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APPLICATION OF FORSTER RESONANCE ENERGY TRANSFER TO INTERACTIONS BETWEEN CELL OR LIPID VESICLE SURFACES[1]

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SUMMARY

Calculations are presented which demonstrate the efficacy of a Förster resonance energy transfer technique to measurement of the aggregation of cells and lipid vesicles.

INTRODUCTION

The use of Förster resonance energy transfer (1) to measure the distance between chromophores is a well established technique (2). The rate of fluorescence energy transfer, k_T, is related to distance between donor and acceptor, R, according to the following equation:

$$(1) \qquad k_T = \frac{1}{\tau}\left(\frac{R_0}{R}\right)^6$$

where τ is the total lifetime of the donor excited state and R_0 is the distance at which fluorescence is 50% quenched.

In the preceding paper (3) we introduced a pair of fluorescent probes which can be used to measure vesicle fusion by Förster resonance energy transfer. Here we demonstrate that this same technique has the potential to measure long range interactions between vesicle or cell surfaces. We have chosen two cases for analysis: the measurement of fluorescence from a unilamilar vesicle suspension; and the measurement of fluorescence from the area of contact between a donor labeled and an acceptor labeled cell (4).

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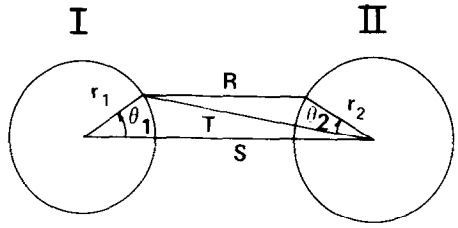

$$R^2 = r_2^2 - 2r_2\, T\cos\theta_2 + T^2$$

$$T^2 = r_1^2 - 2r_1\, S\cos\theta_1 + S^2$$

Figure 1. Definition of geometrical parameters used in the calculation of energy transfer between a donor labeled sphere, I, and an acceptor labeled sphere, II.

RESULTS AND DISCUSSION

We have chosen to model aggregating vesicles as two spheres, one containing randomly distributed fluorescence energy donor probes, I, and the other, II, containing a similar distribution of acceptor probes (Figure 1). We can see that the total rate constant for transfer from a donor at an arbitrary point on surface I will be given as

$$(2) \qquad k_T = \frac{2\pi r_2^2 R_o^6 \sigma_A}{\tau} \int_{\theta_2=0}^{\pi} R^{-6} \sin\theta_2 d\theta_2 \ .$$

where σ_A is the surface density of acceptor probes on II in reciprocal square Ångstroms. This integral can be evaluated to yield

$$(3) \qquad k_T = \left(\frac{2\pi r_2^2 R_o^6 \sigma_A}{\tau}\right)\left((T-r_2)^{-4} - (T+r_2)^{-4}\right) \ .$$

This rate constant is then used to compute a quantum yield

$$(4) \qquad \phi = \frac{k_f}{k_f + k_{nr} + k_T}$$

which must, itself, be integrated over the normalized surface area of the donor sphere:

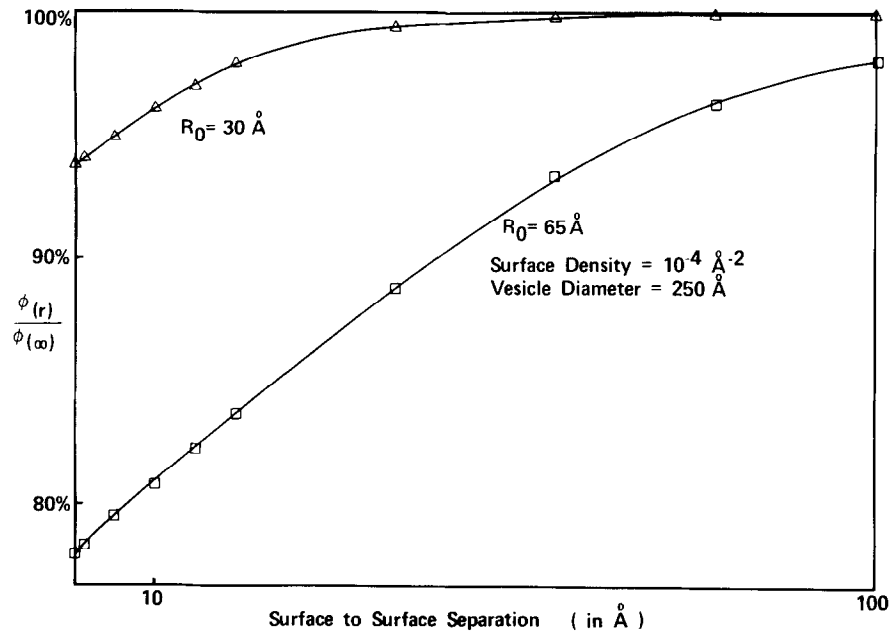

Figure 2. Calculated quantum yield for 250 Å vesicles where the donor has a $\tau = 6 \times 10^{-9}$ sec (as does dansyl-DPPE) and the quencher surface density is 10^{-4} Å^{-2}. The chromophores are assumed to be localized at the vesicle surface.

$$(5) \qquad \langle\phi\rangle = \frac{1}{4\pi r_1^2} \int_{\theta_1=0}^{\pi} \frac{k_f r_1^2 \sin\theta_1 d\theta_1}{k_f + k_{nr} + k_T}$$

k_f and k_{nr} are the rate constants for radiative and non-radiative decay of the excited donor, respectively. Numerical integration of (5) allows prediction of the relative quantum yield as a function of R_0 and surface to surface separation. The results are displayed in Figure 2. This technique can produce measurable amounts of quenching for attainable values of R_0 even at distances where a long range intermembrane potential minimum may occur (5) (40-70 Å).

The vesicle aggregation experiment would involve measurement of emission spectra from bulk phospholipid dispersions. We can, however, envisage another type of experiment which would extend this technique to the study of cell-cell adhesion. A fluorescence microscope could be used to focus

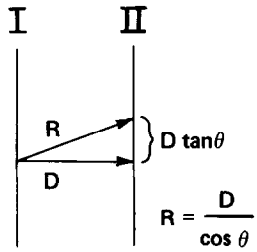

Figure 3. Definition of geometrical parameters used in the calculation of energy transfer between adjacent cell surfaces. I is the donor labeled surface and II is the acceptor labeled surface.

on the interacting surfaces of the two cells eliminating the contribution to the fluorescence from areas remote from each other.

In the case of aggregating cells, opposed surfaces can extend for several microns--a distance large with respect to the Förster process. We have, therefore, chosen to model this as two opposing infinite planes (Figure 3). In this case the integral:

$$(6) \qquad k_T = \frac{2\pi D^2 \sigma_A R_o^6}{\tau} \int_{\theta=0}^{\pi/2} R^{-6} \tan\theta \, d(\tan\theta)$$

can be solved analytically to give:

$$(7) \qquad k_T = \frac{\sigma_A \pi R_o^6}{2\tau D^4}$$

It is interesting to note that this form shows a reduction in the order of the distance dependence from the inverse sixth order, normally associated with the Förster process, to inverse fourth order in D, the perpendicular interplane distance. A similar result was obtained by Bücher et al. (2a) using a different approach, in their study of stacked dye monolayers. Calculation of fluorescent quantum yields for three systems of probes differing only in R_o is displayed in Figure 4. The predicted reduction in quantum yield in this case is quite dramatic even at relatively low surface concentration of acceptor.

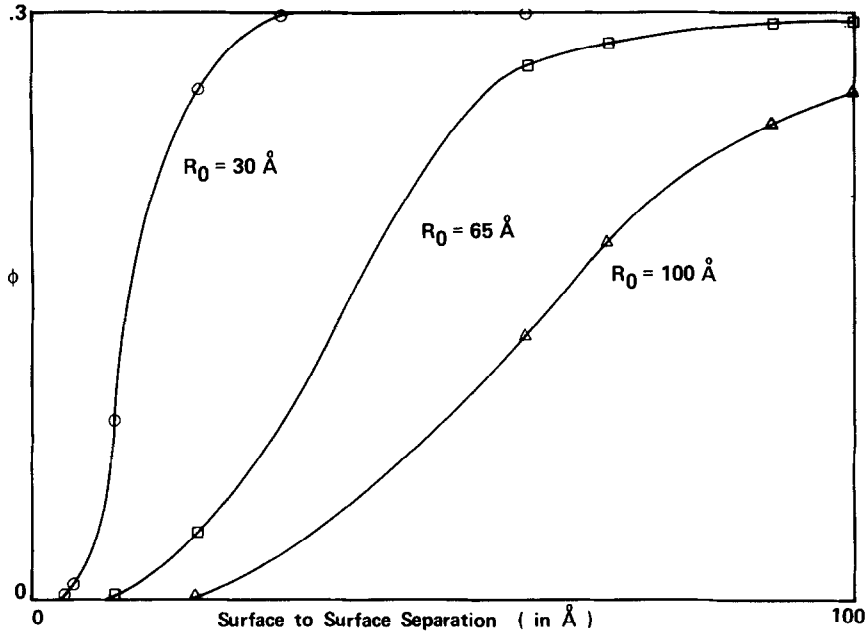

Figure 4. Calculated quantum yield for 2 adjacent cell surfaces. The donor parameters (ϕ = 0.3, τ = 6 x 10^{-9} sec) are those of dansyl-DPPE and the acceptor surface density (σ_A) is 10^{-4} $Å^{-2}$.

In each experimental case the fluorescent probes must be tightly bound to preclude mixing of the probes by dissociation-association. Further, the probes should be held near the surface of the membrane to maximize the Förster interaction. Both of these conditions are well satisfied by the donor-acceptor pair introduced in the preceeding paper (3). This pair, however, has a relatively low R_0 (36 Å) and so we are currently involved in the evaluation of other pairs (6) to facilitate performance of the experiments suggested by the above calculations.

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