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RESONANCE ENERGY TRANSFER IN MEMBRANE SYSTEMS III. PLANAR ACCEPTOR DISTRIBUTION IN TWO MONOLAYERS

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The model of resonance energy transfer in membranes is extended to the case where the donors and acceptors are randomly distributed over three parallel planes (two acceptor ones and a donor one). The model allows for the orientational behavior of chromophores in the membrane and permits expressing the averaged orientation factor for a given donor-acceptor pair via steady-state and fundamental anisotropies of the chromophores in corresponding environment. Criteria for the optimal choice of donor-acceptor pairs providing maximum accuracy of estimation of the donor plane position in the membrane are given.

KEY WORDS: resonance energy transfer, membrane systems, protein-lipid interactions

Resonance energy transfer (RET) is a phenomenon characterized by the inverse sixth power dependence on the separation between energy donor and acceptor [1]. This feature determines wide use of RET as a tool for structural characterization of macromolecular assemblies, particularly, biological membranes [2-5]. Due to the sensitivity of RET to the distance of the donor and acceptor separation, the geometry of the system has a significant influence on the analysis and interpretation of the measurements. In the previous papers of this series we have considered how the transfer efficiency is affected by the dimensionality of donor and acceptor arrays [6] and by the curvature of the surface to which the chromophores are confined [7]. In the present paper we are concerned with the case where the donors and acceptors are randomly distributed over three parallel planes (two acceptor ones and a donor one). Such a situation may take place, for example, when the bilayer contains acceptor-labeled lipids, while donors are represented by tryptophan residues of membrane-bound protein. In the following we present corresponding theoretical model and determine experimental conditions providing the most accurate evaluation of the donor plane position relative to the bilayer mid-plane.

THEORY

As was shown earlier [6,8], in the case of energy transfer to an array of acceptors, general expressions for the relative quantum yield of the donor (Q_r) have the following form:

$$Q_r = \frac{Q_{DA}}{Q_D} = \int_0^\infty \exp\left[-\lambda\right]\left(I(\lambda)\right)^N d\lambda\,, \quad I(\lambda) = \int_{R_e}^{R_d} \exp\left[-\lambda\left(R_o / R\right)^6\right] W(R)dR\,. \tag{1}$$

Here Q_D and Q_{DA} are the donor quantum yields in the absence and presence of acceptors, respectively, λ is the dimensionless time, $\lambda = t/\tau_D$, N is the number of acceptor molecules within certain distance from the donor (R_d), beyond which energy transfer is insignificant, R_e is the distance of closest approach of the donor and acceptor molecules, R_0 is Förster radius for a given donor-acceptor pair, and $W(R)dR$ is the probability of finding an acceptor at the distance $R+dR$ from the donor (normalized to unity in the range $\left[R_e, R_d\right]$). The functional form of $W(R)$ and N depends on the dimensionality and mutual arrangement of the donor and acceptor arrays. In the present study we are primarily concerned with the special case where the donors and acceptors are randomly distributed over three planes parallel to the membrane surface – two acceptor planes, symmetrical relative to the membrane mid-plane, and one donor plane (Fig.1). In this case the probability function is given by

$$W(R) = \begin{cases} \dfrac{R}{R_d^2 - d_c^2 - (0.5d_t)^2}, & \left|d_c - 0.5d_t\right| \le R < d_c + 0.5d_t, \\ \dfrac{2R}{R_d^2 - d_c^2 - (0.5d_t)^2}, & d_c + 0.5d_t \le R < R_d, \end{cases} \tag{2}$$

where d_c is the distance between the donor plane and the bilayer mid-plane, and d_t is the distance between two acceptor planes (Fig.1). The number of acceptor molecules surrounding one donor molecule (N) is related to the surface acceptor concentration (C_A^s) as follows:

$$N = 2\pi[R_d^2 - d_c^2 - (0.5d_t)^2]C_A^s. \quad (3)$$

To account for the orientational effects we represent Förster distance (R_0) as a product of orientation factor (κ^2) and the distance-independent portion (R_{0r}): $R_0^6 = \kappa^2(R)\cdot(R_{0r})^6$, since, as was shown previously [9], orientation factor in membrane systems depends on the donor-acceptor separation. Orientation factor, characterizing mutual orientation of the donor and acceptor transition dipoles, in this case is given by

$$\kappa_{1,2}^2(R) = d_D d_A\left[1-3\left(\frac{d_c \mp 0.5d_t}{R}\right)^2\right]^2 + \frac{1}{3}(1-d_D)+\frac{1}{3}(1-d_A)+\left(\frac{d_c \mp 0.5d_t}{R}\right)^2(d_D - 2d_D d_A + d_A), \quad (4)$$

where d_A and d_D are the donor and acceptor depolarization factors, related to the steady-state r and fundamental r_0 anisotropies of the donor and acceptor [10]:

$$d_{D,A} = \pm(r_{D,A}/r_{0D,A})^{1/2}. \quad (5)$$

Subscripts 1 and 2 in eq.(4) (and corresponding signs in the right-hand side) stand for the different acceptor planes (upper and lower in Fig.1, respectively). Finally, eq.(1, right) can be rewritten as

$$I(\lambda) = \frac{1}{R_d^2 - d_c^2 - (0.5d_t)^2}\left[\int_{|d_c-0.5d_t|}^{R_d} \exp\left[-\lambda\kappa_1^2(R)\left(\frac{R_{0r}}{R}\right)^6\right] RdR + \int_{d_c+0.5d_t}^{R_d} \exp\left[-\lambda\kappa_2^2(R)\left(\frac{R_{0r}}{R}\right)^6\right] RdR\right]. \quad (6)$$

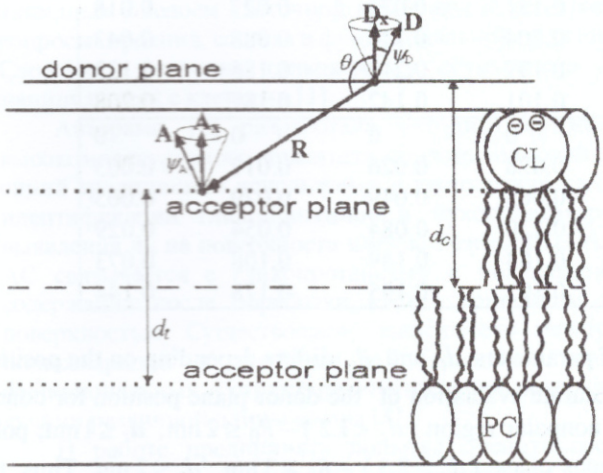

Fig. 1. Relative disposition of the donor and acceptor planes in the lipid bilayer. Axial distributions of the donor (**D**) and acceptor (**A**) transition moments around their mean positions ($\mathbf{D_x}$ and $\mathbf{A_x}$) are also shown (see [9,10] for details).

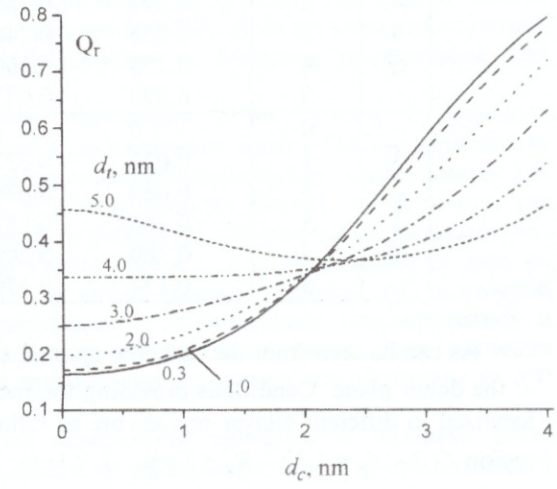

Fig. 2. Relative quantum yield of the donor as a function of the donor plane position for different acceptor plane separations. Surface acceptor concentration was fixed at 0.03 nm^{-2}.

DISCUSSION

The model presented here permits determination of the position of the donor plane in the lipid bilayer (d_c) by nonlinear least squares fitting of fluorescence quenching data. In order to find the optimal experimental conditions providing the most accurate distance estimates we performed numerical simulations using eqs.(1-3) and different sets of parameters d_t, R_0, and d_c. Note that in the present analysis we restricted ourselves to analyzing the case where donors and acceptors are rapidly tumbling and their transition dipoles can adopt all orientations in a time short compared with the transfer time, which corresponds to $d_A = d_D = 0$

and $\kappa^2 = 2/3$. Fig.2 shows typical dependencies of the donor relative quantum yield on the distance between the donor plane and the bilayer mid-plane. Different curves in this figure correspond to different d_t. It is clearly seen that as the distance between acceptor planes (d_t) decreases the curves become steeper. This fact indicates that, when decreasing d_t, the relative quantum yield of the donor (measured experimentally) becomes more sensitive to variation of d_c, thus making the estimates of d_c more accurate. It is also noteworthy that the values of d_c below ~1 nm can not be determined accurately from the fluorescence data, since the curves at low d_c are quite flat. To make the analysis more general we calculated the slope (first derivative, dQ_r/dd_c) of analogous curves for the experimentally important range of Förster radii and distances between acceptor planes (Table). These values represent the sensitivity of RET efficiency with respect to d_c.

Table. Model sensitivity (dQ_r/dd_c) with respect to d_c. Surface acceptor concentration was fixed at 0.03 nm^{-2}.

	d_c,	d_t, nm					
	nm	0.3	1.0	2.0	3.0	4.0	5.0
R_0 = 2 nm	0	0	0	0	0	0	0
	1	0.188	0.169	0.093	- 0.008	- 0.101	- 0.165
	2	0.258	0.233	0.154	0.074	0.006	- 0.059
	3	0.082	0.103	0.162	0.187	0.133	0.064
	4	0.023	0.027	0.046	0.089	0.157	0.184
	5	0.008	0.009	0.013	0.022	0.043	0.088
R_0 = 3 nm	0	0	0	0	0	0	0
	1	0.076	0.077	0.072	0.050	0.003	- 0.062
	2	0.207	0.184	0.131	0.075	0.027	- 0.018
	3	0.256	0.246	0.204	0.144	0.088	0.043
	4	0.153	0.166	0.197	0.212	0.185	0.133
	5	0.070	0.077	0.101	0.142	0.187	0.208
R_0 = 4 nm	0	0	0	0	0	0	0
	1	0.026	0.027	0.028	0.026	0.017	- 0.003
	2	0.080	0.076	0.065	0.049	0.029	0.005
	3	0.166	0.153	0.120	0.084	0.054	0.029
	4	0.209	0.205	0.184	0.149	0.108	0.073
	5	0.174	0.179	0.191	0.195	0.178	0.144

As can be seen from the table the optimal set of parameters d_t and R_0 differs depending on the position of the donor plane. Conditions providing the most accurate evaluation of the donor plane position for donors localized in different bilayer regions are as follows: nonpolar region ($d_c < 1.2$) – $R_0 \le 2$ nm, $d_t \le 1$ nm; polar region ($1.2 < d_c < 2.3$) – $R_0 \le 3$ nm, $d_t \le 1$ nm; aqueous phase ($d_c > 2.3$) – $R_0 \ge 3$ nm, $d_t \le 2$ nm. Thus, the above analysis reveals possibility of choosing the most appropriate donor-acceptor pair for accurate distance determination in a given transverse region of the membrane.

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