CALCULATION OF FLUORESCENCE RESONANCE ENERGY TRANSFER ON SURFACES

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ABSTRACT A general method for estimating fluorescence resonance energy transfer between distributions of donors and acceptors on surfaces is presented. Continued fraction approximants are obtained from equivalent power series expansions of the change in quantum yield in terms of the fluorescent lifetimes or the steady-state fluorescence. These approximants provide analytic equations for the analysis of energy transfer and error bounds for the approximants. Specific approximants are derived for five models of interest for membrane biochemistry: (*a*) an infinite plane, (*b*) parallel infinite planes, (*c*) the surface of a sphere, (*d*) the surfaces of concentric spheres, and (*e*) the surfaces of two separated spheres. Recent experimental results in the literature are analyzed with the equations obtained.

INTRODUCTION

Fluorescence energy transfer is a well-established technique for determining distances in biological systems (1,2). In most applications a single energy donor specifically located on a macromolecule transfers its electronic excitation energy to a single energy acceptor at another specific location. Studies in which multiple donors and acceptors are present have also been carried out (cf. references 3 and 4). Recent studies with membranes and phospholipid vesicles have shown that topographical information can be obtained from energy transfer measurements when a distribution of donors transfers energy to a distribution of acceptors. For example, acceptor surface densities can be determined using randomly oriented donors and acceptors on a membrane or vesicle surface (5–7). This may be useful in studying processes such as phase separations, antibody-receptor clustering, and membrane fusion. Energy transfer between a tryptophan residue on cytochrome b_5 and lipids in phospholipid vesicles has been used to determine the distance between the fluorophore and the vesicle surface (8). The location of specific polypeptide chains of the ATP synthesizing complex from chloroplasts relative to the membrane surface has been studied using polypeptide specific fluorescent antibodies and lipid analogues as energy acceptors (9). These few quantitative studies indicate that fluorescence energy transfer measurements can be a valuable tool in membrane biology.

The theory for energy transfer between distributions of donors and acceptors on surfaces has been based on the work of Förster (10), and application of the theory in its most general form requires extensive numerical integrations (5,6). Relatively simple analyses have been derived for limiting cases where the distance of closest approach of acceptor and donor is either very long (5) or very short (11). Other theoretical analyses have used various approximations (7,12), but analytical expressions of general validity have not yet been presented. In this work, close approximations to the exact theory are developed for the calculation of energy transfer between distributions of surface donors and acceptors that are

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valid over a wide range of conditions and can be readily applied to experimental situations. Both fluorescence lifetime and steady-state measurements are considered, and simple analytical expressions are presented for several specific models. Moreover, upper and lower bounds for the energy transfer can be readily calculated from the approximations. The approach used allows the derivation of simple, accurate approximants and their error bounds for a variety of specific energy transfer problems.

Measurement of Energy Transfer

The most convenient measures of energy transfer are the steady-state quantum yield of the energy donor and the fluorescence lifetime of the energy donor. When multiple energy donors and acceptors are present, the fractional change in quantum yield due to energy transfer can be written as (cf. reference 2)

$$\frac{Q_{\mathrm{DA}}}{Q_{\mathrm{D}}} = \sum_j \frac{1}{1 + \sum_i \tau_{\mathrm{D}} k_{tij}}, \tag{1}$$

where Q_{DA} is the observed quantum yield in the presence of energy acceptors, Q_{D} is the observed quantum yield in their absence, τ_{D} is the fluorescence lifetime of the donor in the absence of energy transfer, k_{tij} is the rate constant for energy transfer, Σ_i is the summation over all acceptors, and Σ_j is the summation over all donors. The Förster theory of singlet-singlet resonance energy transfer shows that (13)

$$k_{tij} = (R_{0ij}/R_{ij})^6/\tau_{\mathrm{D}}, \tag{2}$$

where R_{ij} is the distance between the jth donor and ith acceptor and

$$R_{0ij} = 9.79 \times 10^3(\kappa_{ij}^2 J_{ij} Q_{\mathrm{D}} n^{-4})^{1/6}\ \text{Å}. \tag{3}$$

Here n is the refractive index of the medium, Q_{D} is the quantum yield of the energy donor, J_{ij} is the overlap integral of the donor emission and acceptor absorbance, and κ_{ij}^2 is the orientation factor for dipolar coupling between donor and acceptor. The overlap integral is defined as

$$J_{ij} = \frac{\int \overline{F}_i(\lambda)\epsilon_j(\lambda)\lambda^4 \mathrm{d}\lambda}{\int \overline{F}_i(\lambda)\mathrm{d}\lambda}, \tag{4}$$

where $\overline{F}_i$ is the corrected fluorescence emission spectrum of the donor, ϵ_j is the extinction coefficient of the acceptor, and λ is the wavelength. In this work, we assume that the spectral properties of all donor molecules are identical in the absence of energy acceptors and that the spectral properties of all acceptor molecules are identical so that only a single overlap integral exists. These approximations are valid for the experimental conditions normally encountered. Furthermore, we assume κ_{ij}^2 is identical for all donor-acceptor pairs; this assumption is not strictly necessary, as is discussed later. With these assumptions, the subscript ij on R_0 is superfluous, and Eq. 1 can be written as

$$\frac{Q_{\mathrm{DA}}}{Q_{\mathrm{D}}} = \sum_j \frac{1}{1 + \sum_i (R_0/R_{ij})^6}. \tag{5}$$

If fluorescence lifetimes are measured experimentally rather than steady-state quantum yields, the observed time dependence of the fluorescence, $\langle F(t) \rangle$, is averaged over all donors and can be written as

$$\langle F(t) \rangle \alpha e^{-t/\tau_D} \langle e^{-\Sigma_i k_{tij} t} \rangle, \tag{6}$$

where $\langle\ \rangle$ indicates ensemble averaging, t is the time, and, as before, $k_{tij} = (R_0/R_{ij})^6/\tau_D$. In practice, the complex time dependence of the fluorescence implied by Eq. 6 is difficult to assess experimentally. Instead, the fluorescence decay is usually analyzed in terms of the empirical equation

$$F(t) = \sum A_i e^{-t/\tau_i}, \tag{7}$$

where A_i and τ_i are experimentally determined parameters. Ascribing individual relaxation times obtained from the use of Eq. 7 to specific molecular processes is virtually impossible for complex systems. This dilemma is avoided if the relaxation times are regarded as curve-fitting parameters, and an average lifetime, τ_{AV}, is defined as

$$\tau_{AV} = \sum A_i \tau_i / \sum A_i, \tag{8}$$

then τ_{AV} is equal to the area under the normalized plot of $F(t)$ versus t. This area is proportional to the quantum yield, and

$$\frac{\tau_{AVDA}}{\tau_{AVD}} = \frac{Q_{DA}}{Q_D}. \tag{9}$$

Thus, in principle, measurement of average lifetimes and quantum yields are equivalent. The ratio Q_{DA}/Q_D can be expressed in terms of Eq. 6 by recalling that the steady-state fluorescence is the time dependent fluorescence integrated over time:

$$\frac{Q_{DA}}{Q_D} = \frac{1}{\tau_D} \int_0^\infty e^{-t/\tau_D} \langle e^{-\Sigma_i (R_0/R_{ij})^6 t/\tau_D} \rangle \, dt. \tag{10}$$

General Expansions and Approximants

In this section general approximants are derived for Q_{DA}/Q_D from Eqs. 5 and 10 when uniform distributions of energy donors and acceptors are present on a surface. The steps in the development are as follows. Firstly, Q_{DA}/Q_D is represented as a power series (which is not necessarily convergent). Secondly, Q_{DA}/Q_D is represented as a continuous fraction related to the power series; this representation converges rapidly and gives both upper and lower bounds for Q_{DA}/Q_D. The application of these results to specific cases is given in the next section.

The approximation by a power series of Q_{DA}/Q_D as given by Eq. 10 is considered first. If a uniform distribution of donors and acceptors on a surface is assumed, the summations are replaced by integrals, and the surface area and number of donor molecules on the surface are large, Eq. 10 can be written as (5)

$$\frac{Q_{DA}}{Q_D} = \frac{1}{\tau_D} \int_0^\infty e^{-t/\tau_D} e^{-\sigma S(t)} \, dt$$

with

$$S(t) = \int_S \left(1 - e^{-(t/\tau_D)(R_0/R)^6}\right) dP. \tag{11}$$

In Eq. 11, σ is the surface density of energy acceptors, dP is the probability the acceptor and donor are located a distance between R and R + dR apart, and the integration is over the surface. (Note that k_t is set equal to $(R_0/R)^6/\tau_D$). Eq. 11 is a Laplace integral and can be written as a power series in τ (cf. reference 14):

$$\frac{Q_{DA}}{Q_D} = \sum_{n=0}^{\infty} \tau_D^n \left(\frac{\partial^n e^{-\sigma S(t)}}{\partial t^n}\right)_{t=0} \tag{12}$$

$$= e^{-\sigma S(0)}\{1 - \tau_D \sigma S'(0) + \tau_D^2[\sigma^2 S'(0)^2 - \sigma S''(0)] - \cdot\cdot\cdot\}$$

$$= 1 - \mu_1 + \mu_2 - \mu_3 \ldots \tag{13}$$

Since $S(0) = 0$, the final series begins with unity and then has terms alternating in sign. The μ_i are defined by Eq. 12. This reduces the problem to one of finding expressions for the derivatives of S at one point, $t = 0$. This can be done by expanding the exponential in the expression for $S(t)$, which gives

$$S(t) = \int_S \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n!} \left(\frac{t}{\tau_D}\right)^n \left(\frac{R_0}{R}\right)^{6n} dP. \tag{14}$$

Explicit calculation of the first few terms in Eq. 13 gives

$$Q_{DA}/Q_D = 1 - \sigma \int_S (R_0/R)^6 dP + \left\{\sigma^2 \left[\int_S (R_0/R)^6 dP\right]^2 + \sigma \int_S (R_0/R)^{12} dP\right\} - \ldots. \tag{15}$$

An equivalent expansion of Eq. 1 can be carried out.

$$\frac{Q_{DA}}{Q_D} = \sum_j \frac{1}{1 + \sum_i (R_0/R_{ij})^6}$$

$$= 1 - \sum_j \sum_i (R_0/R_{ij})^6 + \sum_j \left[\sum_i (R_0/R_{ij})^6\right]^2 - \sum_j \left[\sum_i (R_0/R_{ij})^6\right]^3 + \cdot\cdot\cdot$$

$$= 1 - \sum_j \sum_i (R_0/R_{ij})^6 + \left\{\sum_j \sum_i (R_0/R_{ij})^{12} + \sum_i \sum_{\substack{j \\ i \neq i'}} \sum_{i'} (R_0/R_{ij})^6 (R_0/R_{i'j})^6\right\} - \cdot\cdot\cdot \tag{16}$$

If a uniform distribution of donors and acceptors on a surface is assumed and the summations are replaced by an integral,

$$\frac{Q_{DA}}{Q_D} = 1 - \sigma \int_S (R_0/R)^6 dP + \left\{\sigma \int_S (R_0/R)^{12} dP + \sigma^2 \left[\int_S (R_0/R)^6 dP\right]^2\right\} - \cdots . \qquad (17)$$

As required, Eqs. 15 and 17 are identical. This shows the power series expansions of Eqs. 1 and 10 are the same, although the expansion for Eq. 10 is easier to use.

The moment expansion by itself is not very useful in approximating Q_{DA}/Q_D. These expansions do not always converge; this is the case when $R_0 > R$. However, continued fraction approximants to Q_{DA}/Q_D are much better behaved. A moment expansion like Eq. 13 can be represented as a continued fraction (15)

$$\frac{Q_{DA}}{Q_D} = \cfrac{\alpha_1}{1 + \cfrac{\alpha_2}{1 + \cfrac{\alpha_3}{1 + \cfrac{\alpha_4}{1 + \cdots}}}} \qquad (18)$$

The α's are determined by the requirement that the formal expansion of the continued fraction have the same coefficients as the moment expansion. The first few α's are:

$$\alpha_1 = 1$$

$$\alpha_2 = \mu_1$$

$$\alpha_3 = \frac{\mu_2 - \mu_1^2}{\mu_1}$$

$$\alpha_4 = \frac{\mu_1\mu_3 - \mu_2^2}{\mu_1(\mu_2 - \mu_1^2)} .$$

Approximants to Q_{DA}/Q_D are obtained from the truncated continued fraction, A_n, by setting $\alpha_{n+1} = \alpha_{n+2} = \ldots = 0$. These approximants have a useful property which allows error bounds to be calculated. The even approximants form a nondecreasing sequence of lower bounds, while the odd approximants form a nonincreasing sequence of upper bounds. The best approximant is the average of the highest order odd and even approximants, $A_{n,n+1}$:

$$A_{n,n+1} = \frac{1}{2}(A_n + A_{n+1}). \qquad (19)$$

The error, ϵ, for this approximant is

$$\epsilon_{n,n+1} \leq \frac{1}{2}|A_{n+1} - A_n|. \qquad (20)$$

Thus, in addition to providing approximants for Q_{DA}/Q_D, this technique also allows an evaluation of the error of the approximation.

Applications of the General Theory

We now apply the results of the previous section to five particular models of practical interest: energy transfer between uniform distributions of energy donors and acceptors on an infinite plane, on two parallel infinite planes, on the surface of a sphere, and on the surfaces of a spherical bilayer; and energy transfer between a uniform distribution of energy donors on the surface of one sphere and a uniform distribution of acceptors on the surface of another sphere.

INFINITE PLANE For a uniform distribution of energy donors and acceptors on an infinite plane, $dP = 2\pi R dR$. If the distance of closest approach of a donor and acceptor is L and the maximum separation of donor and acceptor is infinity, Eq. 14 can be written as

$$S(t) = \int_L^{\infty} \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n!} \left(\frac{t}{\tau_D}\right)^n \left(\frac{R_0}{R}\right)^{6n} 2\pi R dR$$

$$= \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n!} \left(\frac{t}{\tau_D}\right)^n \left(\frac{R_0}{L}\right)^{6n-2} \frac{2\pi R_0^2}{(6n-2)}. \quad (21)$$

Use of Eqs. 12, 18, and 21 permits calculation of the approximants for Q_{DA}/Q_D. The first three are

$$A_1 = \left[1 + \frac{a_1 \pi \sigma R_0^2}{2}\left(\frac{R_0}{L}\right)^4\right]^{-1} \quad (22)$$

$$A_2 = \frac{1 + \left(\frac{a_2}{a_1}\right)\left(\frac{2}{5}\right)\left(\frac{R_0}{L}\right)^6}{1 + \left(\frac{a_2}{a_1}\right)\left(\frac{2}{5}\right)\left(\frac{R_0}{L}\right)^6 + \frac{a_1 \pi \sigma R_0^2}{2}\left(\frac{R_0}{L}\right)^4} \quad (23)$$

$$A_3 = \frac{1 + \frac{a_1 \pi \sigma R_0^2}{2}\left(\frac{R_0}{L}\right)^4 + \left(\frac{a_3}{a_2}\right)\left(\frac{5}{8}\right)\left(\frac{R_0}{L}\right)^6}{\left[1 + \frac{a_1 \pi \sigma R_0^2}{2}\left(\frac{R_0}{L}\right)^4\right]^2 + \left[1 + \frac{a_1 \pi \sigma R_0^2}{2}\left(\frac{R_0}{L}\right)^4\right]\left(\frac{a_3}{a_2}\right)\left(\frac{5}{8}\right)\left(\frac{R_0}{L}\right)^6 - \frac{a_2 \pi \sigma R_0^2}{5}\left(\frac{R_0}{L}\right)^{10}}. \quad (24)$$

For the infinite plane $a_1 = a_2 = a_3 = 1$, but these factors will be useful in discussing other models.

To illustrate the validity of the approximants derived, Q_{DA}/Q_D is plotted versus σR_0^2 for several values of R_0/L. The lines are exact solutions obtained by numerical integration or from the tables of Wolber and Hudson (11). The points are the approximants A_1, $A_{2,3}$, and $A_{3,4}$. The convergence of the approximants to the actual curve is apparent. The approximant A_1 is the limiting value of Q_{DA}/Q_D when $R_0 \ll L$ (cf. reference 5). The range of validity of the approximants depends on both R_0/L and σR_0^2. For $\sigma R_0^2 = 0.5$, $A_{2,3}$ is a good approximation for Q_{DA}/Q_D if $R_0/L \lesssim 1.4$. For $\sigma R_0^2 = 0.5$ and $R_0/L = 2.0$, $A_{2,3}$ is ~ 50% higher than the actual

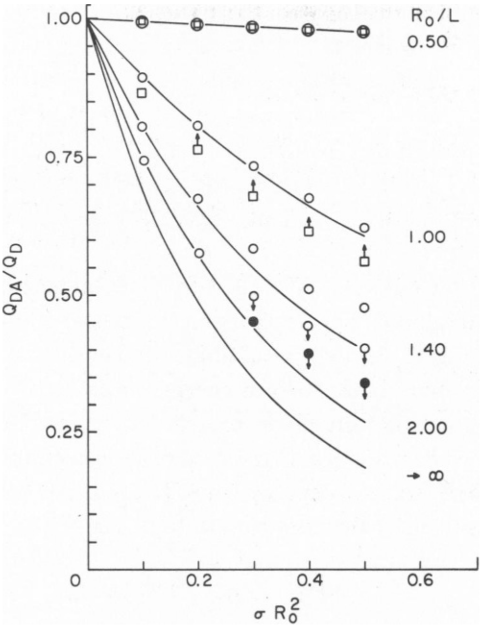

FIGURE 1 Relative quantum yield, Q_{DA}/Q_D, versus σR_0^2 for randomly oriented energy donors and acceptors on an infinite plane. Q_{DA} is the quantum yield in the presence of energy acceptors, Q_D is the quantum yield in the absence of energy acceptors, σ is the surface density of energy acceptors, and R_0 is defined by Eq. 3. Solid lines, numerical integration of Eq. 11; □, A_1; ○, $A_{2,3}$; ●, $A_{3,4}$.

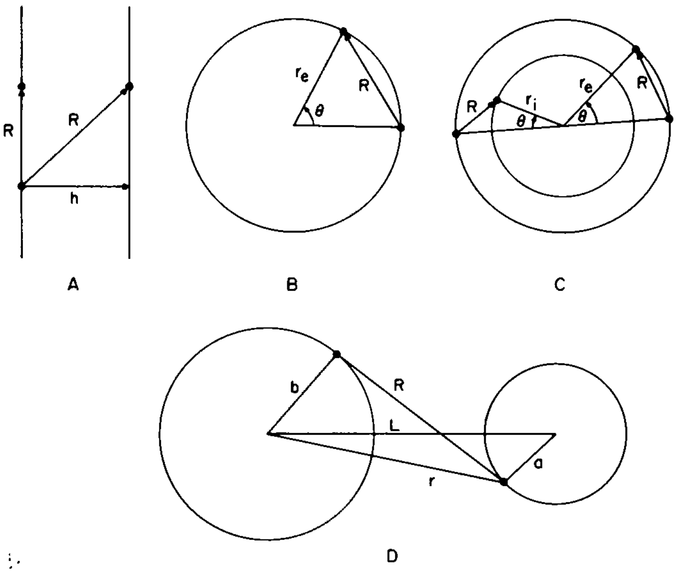

FIGURE 2 Models for energy transfer calculations: (*A*) infinite parallel planes separated by a distance *h*; (*B*) sphere of radius r_e; (*C*) concentric spheres with an inner sphere of radius r_i and an outer sphere of radius r_e; and (*D*) spheres of radii *a* and *b* whose centers are separated by a distance *L*. *R* is the distance between an energy donor and acceptor.

value of Q_{DA}/Q_D; $A_{3,4}$ is ~ 25% higher than the actual value. The values of Q_{DA}/Q_D versus σR_0^2 are also shown for the limit where $L \ll R_0$. A simple series approximation for this case has been derived (11). The results presented indicate that continued fractions give a good representation of Q_{DA}/Q_D over a wide range of values of σR_0^2 and R_0/L, but that as σR_0^2 and/or R_0/L become large, approximants of high order are necessary. Also, present experimental limitations require that $Q_{DA}/Q_D \lesssim 0.95$ for reliable measurements. Thus the curve for $R_0/L = 0.5$ in Fig. 1 is at the limit of experimental feasibility (or somewhat beyond) for the range of σR_0^2 considered.

PARALLEL INFINITE PLANES Models with different geometries can be easily treated with the methodology developed. Several possible models are illustrated in Fig. 2. For the infinite planar bilayer (Fig. 2 *A*), two possibilities are considered. If the energy donors are uniformly distributed on one plane and the energy acceptors on the other, the analysis is identical to that for the single infinite plane, except that the distance of closest approach L is now the distance between the planes, h. If the donors and acceptors are uniformly distributed on both planes, two contributions to energy transfer exist, one for energy transfer within a plane and one for energy transfer between planes. In this case

$$S(t) = S_1(t) + S_2(t)$$

where

$$S_1(t) = \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n!} \left(\frac{t}{\tau_D}\right)^n \frac{2\pi R_0^2}{6n-2} \left(\frac{R_0}{L}\right)^{6n-2}$$

$$S_2(t) = \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n!} \left(\frac{t}{\tau_D}\right)^n \frac{2\pi R_0^2}{6n-2} \left(\frac{R_0}{h}\right)^{6n-2},$$

where $S_1(t)$ is due to energy transfer within a plane and $S_2(t)$ is due to energy transfer between planes. These two sums can be combined to give

$$S(t) = \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n!} \left(\frac{t}{\tau_D}\right)^n \frac{2\pi R_0^2}{6n-2} \left(\frac{R_0}{L}\right)^{6n-2} a_n \qquad (25)$$

with

$$a_n = 1 + \left(\frac{L}{h}\right)^{6n-2}$$

and the approximants to Q_{DA}/Q_D are as given by Eqs. 22–24. The range of validity of the approximants is similar to that for the single infinite plane if $h \gtrsim L$.

SPHERICAL SURFACE For a uniform distribution of energy donors and acceptors on the surface of a sphere of radius r_e (Fig. 2 *B*),

$$S(t) = \int_{\theta_L}^{\pi} (1 - e^{-(t/\tau_D)(R_0/R)^6}) 2\pi R^2 \sin\theta \, d\theta, \qquad (26)$$

where θ_L is the angle of closest approach. From simple geometry

$$R^2 = 2r_e^2(1 - \cos\theta)$$

$$\cos\theta_L = (1 - L^2/2r_e^2).$$

Expanding the exponential and integrating gives

$$a_n = 1 - (L/2r_e)^{6n-2}. \tag{27}$$

The validity of the approximants again is similar to that for the infinite plane, since $L/2r_e < 1$ for cases of practical interest.

SPHERICAL BILAYER For a uniform distribution of energy donors and acceptors on the surface of two concentric spheres (Fig. 2 *C*), four contributions to energy transfer must be considered: energy transfer between donors and acceptors on the same surface and between donors and acceptors on the two different surfaces. The contributions due to energy transfer on the surface of the external sphere are given by Eq. 27. The contribution due to energy transfer between donors on the external surface and acceptors on the internal surface can be calculated by referring to Fig. 2 *C*.

Again $S(t)$ is given by Eq. 26 but now

$$R^2 = (r_e^2 + r_i^2 - 2r_e r_i \cos\theta)$$

$$dP = 2\pi r_e^2 \sin\theta \, d\theta$$

and $\theta_L = 0$. Expansion and integration as done previously gives

$$a_n = L^{6n-2}(r_e/r_i)\left[\frac{1}{(r_e - r_i)^{6n-2}} - \frac{1}{(r_e + r_i)^{6n-2}}\right]. \tag{28}$$

For the donors on the external sphere the coefficients a_n for the complete expansion are the sum of Eqs. 27 and 28. If the surface on the internal sphere has the same density of donors and acceptors as the external surface, coefficients identical to Eqs. 27 and 28 are obtained, except that r_e and r_i must be interchanged. Before combining the four coefficients, one must take into account that the surface area of the external sphere is greater than the surface area of the internal sphere. The fraction of donors on the external sphere is $r_e^2/(r_i^2 + r_e^2)$ and that on the internal sphere is $r_i^2/(r_e^2 + r_i^2)$. When the coefficients a_n are multiplied by these fractions, the coefficients that include all contributions to energy transfer are

$$a_n = 1 - L^{6n-2}\left\{\frac{r_e^2}{r_e^2 + r_i^2}\left[\frac{1}{(2r_e)^{6n-2}} + \frac{(r_e/r_i)}{(r_e - r_i)^{6n-2}} - \frac{(r_e/r_i)}{(r_e + r_i)^{6n-2}}\right] + \frac{r_i^2}{r_e^2 + r_i^2}\left[\frac{1}{(2r_i)^{6n-2}} + \frac{(r_i/r_e)}{(r_e - r_i)^{6n-2}} - \frac{(r_i/r_e)}{(r_e + r_i)^{6n-2}}\right]\right\}. \tag{29}$$

SEPARATED SPHERES One further case of practical interest is considered, namely, energy transfer between a uniform distribution of energy donors on a sphere of radius a and a uniform distribution of energy acceptors on a separate sphere of radius b (Fig. 2 *D*). The centers of the two spheres are separated by a distance L and $L - (a + b) > 0$. An approximate solution to this problem has been presented previously (9). The appropriate series expansion of $Q_{\mathrm{DA}}/Q_{\mathrm{D}}$ is given by Eq. 16. In this case consecutive integrations must be done over the donor and acceptor spheres. Thus

$$\mu_1 = \frac{N_{\mathrm{A}} R_0^6 \int_{S_{\mathrm{A}}} \int_{S_{\mathrm{D}}} \frac{1}{R^6} \mathrm{d}S_{\mathrm{A}} \mathrm{d}S_{\mathrm{D}}}{\int_{S_{\mathrm{A}}} \int_{S_{\mathrm{D}}} \mathrm{d}S_{\mathrm{A}} \mathrm{d}S_{\mathrm{D}}},$$

where N_{A} is the number of acceptors on the surface of the sphere and $\mathrm{d}S_{\mathrm{A}}$ and $\mathrm{d}S_{\mathrm{D}}$ are the surface differentials for the acceptor and donor spheres. Integration over the acceptor sphere can be readily carried out with the relationships $\mathrm{d}S_{\mathrm{A}} = 2\pi b^2 \sin\theta \mathrm{d}\theta$ and $R^2 = r^2 + b^2 - 2br \cos\theta$:

$$\mu_1 = \frac{N_{\mathrm{A}} R_0^6 \int_{S_{\mathrm{D}}} \int_0^{\pi} \frac{1}{R^6} 2\pi b^2 \sin\theta \mathrm{d}\theta}{(4\pi b^2)(4\pi a^2)} = \frac{N_{\mathrm{A}} R_0^6}{(4\pi a^2)} \int_{S_{\mathrm{D}}} \left[\frac{2b^2}{(r^2 - b^2)^4} + \frac{1}{(r^2 - b^2)^3}\right] \mathrm{d}S_{\mathrm{D}}.$$

Integration over the donor sphere then follows with $r^2 = L^2 + a^2 - 2aL \cos\theta$ and $\mathrm{d}S_{\mathrm{D}} = 2\pi a^2 \sin\theta \mathrm{d}\theta$. The final result is

$$\mu_1 = N_{\mathrm{A}} R_0^6 \left\{\frac{L^6 - L^4(a^2 + b^2) + L^2\left[\frac{8}{3} a^2 b^2 - (b^2 - a^2)^2\right] + (b^2 - a^2)^2(a^2 + b^2)}{[L^4 - 2L^2(a^2 + b^2) + (b^2 - a^2)^2]^3}\right\}. \quad (30)$$

The second moment is

$$\mu_2 = \frac{N_{\mathrm{A}}^2 R_0^{12}}{2} \int_0^{\pi} \left[\frac{2b^2}{(r^2 - b^2)^4} + \frac{1}{(r^2 - b^2)^3}\right]^2 \sin\theta \mathrm{d}\theta$$

$$= \frac{N_{\mathrm{A}}^2 R_0^{12}}{4aL} \left[\frac{\left(\frac{4}{7}\right) b^4 (y^7 - x^7)}{y^7 x^7} + \frac{\left(\frac{2}{3}\right) b^2 (y^6 - x^6)}{y^6 x^6} + \frac{\left(\frac{1}{5}\right) (y^5 - x^5)}{y^5 x^5}\right]$$

with

$$y = L^2 - b^2 + a^2 + 2aL \quad \text{and} \quad x = L^2 - b^2 + a^2 - 2\mathrm{aL}. \quad (31)$$

To illustrate the range of validity of the approximants for $Q_{\mathrm{DA}}/Q_{\mathrm{D}}$, the curve obtained by numerical integration is shown for a particular case together with the approximants A_1 and $A_{1,2}$ in Fig. 3. The approximant $A_{1,2}$ falls quite close to the results of the numerical integration.

Orientation of Transition Dipoles (κ^2) and Donor-Acceptor Distributions

The explicit assumption has been made that κ^2 is the same for all donor-acceptor pairs. Customarily κ^2 is assigned a value of $2/3$, the appropriate value when both the donor and acceptor rotate freely and rapidly relative to the fluorescence lifetime. This assumption is not likely to produce a serious error in the interpretation of energy transfer measurements,

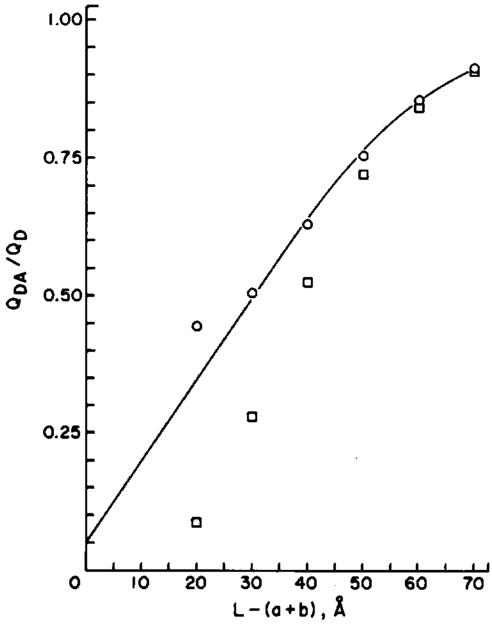

FIGURE 3 Relative quantum yield for separated spheres, Q_{DA}/Q_D, versus $L - (a + b)$ for the case of $a = b = 25$ Å, $R_0 = 35$ Å, and $N_A = 100$. Q_{DA} is the quantum yield in the presence of energy acceptors, Q_D is the quantum yield in the absence of energy acceptors, R_0 is defined by Eq. 3, N_A is the number of acceptors on the sphere of radius b, L is the distance between the centers of the spheres, and a and b are the radii of the spheres (Fig. 2 *D*). Solid line, numerical integration of Eq. 16; □, A_1; ○, $A_{1,2}$.

because R_0 depends on the one-sixth power of κ^2, energy donors and acceptors usually have some rotational mobility, and multiple electronic transitions are often involved in donor fluorescence (cf. references 1 and 16). Bounds for κ^2 can be established from fluorescence polarization measurements; extensive theoretical treatments of this problem have been developed (cf. references 11 and 17). A similar theoretical treatment can be applied to the approximants calculated here; the result will be a scaling factor for R_0 with upper and lower bounds. A detailed analysis of this problem is not of practical value at this time.

For most experiments a distribution of acceptor surface densities exists within the system. The number of acceptors (N_A or σ) occurs linearly or raised to integral powers in the power series approximations so that use of a distribution function is not difficult. If the distribution is Poisson, then the correct values of σ and N_A to use are the averages, as is normally assumed. (Note that for a Poisson distribution $\langle N_A^p \rangle = \langle N_A \rangle^p$ where p is an integer and the brackets indicate an average.) The use of a Poisson distribution with the exact equations for Q_{DA}/Q_D requires a complex numerical analysis.

DISCUSSION

The approximants developed here offer a practical and general method for estimating energy transfer between distributions of donors and acceptors on surfaces. In addition to the simple analytical form of the lower order approximants, this method has the advantage of estimating error bounds for the approximation. The approximants also are convenient if a specific distribution in R_{ij} is used, since the distribution function always is multiplied by $1/R_{ij}$ raised to some power, resulting in relatively simple integrals. The approximant $A_{2,3}$ generally is adequate if $R_0/L \lesssim 1.5$. Moreover, reference to Fig. 1 indicates that if $R_0/L > \sim 2$, the series approximation derived for $R_0/L \rightarrow \infty$ is adequate (11). To test the utility of the method, experimental data in the literature were analyzed using $Q_{DA}/Q_D = A_{2,3}$. The infinite plane models were used, since corrections due to radius of curvature are small for the systems studied.

For energy transfer between small fluorescent lipid labels, distances of closest approach are ~ 10 Å. Since most R_0 values lie between 20 and 40 Å, most of the data of Fung and Stryer (6) for energy transfer between modified lipid molecules on the surface of phospholipid vesicles can be fit with the series approximation of Wolber and Hudson (11). With this approximation Q_{DA}/Q_D depends only on σ and R_0; it does not depend on L. Fung and Stryer noted that Q_{DA}/Q_D was relatively insensitive to the choice of L. Our approximants adequately fit the data only for the case where $R_0 = 25.5$ Å. In this case, a distance of closest approach of 13 Å was required. Estep and Thompson (7) studied energy transfer on phospholipid vesicles using anthracene as the energy donor and perylene as the energy acceptor. Their data are well described by $A_{2,3}$ with $L = 17$ Å. This is a reasonable distance of closest approach for the bulky donor and acceptor molecules.

Energy transfer between a tryptophan on cytochrome b_5 and trinitrophenyl or dansyl acceptors on the surface of a phospholipid vesicle has been measured (8). The distance from tryptophan to the vesicle surface was estimated to be 20–22 Å by use of an approximate theory (12). With the parallel infinite planes model and $A_{2,3}$, the data are fit best with a distance of closest approach of 18 Å. Energy transfer between a fluorescent donor embedded ~15 Å from the outer surface of the red blood cell membrane and hemoglobin bound to the inside of the membrane has been measured (5). The data were analyzed with the parallel infinite planes model and the approximation $R_0 \ll L$, which is equivalent to using A_1; a value of 42 Å was obtained for L. A more exact analysis with $A_{2,3}$ gives a value of 34 Å for L.

In conclusion, the approximants derived here appear to be useful in analyzing most of the energy transfer measurements done thus far on membrane-like surfaces.

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REFERENCES

1. Stryer, L. 1978. Fluorescence energy transfer as a spectroscopic ruler. *Annu. Rev. Biochem.* **47**:819.
2. Hammes, G. G. 1981. Fluorescence methods. In Protein-Protein Interactions. C. Frieden and L. W. Nichol, editors. Wiley-Interscience. In press.
3. Hahn, L.-H. E., and G. G. Hammes. 1978. Structural mapping of aspartate transcarbamoylase by fluorescence energy-transfer measurements: determination of the distance between catalytic sites of different subunits. *Biochemistry.* **17**:2423.
4. Angelides, K., and G. G. Hammes. 1979. Fluorescence studies of the pyruvate dehydrogenase multienzyme complex from *Escherichia coli. Biochemistry.* **18**:1223.
5. Shaklai, N., J. Yguerabide, and H. M. Ranney. 1977. Interaction of hemoglobin with red blood cell membranes as shown by a fluorescent chromophore. *Biochemistry.* **16**:5585.

6. Fung, B. K., and L. Stryer. 1978. Surface density determination in membranes by fluorescence energy transfer. *Biochemistry*. **17**:5241.

7. Estep, T. N., and T. E. Thompson. 1979. Energy transfer in lipid bilayers. *Biophys. J.* **26**: 195.

8. Fleming, P. J., D. E. Koppel, and P. Strittmatter. 1979. Intramembrane position of the fluorescent tryptophanyl residue in membrane-bound cytochrome b_5. *Biochemistry* **18**:5458.

9. Baird, B., U. Pick, and G. G. Hammes. 1979. Structural investigations of reconstituted chloroplast ATPase with fluorescence measurements. *J. Biol. Chem.* **254**:3818.

10. Förster, T. 1949. Experimentelle und theoretische Untersuchung des zwischenmolekularen Übergangs von Elektronenanregungsenergie. *Z. Naturforsch. A. Astrophys. Phys. Phys. Chem.* **4**:321.

11. Wolber, P. K., and B. S. Hudson. 1979. An analytic solution to the Förster energy transfer problem in two dimensions. *Biophys. J.* **28**:197.

12. Koppel, D. E., P. J. Fleming, and P. Strittmatter. 1979. Intramembrane positions of membrane bound chromophores determined by excitation energy transfer. *Biochemistry*. **18**:5450.

13. Förster, T. 1956. Delocalized excitation and excitation transfer. *In* Modern Quantum Chemistry, Part III. O. Sinanoglu, editor. Academic Press, Inc., New York. 93.

14. Olver F. W. J. 1974. Asymptotics and Special Functions. Academic Press, Inc., New York. 66.

15. Gordon, R. G. 1968. Error bounds in equilibrium statistical mechanics. *J. Math. Phys.* **9**:655.

16. Haas, E., E. Katchalski-Katzir, and I. Z. Steinberg. 1978. Effect of the orientation of donor and acceptor on the probability of energy transfer involving electronic transitions of mixed polarization. *Biochemistry. 17:5064.*

17. Dale, R. E., J. Eisinger, and W. E. Blumberg. 1979. The orientational freedom of molecular probes. *Biophys. J.* **26**:161.