrt{3})\sqrt{\frac{k}{3}} & 0 \leq k \leq 1 \\ \ln(2+\sqrt{3})\sqrt{\frac{k}{3}} + \frac{\sqrt{k-1} - \sqrt{k}\ln(\sqrt{k} + \sqrt{k-1})}{\sqrt{3}} & 1 < k \leq 4 \end{cases}$$

For future reference, we mention that the autocorrelation function of $\delta\kappa^2$ normalized so that it is unity at $t=0$ can be shown to be (see Appendix)

$$C(t) = \frac{\langle \delta\kappa^2(t)\delta\kappa^2(0) \rangle}{\langle (\delta\kappa^2)^2 \rangle} = \frac{19}{29}e^{-12Dt} + \frac{10}{29}e^{-6Dt} \quad (12)$$

The corresponding correlation time τ_c is the area under this function:

$$\tau_c = \int_0^\infty C(t)dt = \frac{13}{116D} \quad (13)$$

Determination of $D(\kappa^2)$

One of us⁸ tried to find a formula for $D(\kappa^2)$ by requiring the normalized autocorrelation function of $\delta\kappa^2$, calculated using eq 3, to be exact. Unfortunately, the final result for $D(\kappa^2)$ was based on the misconception that this correlation function was a single-exponential, $\exp(-12Dt)$, rather than the correct expression given in eq 12. Using such an approach, the best one can do is to find a κ^2 -dependent diffusion coefficient, which we denote here as $D_1(\kappa^2)$, that leads to an autocorrelation function equal to $\exp(-t/\tau_c)$, where τ_c is given by eq 13. A simple way of doing this is based on the observations⁹ that the autocorrelation functions of the left eigenfunctions of the diffusion operator are single exponentials with a decay rate equal to the corresponding eigenvalues, and that all crosscorrelation functions vanish. Thus all one has to do is to determine $D_1(\kappa^2)$ so that $\delta\kappa^2 = \kappa^2 - \langle \kappa^2 \rangle$ is the left eigenfunction of the diffusion operator in eq 3 with eigenvalue equal to $-1/\tau_c$:

$$\frac{1}{p_{\text{eq}}(\kappa^2)} \frac{\partial}{\partial \kappa^2} D_1(\kappa^2) p_{\text{eq}}(\kappa^2) \frac{\partial}{\partial \kappa^2} (\kappa^2 - \langle \kappa^2 \rangle) = -\frac{\kappa^2 - \langle \kappa^2 \rangle}{\tau_c}$$

or equivalently

$$\frac{\partial}{\partial \kappa^2} D_1(\kappa^2) p_{\text{eq}}(\kappa^2) = \frac{\langle \kappa^2 \rangle - \kappa^2}{\tau_c} p_{\text{eq}}(\kappa^2)$$

Integrating both sides, we have

$$D_1(\kappa^2) p_{\text{eq}}(\kappa^2) = \int_0^{\kappa^2} \frac{\langle k^2 \rangle - k^2}{\tau_c} p_{\text{eq}}(k^2) dk^2$$

Using eqs 10, 11, and 13, and evaluating the integral we find

$$D_1(\kappa^2) = \begin{cases} \frac{232}{39} D \kappa^2 (2 - \kappa^2), & 0 \leq \kappa^2 \leq 1 \\ \frac{232}{117} D \left(3\kappa^2 (2 - \kappa^2) + \frac{(4 - \kappa^2) \sqrt{\kappa^2 (\kappa^2 - 1)}}{\ln[(2 + \sqrt{3})(\sqrt{\kappa^2} - \sqrt{\kappa^2 - 1})]} \right), & 1 < \kappa^2 \leq 4 \end{cases} \quad (14)$$

The functional form of $D_1(\kappa^2)$ is shown in Figure 1.

The fact that $D(\kappa^2)$ vanishes at the two boundaries, $\kappa^2 = 0$ and 4, is not unexpected, in light of similar behavior in the case of simple rotational diffusion. Specifically, the transformation $x = \cos \theta$ converts the rotational diffusion equation $p(\theta, t)/t = (\sin \theta)^{-1} D_0 / \theta \sin \theta p(\theta, t)/\theta$ into $p(x, t)/t = 1/x D(x) p(x, t)/x$, $-1 \leq x \leq 1$, where the position-dependent diffusion coefficient, $D(x) = (1 - x^2)D_0$, also vanishes at the two boundaries.

Our second procedure is based on projecting the multidimensional rotational diffusion equation (eq 4) onto κ^2 by using the local equilibrium approximation. The fact that $p(\kappa^2, t)$ is exactly given by

$$p(\kappa^2, t) = \int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu} | \boldsymbol{\nu})) p(\boldsymbol{\mu}, \boldsymbol{\nu}, t) \quad (15)$$

suggests that we multiply both sides of eq 4 by $\delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu}))$ and integrate over all $\boldsymbol{\mu}$ and $\boldsymbol{\nu}$. Because of the symmetry between $\boldsymbol{\mu}$ and $\boldsymbol{\nu}$, we have

$$\frac{\partial p(\kappa^2, t)}{\partial t} = 2D \int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu} | \boldsymbol{\nu})) \boldsymbol{\nu} \times \frac{\partial}{\partial \boldsymbol{\nu}} \cdot \boldsymbol{\nu} \times \frac{\partial}{\partial \boldsymbol{\nu}} p(\boldsymbol{\mu}, \boldsymbol{\nu}, t)$$

Integrating by parts and using the identity

$$\boldsymbol{\nu} \times \frac{\partial}{\partial \boldsymbol{\nu}} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})) = -\frac{d}{d\kappa^2} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})) \boldsymbol{\nu} \times \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}}$$

we have

$$\frac{\partial p(\kappa^2, t)}{\partial t} = 2D \frac{d}{d\kappa^2} \int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}|\boldsymbol{\nu})) \boldsymbol{\nu} \times \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} \cdot \boldsymbol{\nu} \times \frac{\partial}{\partial \boldsymbol{\nu}} p(\boldsymbol{\mu}, \boldsymbol{\nu}, t)$$

which is exact but not closed. To close it we make the local equilibrium approximation that

$$\begin{aligned} p(\boldsymbol{\mu}, \boldsymbol{\nu}, t) &= p_{\text{eq}}(\boldsymbol{\mu}, \boldsymbol{\nu}) \frac{\int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}|\boldsymbol{\nu})) p(\boldsymbol{\mu}, \boldsymbol{\nu}, t)}{\int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}|\boldsymbol{\nu})) p_{\text{eq}}(\boldsymbol{\mu}, \boldsymbol{\nu})} \\ &= \frac{\int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}|\boldsymbol{\nu})) p(\boldsymbol{\mu}, \boldsymbol{\nu}, t)}{\int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}|\boldsymbol{\nu}))} \end{aligned}$$

Substituting this into eq 16, using the chain rule to carry out the differentiation with respect to $\boldsymbol{\nu}$, and the identities eqs 6 and 16, we note that the resulting equation can be written in the form of eq 3 if we define

$$D_2(\kappa^2) p_{\text{eq}}(\kappa^2) = 2D \int \int d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}|\boldsymbol{\nu})) \boldsymbol{\nu} \times \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} \cdot \boldsymbol{\nu} \times \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} \quad (16)$$

Now using the identity $(\boldsymbol{a} \times \boldsymbol{b}) \cdot (\boldsymbol{c} \times \boldsymbol{d}) = (\boldsymbol{a} \cdot \boldsymbol{c})(\boldsymbol{b} \cdot \boldsymbol{d}) - (\boldsymbol{a} \cdot \boldsymbol{d})(\boldsymbol{b} \cdot \boldsymbol{c})$ we have

$$\boldsymbol{\nu} \times \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} \cdot \boldsymbol{\nu} \times \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} = \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} \cdot \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} - \left(\boldsymbol{\nu} \cdot \frac{\partial \kappa^2}{\partial \boldsymbol{\nu}} \right)^2$$

Differentiating eq 2 with respect to κ^2 , one finds that $\partial \kappa^2 / \partial \boldsymbol{\nu} = \pm 2\sqrt{\kappa^2}(\boldsymbol{\mu} - 3\hat{\boldsymbol{r}}(\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}}))$. Thus $\boldsymbol{\nu} \cdot \kappa^2 / \boldsymbol{\nu} = 2\kappa^2$ and $\partial \kappa^2 / \partial \boldsymbol{\nu} \cdot \partial \kappa^2 / \partial \boldsymbol{\nu} = 4\kappa^2(1 + 3(\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}})^2)$. Combining all this and using eq 6, we find

$$D_2(\kappa^2) = 8D\kappa^2(1 - \kappa^2 + I/p_{\text{eq}}(\kappa^2)) \quad (17)$$

where we have defined

$$I = 3 \int \int d\boldsymbol{\mu} d\boldsymbol{\nu} (\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}})^2 \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu}))$$

If we use the Steinberg representation of κ^2 in eq 7, it can be seen that all we have to do to evaluate the above integral is to add a factor x^2 to the integrals in eqs 10 and 11 since $x = \boldsymbol{\mu} \cdot \hat{\boldsymbol{r}}$. Thus

$$I = \frac{3}{2\sqrt{\kappa^2}} \int_{f(\kappa^2)}^1 \frac{x^2 dx}{\sqrt{1 + 3x^2}},$$

where $f(\kappa^2) = 0$ for $0 \leq \kappa^2 < 1$ and $f(\kappa^2) = \sqrt{(\kappa^2 - 1)/3}$ for $1 \leq \kappa^2 \leq 4$. Evaluating these integrals, it follows from eq 17 that

$$D_2(\kappa^2) = \begin{cases} 4D\kappa^2 \left(1 - 2\kappa^2 + \frac{2\sqrt{3}}{\ln(2 + \sqrt{3})} \right), & 0 \leq \kappa^2 \leq 1 \\ 4D\kappa^2 \left(1 - 2\kappa^2 + \frac{2\sqrt{3} - \sqrt{\kappa^2(\kappa^2 - 1)}}{\ln[(2 + \sqrt{3})(\sqrt{\kappa^2} - \sqrt{\kappa^2 - 1})]} \right), & 1 \leq \kappa^2 \leq 4 \end{cases} \quad (18)$$

$D_2(\kappa^2)$ is compared to $D_1(\kappa^2)$ in Figure 1. The two κ^2 -dependent diffusion coefficients agree quite closely, exhibiting in particular similar singular behavior at $\kappa^2 = 0, 1$, and 4 .

Numerical Solution of the Diffusion Equation

To solve eq 3, we discretize space by dividing the interval $0 \leq \kappa^2 \leq 4$ into M bins of size $\Delta = 4/M$. We define the grid points as $\kappa_i^2 = (i - 1/2)\Delta$, $i = 1, 2, \dots, M$, so that an integral of the form $I = \int_0^4 f(\kappa^2) d\kappa^2$ can be evaluated using the midpoint rule (i.e., $I = \sum_{i=1}^M f(\kappa_i^2) \Delta$). (Here we set M a multiple of four, so that the singular point $\kappa^2 = 1$ falls in the middle of two grid points.) By discretizing the diffusion operator using the theory of finite differences one can reduce the solution of the differential equation 3 to a set of rate equations,

$$\frac{d\mathbf{p}}{dt} = \mathbf{K}\mathbf{p}$$

where $\mathbf{p}$ is a vector with elements $p_i = p(\kappa_i^2, t)$. $\mathbf{K}$ is an $M \times M$ matrix with elements,⁸

$$K_{i \pm 1, i} = \frac{D(\kappa_{i \pm 1/2}^2) p_{\text{eq}}(\kappa_{i \pm 1/2}^2)}{p_{\text{eq}}(\kappa_i^2) \Delta^2} \quad (19)$$

with $K_{01} = K_{M+1, M} = 0$ and $K_{ii} = -K_{i-1, i} - K_{i+1, i}$. All other elements are zero. One way of obtaining this relation is to start with Kramers' formula for the rate,

$$k_{\text{Kramers}} = \left[\left(\int_{\cup} p_{\text{eq}}(x) dx \right) \left(\int_{\cap} \frac{dx}{D(x) p_{\text{eq}}(x)} \right) \right]^{-1}$$

and evaluate the integral over the “well” (i.e., site i) by using the midpoint rule, $\int_{\cup} p_{\text{eq}}(x) dx = p_{\text{eq}}(x_i) \Delta$. For the “dividing surface” in the middle of two adjacent sites, we have $\int_{\cap} (D(x) p_{\text{eq}}(x))^{-1} dx = (D(x_{i \pm 1/2}) p_{\text{eq}}(x_{i \pm 1/2}))^{-1} \Delta$. (We note that the discretization of Bicout and Szabo¹⁰ is recovered if D at the half-point is approximated by the algebraic mean, and p_{eq} by the geometric mean.) The autocorrelation function of κ^2 is obtained as

$$\begin{aligned}\langle \kappa^2(t) \kappa^2(0) \rangle &= \sum_{i,j} \kappa_i^2 p(\kappa_i^2, t | \kappa_j^2, 0) \kappa_j^2 p_{\text{eq}}(\kappa_i^2) \Delta \\ &= \sum_{i,j} \kappa_i^2 (\exp \mathbf{K} t)_{ij} \kappa_j^2 p_{\text{eq}}(\kappa_i^2) \Delta\end{aligned}$$

where $p(\kappa_i^2, t | \kappa_j^2, 0)$ is the probability of being at grid point i at time t , having started at grid point j at time $t = 0$.

A potential problem here is that $p_{\text{eq}}(\kappa^2)$ has an integrable singularity at $\kappa^2 = 0$. The simplest way of handling this is to replace $p_{\text{eq}}(\kappa_i^2) \Delta$ in eq 19 by $\int_{\kappa_i^2 - \Delta/2}^{\kappa_i^2 + \Delta/2} p_{\text{eq}}(\kappa^2) d\kappa^2$. Alternately, a variable transformation that removes this singularity is $x = \sqrt{\kappa^2}$. The corresponding distribution functions are $p'(x, t) = 2x p(\kappa^2 = x^2, t)$, and the diffusion coefficient of x is $D'(x) = D(\kappa^2 = x^2)/(4x^2)$.

Reorientational Dynamics and FRET Efficiency

In the absence of dynamics (κ^2 and r are fixed), the donor fluorescence intensity, $I(t)$, following a δ -function excitation is

$$I(t)/I(0) = \exp(-(k_D + k_{\text{ET}})t) \quad (20)$$

The FRET efficiency E is the probability that the excited donor decays by transferring its energy to the acceptor rather than returning to the ground state,

$$E = \frac{k_{\text{ET}}}{k_D + k_{\text{ET}}} = 1 - k_D \tau_{\text{DA}} \quad (21)$$

where $\tau_{\text{DA}} = (k_D + k_{\text{ET}})^{-1}$ is the donor lifetime in the presence of the acceptor. When the energy transfer rate, $k_{\text{ET}}(t)$, fluctuates with time t , the donor fluorescence intensity can be written as a path integral,

$$I(t)/I(0) = e^{-k_D t} \left\langle e^{-\int_0^t k_{\text{ET}}(\tau) d\tau} \right\rangle \quad (22)$$

where the average is over all trajectories starting from the equilibrium distribution. Now the FRET efficiency, which can be thought of as a splitting or commitment probability, can be found by integrating the flux through the energy transfer channel over time from zero to infinity,

$$\begin{aligned}
 E &= \int_0^\infty e^{-k_D t} \left\langle k_{ET}(t) e^{-\int_0^t k_{ET}(\tau) d\tau} \right\rangle dt \\
 &= - \int_0^\infty e^{-k_D t} \frac{d}{dt} \left\langle e^{-\int_0^t k_{ET}(\tau) d\tau} \right\rangle dt
 \end{aligned}$$

Integrating by parts, E can be written in the form of eq 21 with

$$\tau_{DA} = \int_0^\infty e^{-k_D t} \left\langle e^{-\int_0^t k_{ET}(\tau) d\tau} \right\rangle dt \quad (23)$$

This was first derived by Makarov and Plaxco.¹¹ When k_{ET} is a constant, this reduces to the result given in eq 21. This expression provides a simple way of calculating the FRET efficiency from a simulated trajectory.¹² It should be noted that τ_{DA} is the excited-state lifetime of the donor in the presence of the acceptor. In general, τ_{DA} is not equal to the fluorescence lifetime, which is the lifetime on condition that the donor decays by emitting a photon rather than by energy transfer.¹³

Let us now assume that r is fixed and it is only κ^2 that fluctuates. The simple formulas for the FRET efficiency can be found if the dynamics is much faster or much slower than the donor fluorescence lifetime, $(k_D)^{-1}$. When it is much faster, all we have to do is replace κ^2 in eq 1 by its average and use the result in eq 8. In this way, we obtain the familiar result

$$E = \frac{R_0^6}{R_0^6 + r^6} \quad (24)$$

On the other hand, when the fluctuations of κ^2 are very slow, then we use eq 1 in eq 21 and average over the equilibrium distribution of κ^2 ,

$$E = \int_0^4 p_{eq}(\kappa^2) \frac{3\kappa^2/2 d\kappa^2}{3\kappa^2/2 + (r/R_0)^6}$$

In the intermediate regime, one has to evaluate the path average in eq 23. If we assume that the dynamics of κ^2 is determined by eq 3, then⁸ using the Feynman-Kac theorem

$$E = 1 - k_D \int_0^\infty dt \int_0^4 d\kappa^2 p(\kappa^2, t) \quad (25)$$

where

$$\frac{\partial p(\kappa^2, t)}{\partial t} = \frac{\partial}{\partial \kappa^2} D(\kappa^2) p_{\text{eq}}(\kappa^2) \frac{\partial}{\partial \kappa^2} \frac{p(\kappa^2, t)}{p_{\text{eq}}(\kappa^2)} - k_D \left(1 + \frac{3}{2} \kappa^2 \left(\frac{R_0}{r} \right)^6 \right) p(\kappa^2, t)$$

with initial condition $p(\kappa^2, 0) = p_{\text{eq}}(\kappa^2)$. In discretized form, this diffusion equation with a sink term can be written as

$$\frac{\partial \mathbf{p}}{\partial t} = (\mathbf{K} - \mathbf{S})\mathbf{p}$$

where $\mathbf{S}$ is a diagonal matrix with elements $S_{ii} = \kappa_D (1 + \frac{3}{2} \kappa_i^2 (R_0/r)^6)$. Solving this equation, we have $\mathbf{p}(t) = \exp((\mathbf{K} - \mathbf{S})t) \mathbf{p}_{\text{eq}}$, where $\mathbf{p}_{\text{eq}}$ is a column vector with elements $(p_{\text{eq}})_i = p_{\text{eq}}(\kappa_i^2) / \sum_j p_{\text{eq}}(\kappa_j^2)$. Substituting this expression for $\mathbf{p}(t)$ into eq 25, carrying out the integral over time, and using the midpoint rule to evaluate the integral over κ^2 , we have

$$E = 1 - k_D \mathbf{1}^T (\mathbf{S} - \mathbf{K})^{-1} \mathbf{p}_{\text{eq}} \quad (26)$$

where $\mathbf{1}^T$ is a row vector with all elements equal to unity, in agreement with eq 117 of ref 8.

RESULTS AND DISCUSSION

Autocorrelation Function.

Figure 2 compares the exact analytical expression for the normalized autocorrelation function $C(t)$ in eq 12 to the predictions for diffusion along κ^2 with diffusion coefficients $D_1(\kappa^2)$ and $D_2(\kappa^2)$. For $D_2(\kappa^2)$, we calculated the correlation function by solving the discretized diffusion equation on a grid with 256 equally spaced points in $0 < \kappa^2 < 4$. We find that $D_2(\kappa^2)$ gives the right short-time behavior, but deviates somewhat for times $t \gtrsim \tau_c$. $D_1(\kappa^2)$ gives a more balanced approximation, producing a $C(t)$ that decays a bit too slowly at short, and a bit too fast at long times. Also shown is the result for $C(t)$ calculated directly from Brownian dynamics simulations using the integrator eq 5 with a timestep $D\delta t = 0.001 \approx 0.01 \tau_c$. We find that simulations and theory agree well.

Comparison to Numerically Estimated $D(\kappa^2)$

A third way of obtaining $D_3(\kappa^2)$ uses exact $\kappa^2(t)$ trajectories as input (see inset in Figure 1), and then attempts to match them as closely as possible by a one-dimensional diffusion process on the potential of mean force, $-\ln p_{\text{eq}}(\kappa^2)$, with a κ^2 -dependent diffusion coefficient. To obtain $D_3(\kappa^2)$, we use Bayesian inference.³ The likelihood function entering the Bayesian posterior requires the calculation of propagators (Green's functions) $p(\kappa_i^2, t | \kappa_j^2, 0)$. This propagator is calculated for the discretized diffusion equation using spectral methods.¹⁰ The values of the diffusion coefficient at half-grid points, $D(\kappa_{\pm 1/2}^2)$, and

of the equilibrium populations, $p_{\text{eq}}(\kappa_i^2)$, are then obtained by sampling over the posterior, using uniform priors on $\ln D(\kappa^2_{\pm 1/2})$ and $p_{\text{eq}}(\kappa_i^2)$.

Figure 1 shows the results of the Bayesian inference approach as filled symbols, obtained for a lag time of $D t = 0.01$, where D is the rotational diffusion coefficient. We find that for this relatively short time, about $0.1 \tau_c$, the Bayesian estimates agrees closely with $D_2(\kappa^2)$. The only exception is the region immediately around the singular point $\kappa^2 = 1$, where the Bayesian estimate is above (below) $D_2(\kappa^2)$ just below (above) $\kappa^2 = 1$. At a longer lag time $D t = 0.1 \approx \tau_c$, the diffusion coefficient from Bayesian inference moves somewhat closer to $D_1(\kappa^2)$ (see Figure 1), as would be expected, since $D_1(\kappa^2)$ gives the correlation time correctly. However, at the singularity $\kappa^2 = 1$, the discretized $D_3(\kappa^2)$ appears to become discontinuous, rising nearly linearly for $\kappa^2 < 1$, dropping sharply at $\kappa^2 = 1$, and then becoming approximately parabolic in κ^2 for $1 < \kappa^2 < 4$. A reason for this apparent discontinuity in $D_3(\kappa^2)$ sampled on a grid emerges from the following comparison of the calculated and simulated propagators.

Propagators.

Figure 3 shows the propagator $p(\kappa^2, t | \kappa_0^2, 0)$ for different κ_0^2 from Brownian dynamics simulation and from solutions of the diffusion equation with different $D(\kappa^2)$. For the lag time of $D t = 0.1 \approx \tau_c$, we find that one-dimensional diffusion with $D_1(\kappa^2)$ or $D_2(\kappa^2)$ produces overall accurate distributions of κ^2 . In particular, if the starting point κ_0^2 is in the more populated region $\kappa^2 < 1$, the predictions for the distribution of positions κ^2 after the lag time matches the simulation result very well. For starting points $\kappa_0^2 \gtrsim 2$, a region with less than 10 % population at equilibrium, we notice some deviations between prediction and simulation results. In particular, the one-dimensional diffusion models predict a cusp-like buildup of probability density at $\kappa^2 = 1$ that is not seen in the simulation results. It is this feature that the Bayesian fit in Figure 1 at the corresponding lag time attempts to correct. Its comparably large $D_3(\kappa^2)$ for $\kappa^2 \lesssim 1$ and small $D_3(\kappa^2)$ for $\kappa^2 \gtrsim 1$ partly prevent the probability density from piling up around κ^2 for $\kappa_0^2 > 1$.

Influence of the Reorientational Dynamics on the FRET Efficiency.

In Figure 4, we plot the mean FRET efficiency E vs r/R_0 for different values of the ratio of the rotational correlation time, $\tau_r = (6D)^{-1}$, to the lifetime $\tau_D = 1/k_D$ of the donor in the absence of acceptor, $\tau_r/\tau_D = k_D/(6D) = 0, 0.2, 1, 5, \infty$. For intermediate values of this ratio, we calculated E by using eq 26 with 256 grid points for diffusion coefficients $D_1(\kappa^2)$ and $D_2(\kappa^2)$. Also shown are reference values obtained from Brownian dynamics simulations using the path integral eq 23. We find that diffusion with $D_1(\kappa^2)$ and $D_2(\kappa^2)$ give nearly indistinguishable FRET efficiencies that are both in excellent agreement with the “exact” values obtained from the path integral.

The magnitude of the dynamic correction in turning FRET efficiencies E into estimates of the dye distances r/R_0 is largest for large E (small r/R_0). When the rotational correlation time is equal to the fluorescence lifetime, $\tau_r/\tau_D = 1$, the actual interdy distance and the distance

inferred from the FRET efficiency E without dynamic correction, eq 24, differ by about 5 % for $E = 0.5$, and by about 10 % for $E = 0.75$. At the most extreme, $\tau_r/\tau_D = \infty$, these differences double. For precision estimates of interdye distances in the determination of molecular structures from FRET measurements,^{14–18} it is thus important to quantify and, if possible, correct for dynamic effects.

CONCLUDING REMARKS

We studied dynamic corrections to the FRET efficiency using diffusion models. One-dimensional diffusion equations for the time evolution of the orientational factor, κ^2 , in the Förster energy transfer rate, k_{ET} , were found to capture the dynamics obtained from Brownian dynamics simulations of the multidimensional orientational motion. The effective diffusion coefficient entering the diffusion equation depends strongly on κ^2 , vanishing at the extremes of $\kappa^2 = 0$ and 4, and exhibiting singular behavior at $\kappa^2 = 1$. Nonetheless, since $p_{eq}(\kappa^2)$ is equally singular, and $p_{eq}(\kappa^2)D(\kappa^2)$ is once differentiable for our two analytic estimates of the diffusion coefficient, the autocorrelation function of κ^2 is well-behaved. Indeed, $D_1(\kappa^2)$ was derived precisely so that the κ^2 autocorrelation function is single-exponential with the exact correlation time. By contrast, $D_2(\kappa^2)$ was obtained under the local equilibrium approximation, and gives the exact autocorrelation function in the limit of small times.

We find that dynamics can affect estimates of interdye distances by 10-20 % in relevant regimes even when the average $\langle \kappa^2 \rangle$ is exactly 2/3, the value expected for isotropic orientational distributions. Just as one-dimensional diffusion of pair distances provides an approximate yet powerful description of conformational dynamics in molecular systems,¹ the magnitude of the dynamic corrections to the FRET efficiency can be estimated from one-dimensional diffusion in the space of κ^2 using the models presented here.

Experimentally, time-resolved fluorescence anisotropy decay measurements (ref?) can be used to estimate the rotational diffusion coefficient D . For isotropic rotational diffusion, the anisotropy decays exponentially as $\exp(-6Dt)$ (ref?). The ratio between the corresponding rotational correlation time, $\tau_r = 1/(6D)$, and the donor lifetime, $\tau_D = 1/k_D$, determines the effects of orientational dynamics, as shown in Figure 4. This ratio covers a wide range, and situations can arise where the two times are comparable, in the 1-nanosecond range at room temperature (ref?). In such cases, turning the FRET signal into accurate distances requires modeling also of the dye orientational dynamics. Similar considerations apply to interdye translational motions on the time-scale of the fluorescence lifetime.

In complex systems, the modeling of FRET from molecular dynamics simulations is non-trivial^{12,16–20} since the simulated dynamics is statistically noisy and the sampling of orientational and translational space may often be incomplete. Therefore, even if one has access to molecular dynamics trajectories of systems with attached dyes, it may be advantageous to regularize the problem by first constructing a low-dimensional diffusion model (e.g., by using the maximum-likelihood or Bayesian formalism in refs 3 and 21) and

then use this model in a second step to evaluate the dynamic corrections to the FRET efficiency.

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APPENDIX:: AUTOCORRELATION FUNCTION OF κ^2

The orientational factor κ^2 can be expressed as sums and products of second-order spherical harmonics involving angles of μ and ν . For a single isotropic rotor the autocorrelation function of each second-order spherical harmonic is a single exponential with relaxation time $(6D)^{-1}$. Thus the normalized autocorrelation function $C(t) = \langle \delta\kappa^2(t)\delta\kappa^2(0) \rangle / \langle (\delta\kappa^2)^2 \rangle$ in eq 12 can have only two relaxation times, $(6D)^{-1}$ and $(12D)^{-1}$. Now since by construction $C(0) = 1$ and $C(\infty) = 0$, it follows that

$$C(t) = Ae^{-12Dt} + (1 - A)e^{-6Dt} \quad (.27)$$

where the constant A remains to be determined. To find A , we need to know a single property of $C(t)$ exactly. Below we will calculate the time derivative of $C(t)$ at $t = 0$, $\dot{C}(0)$, and then determine A from the time derivative of eq .27 at $t = 0$, i.e., $\dot{C}(0) = -(6 + 6A)D$.

By definition,

$$\langle \kappa^2(t)\kappa^2(0) \rangle = \iint d\mu d\nu d\mu_0 d\nu_0 \kappa^2(\mu, \nu) p(\mu, \nu, t | \mu_0, \nu_0, 0) \kappa^2(\mu_0, \nu_0)$$

since $p_{eq}(\mu, \nu) = 1$. Here the propagator of Green's function is a solution of the multidimensional rotation diffusion equation, eq 4, subject to the initial condition $p(\mu, \nu, 0 | \mu_0, \nu_0, 0) = \delta(\mu - \mu_0)\delta(\nu - \nu_0)$. Differentiating both sides of the preceding equation with respect to time, and using the fact that the propagator satisfies eq 4, we have

$$\frac{d\langle \kappa^2(t)\kappa^2(0) \rangle}{dt} = D \iint d\mu d\nu d\mu_0 d\nu_0 \kappa^2(\mu, \nu) \left(\mu \times \frac{\partial}{\partial \mu} \cdot \mu \times \frac{\partial}{\partial \mu} + \nu \times \frac{\partial}{\partial \nu} \cdot \nu \times \frac{\partial}{\partial \nu} \right) \quad (.28)$$

$$* p(\mu, \nu, t | \mu_0, \nu_0, 0) \kappa^2(\mu_0, \nu_0)$$

[GH: added D in front of integral. What should we do about the line continuation?] Since at $t = 0$, $p(\mu, \nu, 0 | \mu_0, \nu_0, 0) = \delta(\mu - \mu_0)\delta(\nu - \nu_0)$, we can immediately carry out the integration over μ_0 and ν_0 , and thus find

$$\left. \frac{d\langle \kappa^2(t)\kappa^2(0) \rangle}{dt} \right|_{t=0} = 2D \iint d\boldsymbol{\mu} d\boldsymbol{\nu} \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu}) \left(\boldsymbol{\mu} \times \frac{\partial}{\partial \boldsymbol{\mu}} \cdot \boldsymbol{\mu} \times \frac{\partial}{\partial \boldsymbol{\mu}} \right) \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})$$

where we have exploited the symmetry between $\boldsymbol{\mu}$ and $\boldsymbol{\nu}$ (and hence the factor of 2).

Integrating by parts, we have

$$\begin{aligned} \left. \frac{d\langle \kappa^2(t)\kappa^2(0) \rangle}{dt} \right|_{t=0} &= 2D \iint d\boldsymbol{\mu} d\boldsymbol{\nu} \left(\boldsymbol{\mu} \times \frac{\partial \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})}{\partial \boldsymbol{\mu}} \right) \cdot \left(\boldsymbol{\mu} \times \frac{\partial \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})}{\partial \boldsymbol{\mu}} \right) \\ &= -2D \int_0^4 d\kappa^2 \iint d\boldsymbol{\mu} d\boldsymbol{\nu} \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})) \left(\boldsymbol{\mu} \times \frac{\partial \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})}{\partial \boldsymbol{\mu}} \right) \cdot \left(\boldsymbol{\mu} \times \frac{\partial \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})}{\partial \boldsymbol{\mu}} \right) \end{aligned} \quad (.29)$$

where to get the last line we simply inverted the identity $\int_0^4 d\kappa^2 \delta(\kappa^2 - \kappa^2(\boldsymbol{\mu}, \boldsymbol{\nu})) = 1$ under the integral sign. Using eq 16 of the main text, this can be written as

$$\begin{aligned} \left. \frac{d\langle \kappa^2(t)\kappa^2(0) \rangle}{dt} \right|_{t=0} &= - \int_0^4 d\kappa^2 D_2(\kappa^2) p_{\text{eq}}(\kappa^2) \quad (.30) \\ &= - \frac{128D}{25} \end{aligned}$$

[GH: I added D in the last line] where in the last step we evaluated the integral over κ^2 for $D_2(\kappa^2)$ in eq 17 and $p_{\text{eq}}(\kappa^2)$ in eqs 10 and 11. Since $\langle (\delta\kappa^2)^2 \rangle = \langle \kappa^4 \rangle - \langle \kappa^2 \rangle^2$, and $\langle \kappa^4 \rangle = 24/25$ and $\langle \kappa^2 \rangle^2 = 2/3$, it follows from eq .30 that $\dot{C}(0) = -288D/29$ and consequently that $A = 19/29$, in agreement with eq 12 of the main text.

To provide an alternate, perhaps more direct derivation, it is useful to imagine that $\boldsymbol{\mu}$ and $\boldsymbol{\nu}$ have different rotational diffusion coefficients, D_μ and D_ν , respectively. We will need the following identities

$$\begin{aligned} \langle P_2(\boldsymbol{\mu}(t) \cdot \boldsymbol{\mu}(0)) \rangle &= e^{-6D_\mu t} \quad (.31) \\ \langle P_2(\boldsymbol{\nu}(t) \cdot \boldsymbol{\nu}(0)) \rangle &= e^{-6D_\nu t} \\ \langle P_2(\boldsymbol{\mu}(t) \cdot \boldsymbol{a}) P_2(\boldsymbol{\mu}(0) \cdot \boldsymbol{b}) \rangle &= \frac{1}{5} P_2(\boldsymbol{a} \cdot \boldsymbol{b}) \langle P_2(\boldsymbol{\mu}(t) \cdot \boldsymbol{\mu}(0)) \rangle \end{aligned}$$

where $\boldsymbol{a}, \boldsymbol{b}$ are unit vectors and $P_2(x) = (3x^2 - 1)/2$ is the second Legendre polynomial. Since κ^2 can be expressed in terms of second-order spherical harmonics, the normalized correlation function of $\delta\kappa^2$ must have the form

$$C(t) = A \langle P_2(\boldsymbol{\mu}(t) \cdot \boldsymbol{\mu}(0)) \rangle \langle P_2(\boldsymbol{\nu}(t) \cdot \boldsymbol{\nu}(0)) \rangle + \frac{1-A}{2} \langle P_2(\boldsymbol{\mu}(t) \cdot \boldsymbol{\mu}(0)) \rangle + \langle P_2(\boldsymbol{\nu}(t) \cdot \boldsymbol{\nu}(0)) \rangle \quad (.32)$$

since $P_2(1) = 1$ and κ^2 is symmetric with respect to the interchange of $\boldsymbol{\mu}$ and $\boldsymbol{\nu}$. This is just the generalization of eq .27. Now in the limit that $D_{\nu} \rightarrow \infty$, $C(t)$ approaches

$$C_{\infty}(t) = \frac{1-A}{2} \langle P_2(\boldsymbol{\mu}(t) \cdot \boldsymbol{\mu}(0)) \rangle \quad (.33)$$

for $t > 0$. But $C_{\infty}(t)$ is nothing but the correlation function of $\delta\kappa^2$ averaged over all values of $\boldsymbol{\nu}$, $\overline{\delta\kappa^2} = \int \delta\kappa^2 d\boldsymbol{\nu}$, i.e.,

$$C_{\infty}(t) = \frac{\langle \overline{\delta\kappa^2(t)} \overline{\delta\kappa^2(0)} \rangle}{\langle (\overline{\delta\kappa^2})^2 \rangle}$$

Now using the identity

$$\int d\boldsymbol{\nu} (a | \boldsymbol{\nu})(b | \boldsymbol{\nu}) = \frac{a \cdot b}{3}$$

it is easy to show that

$$\overline{\delta\kappa^2} = \int \delta\kappa^2 d\boldsymbol{\nu} = \frac{2}{3} P_2(\boldsymbol{\mu} \cdot \hat{\boldsymbol{r}})$$

Thus

$$\begin{aligned} C_{\infty}(t) &= \frac{4}{9} \frac{\langle P_2(\boldsymbol{\mu}(t) \cdot \hat{\boldsymbol{r}}) P_2(\boldsymbol{\mu}(0) \cdot \hat{\boldsymbol{r}}) \rangle}{\langle (\overline{\delta\kappa^2})^2 \rangle} \\ &= \frac{4}{45} \frac{\langle P_2(\boldsymbol{\mu}(t) \cdot \boldsymbol{\mu}(0)) \rangle}{\langle (\overline{\delta\kappa^2})^2 \rangle} \end{aligned}$$

where we have used the last identity in eq .31. Comparing this with eq .32 it follows that $A = 10/19$ as before.

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Figure 1: Diffusion coefficient $D(\kappa^2)$ as a function of κ^2 . The analytical approximations $D_1(\kappa^2)$ (magenta) and $D_2(\kappa^2)$ (green) are compared to the numerical estimates $D_3(\kappa^2)$ from Bayesian inference³ for lag times $D\Delta t = 0.01$ (red filled circles) and $D\Delta t = 0.1$ (black open triangles). Error bars indicate a 95 % credibility range of the marginalized posterior. The inset shows a trajectory of $\kappa^2(t)$, obtained from simulations of the rotational diffusion of two dipole unit vectors using eq 5, as a function of dimensionless time Dt .

Figure 2:

Normalized autocorrelation function of κ^2 . The exact result eq 12 (orange) is compared to the results for one-dimensional diffusion on the potential of mean force for κ^2 with diffusion coefficients $D_1(\kappa^2)$ (magenta) and $D_2(\kappa^2)$ (green), obtained for the latter by solving the discretized diffusion equation for 256 grid points with matrix elements eq 19, with $p_{\text{eq}}(\kappa_i^2)\Delta$

replaced by $\int_{\kappa_i^2 - \Delta}^{\kappa_i^2 + \Delta} \frac{1}{2} p_{\text{eq}}(\kappa^2) d\kappa^2$. **Symbols show the results of Brownian dynamics**

simulations with the integrator eq 5 for a time step $D\delta t = 0.001$. The inset shows the autocorrelation function on a logarithmic scale.

Figure 3:

Propagator $p(\kappa^2, \Delta t | \kappa_0^2, t=0)$ after a lag time $D \Delta t = 0.1 \approx \tau_c$, as determined from Brownian dynamics simulations (yellow bars) and by solving the one-dimensional diffusion equation with diffusion coefficients $D_1(\kappa^2)$ (magenta line) and $D_2(\kappa^2)$ (green line). The thick black bar indicates the starting bin, centered on κ_0^2 . For the numerical solution of the diffusion equation, we used 256 grid points. To be consistent with the simulations using 32 bins, we imposed at time $t=0$ a local equilibrium distribution on the 8 grid points within the bin

centered on κ_0^2 , and at time t summed the probabilities of the 8 grid points centered around κ^2 . The black line shows the overall equilibrium distribution, eq 11, multiplied by the bin width. Distributions are normalized to one.

Figure 4:

Mean FRET efficiency as a function of the interdyne distance r/R_0 for different ratios of the rotational correlation time of a dye to the donor lifetime, $\tau_r/\tau_D = k_D/(6D) = 0$ (red), 0.2 (orange), 1 (green), 5 (cyan), and ∞ (blue; curves right to left). Solid and dashed lines show the results obtained for one-dimensional diffusion on the potential of mean force of κ^2 with diffusion coefficients $D_1(\kappa^2)$ and $D_2(\kappa^2)$, respectively. Symbols show the results obtained from Brownian dynamics simulations for intermediate values of τ_r/τ_D using the path-integral expression for the lifetime in eq 23.