Published in final edited form as:

Chem Phys Lipids. 1999 September ; 101(2): 243–253. doi:10.1016/s0009-3084(99)00066-3.

Resonance energy transfer study using a rhenium metal–ligand lipid conjugate as the donor in a model membrane

Li Li, Ignacy Gryczynski, Joseph R. Lakowicz*

Center for Fluorescence Spectroscopy, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 725 West Lombard Street, Baltimore, MD 21201, USA

Abstract

We measured steady state and time-resolved resonance energy transfer between donors and acceptors in model membranes. The donor was a long lifetime rhenium–lipid complex, which displayed a mean lifetime of 1 μ s and lifetime components as long as 3 μ s in the labeled DOPC membranes. The transfer efficiencies were found to be substantially larger than those predicted without consideration of lateral diffusion. The larger transfer efficiencies are consistent with a mutual lateral diffusion coefficient in the membrane near 2×10^{-8} cm²/s. These results demonstrate that lateral diffusion in membranes can be detected with μ s lipid probes.

Keywords

Resonance energy transfer; Metal–ligand lipid conjugate; Model membrane

1. Introduction

Resonance energy transfer (RET) between luminescent donors and suitable acceptors occurs over distances ranging from 20 to 90 Å. These distances are comparable to the dimensions of biological macromolecules and assemblies. Hence, RET often serves as a ‘spectroscopic ruler’ which allows quantitative measurements of distances between stationary energy donors and acceptors (Stryer, 1978; Lakowicz, 1999). This method has frequently been used to obtain structural and conformational information about macromolecules.

Until now, most studies of RET were accomplished using fluorophores with ns lifetimes. For labeled proteins and membranes the distance between a donor and an acceptor is usually constant during the ns lifetime of the excited donor. One exception are the fluorescent lanthanides such as Tb³⁺, with ms lifetimes. Luminescent lanthanides have been used as donors to attain resonance energy transfer in the rapid-diffusion limit (Thomas, Caslsen and Stryer, 1978; Stryer, Thomas and Meares, 1982). Diffusion enhances the extent of energy transfer, and the transfer efficiency becomes limited by the distance of closest approach between the donor and acceptor.

*Corresponding author.

Another interesting regime is when the donor decay time allows diffusion during the excited state lifetime, but diffusive overlaying is not complete. As shown theoretically by Steinberg et al., (Steinberg and Katchalski, 1968) energy transfer is greatly enhanced if diffusion changes the distances between donors and acceptors during the excited state lifetime of the donor. However, there is little experimental data to show the effect of site-to-site motion in macromolecules on energy transfer because of suitable fluorophores not been available. Clearly, it is essential to develop intermediate lifetime donors ($\sim 1 \mu\text{s}$) to allow energy transfer to be sensitive to lateral diffusion in membranes and domain motions in proteins.

Metal–ligand complexes (MLCs), as a class of luminescent probes, are unique in that their biophysical properties, such as maximum emission wavelength, excited state lifetime, and quantum efficiency, can be varied by changing the coordinate ligands. Importantly, the lifetimes can be varied from tens of ns to $10 \mu\text{s}$. Through engineering the ligands, complexes with suitable spectral properties can be obtained. Recently, it has been proven in this laboratory that luminescent metal–ligand complexes (MLCs) can serve as donors in analytical assays based on energy transfer (Youn, Terpetschnig, Szmecinski and Lakowicz, 1995; Tolosa, Szmecinski, Rao and Lakowicz, 1997).

There is considerable interest in the role of lipid self-organization and diffusion in the regulation of membrane-mediated events. An important contribution to the study of membranes by fluorescence is the work of Fung and Stryer (1978) who investigated the dependence of transfer efficiency upon the surface density of unassociated donors and acceptors. Resonance energy transfer has also been used to determine the location of DPH in bilayer vesicles (Davenport, Dale, Bisby and Cundall, 1985) and to investigate associations between proteins and membranes (Rehorek, Dencher and Heyn, 1983; Kleinfeld and Lukacovic, 1985).

In this paper, we report the study of energy transfer between luminescent-labeled phospholipids in model membrane vesicles composed of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine(DOPC). The reactivity of the primary amino group of the phosphatidyl ethanolamine was used to synthesize a luminescent phospholipid derivative (Re–PE) of $[\text{Re}(4,7\text{-Me}_2\text{phen})(\text{CO})_3(4\text{-COO HPy})](\text{PF}_6)$, where 4,7-Me₂phen is 4,7-dimethyl-1,10-phenanthroline, 4-COOHPy is isonicotinic acid, and PE stands for dipalmitoyl-L-phosphatidyl ethanolamine (Fig. 1). The μs lifetime rhenium–lipid conjugate (Re–PE) serves as the energy donor and a commercially available fluorescent lipid derivative, *N*-(Texas Red sulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Tr–PE), serves as an acceptor. These two lipid analogues allow minimal membrane perturbation. They are randomly distributed in the plane of the membrane and the chromophores are probably located at the lipid–water interface. The Förster distance (R_0) at which energy transfer is 50% efficient is $35.3 \text{ \AA}$ for this donor–acceptor pair. Since donor and acceptor are probably localized in the head group region, and membrane thickness of the small unilamellar vesicles ($\sim 50 \text{ \AA}$) is larger than R_0 , resonance energy transfer is expected between donors and acceptors in the same side of the bilayer. We have found that the extent of the energy transfer highly depends on the surface density of the acceptor. However, there is a discrepancy for the transfer efficiency from our experiment data and the theoretical prediction based on the method of Fung and Stryer (Fung and Stryer, 1978). This theory provides the general

solution in a two-dimensional system in the absence of lateral diffusion. The present experiments indicates that lateral diffusion enhances the transfer efficiencies over that predicted from Fung and Stryer. This increase in transfer efficiency was used to estimate the diffusion coefficient in the DOPC membranes. Further development of theory and software is needed for precise calculation of the lateral diffusion coefficient.

2. Theory

A general solution of resonance energy transfer on a two-dimensional membrane surface in the absence of diffusion has been derived by Fung and Stryer (1978). The rate of energy transfer k_T from a donor to an acceptor separated by a distance r is

$$k_T = \frac{1}{\tau_D} \left(\frac{R_0}{r} \right)^6 \quad (1)$$

where τ_D is the excited state lifetime of the donor in the absence of acceptor and R_0 is the Förster distance at which the rate of transfer is equal to the decay rate of the donor (τ_D^{-1}) (Förster, 1948). One simple expression of the distance (in Å) is given by (Lakowicz, 1999)

$$R_0 = 9.79 \times 10^3 (\kappa^2 n^{-4} Q_D J)^{1/6} \quad (2)$$

where κ^2 is a factor describing the relative orientation in space of the transition dipoles of the donor and acceptor, n is the refractive index of the medium, Q_D is the quantum yield of the donor in the absence of the acceptor, and J is the overlap integral expressing the degree of spectral overlap between the donor emission and acceptor absorption (in $M^{-1} cm^3$). Assuming no transfer between energy donors, and no diffusion during the excited state lifetime of the donor, the fluorescence intensity decay of the donor is as follows

$$I_D(t) = I_D(0) e^{-\sigma S(t)} \quad (3)$$

$$S(t) = \int_{r_c}^{\infty} [1 - e^{(-t/\tau_D)(R_0/r)^6}] 2\pi r dr. \quad (4)$$

In these equations $e^{-\sigma S(t)}$ is the energy transfer term, σ is the surface density of the acceptor, and r_c is the distance of closest approach between the donor and acceptor. The energy transfer efficiency is obtained by

$$E = 1 - \frac{\int_0^{\infty} e^{-t/\tau_D} e^{-\sigma S(t)} dt}{\int_0^{\infty} e^{-t/\tau_D} dt}. \quad (5)$$

Two features are worth a mention. The transfer efficiency increases with the surface density of the acceptor, and R_0 and is independent of the surface concentration of the donor. For the

donor–acceptor pair with $R_0=35.3 \text{ \AA}$ in the present study, the theoretical transfer efficiency was obtained by numerical integration of Eqs. (3)–(5) for σ ranging from 0 to 0.0145 acceptors per phospholipid. The efficiency curve is shown later in the paper (see Fig. 7) for comparison with experimental data. The distance of closest approach here is assumed to be $8.6 \text{ \AA}$, which is the square root of the area occupied by single DOPC molecule (Beitinger, Vogel, Möbius and Rahmann, 1989).

3. Materials and methods

The syntheses of $[\text{Re}(4,7\text{-Me}_2\text{phen})(\text{CO})_3(4\text{-COOHPy})](\text{PF}_6)$, where 4,7-Me₂phen is 4,7-dimethyl-1,10-phenanthroline and 4-COOHPy is isonicotinic acid, and its phospholipid analogue (Re–PE) serving as the energy donor were described in the previous report (Li, Castellano, Gryczynski and Lakowicz, 1999). The energy acceptor, *N*-(Texas Red sulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Tr-PE), was obtained from Molecular Probes and used as received. 1,2-dioleoyl-*sn*-glycero-3-phosphocholine(DOPC) was from Sigma Chemical Co. All solvents and reagents were obtained from Aldrich and used without further purification. Water was deionized with a Milli-Q purification system.

3.1. Preparation of phospholipid vesicles

For vesicle preparation, aliquots of the donor and acceptor phospholipids and DOPC in CHCl_3 were taken from stock solutions, and the solvent was removed by a stream of argon. The molar ratio of Re–PE to Tr–PE was kept constant at 4.5:1 while the amount of DOPC was varied to obtain molar ratios of Tr–PE to DOPC ranging from 0 to 0.0145. Vesicles were prepared by sonication under an atmosphere of argon in 0.1 M sodium phosphate buffer, pH 7.2, at final lipid concentrations ranging from 0.5 to 7.0 mg/ml. Using this preparation procedure, the vesicle diameter is between 200 and 250 $\text{\AA}$ was determined through anisotropy measurements using the long lifetime ruthenium complex lipid (Li, Szmazinski and Lakowicz, 1997).

3.2. Instrumentation

Absorption and emission spectra were recorded on a HP 8453 diode array spectrophotometer and a SLM AB2 fluorimeter under magic angle polarization conditions, respectively. The frequency-domain fluorimeter (ISS, Koala) utilized 325 nm excitation from a HeCd laser (Liconix, 20 mW). This laser was passed through a Pockels cell operated from an ISS low frequency amplifier (K2.LF) which provided cw, modulated light from 3 kHz–2.5 MHz. Two PTS frequency synthesizers (PTS-500) were used to modulate the Pockels cell and detection system. For fluorescence intensity measurements, a 500 nm cut-off filter (500FH90–50S) and two short-wavelength pass filters (550FL07–50S) from Andover were used to isolate the emission of the energy donor from that of the acceptor.

The frequency-domain intensity decays were best fit to a three-exponential decay law

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \quad (6)$$

where α_i is the amplitude of the intensity decay time τ_i , with $\sum \alpha_i = 1.0$. Two types of average lifetimes (Sillen and Engelborghs, 1998) are in widespread use. The intensity average lifetime is defined as

$$\bar{\tau} = \sum_i \alpha_i \tau_i^2 / \sum_i \alpha_i \tau_i, \quad (7)$$

and amplitude average lifetime defined as

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i / \sum_i \alpha_i \quad (8)$$

For calculation of the transfer efficiency the value of $\langle \tau \rangle$ should be used (Wu and Brand, 1994; Lakowicz, 1999).

4. Results

The molecular structures of the energy donor (Re-PE) and acceptor (Tr-PE) are given in Fig. 1. The absorption spectra of Re-PE, Tr-PE, and both Re-PE and Tr-PE in DOPC vesicles at 20°C are shown in Fig. 2. During these measurements, the amount of either donor or acceptor alone was kept the same as that when both donor and acceptor were present with the molar ratio of 4.5/1. The ratio of Tr-PE to DOPC is 0.00676 (Fig. 2). The strong UV band at 280 nm and the broad shoulder absorbance above 300 nm in the presence of Re-PE are due to the ligand-centered transition and MLCT transition, respectively (Wallace and Rillema, 1993). Importantly, the absorption spectrum with both Re-PE and Tr-PE present closely approaches to the sum of the spectra of Re-PE and Tr-PE alone in bilayer membranes, which suggests that there is no ground state interaction between the donor (Re-PE) and acceptor (Tr-PE).

For resonance energy transfer studies, we obtained the Förster distance, R_0 , by means of the spectral overlap based on Eq. (2) (Fig. 3). Using $n=1.4$, the donor quantum yield of 0.075, and assuming $\kappa^2=2/3$, R_0 was calculated to be 35.3 Å from the spectral overlap integral of $2.07 \times 10^{-13} \text{ M}^{-1} \text{ cm}$. Since the energy acceptor, Tr-PE, highly fluoresces, it is important to enhance the emission of the donor (Re-PE) relative to that of Tr-PE. In the experimental design, the molar ratio of Re-PE to Tr-PE of 4.5/1 was chosen, and a set of filters were employed with which only emission of Re-PE was detected (Fig. 4).

The emission spectra of DOPC vesicles labeled with both Re-PE and Tr-PE, in the absence of combined filters stated above, are shown as a function of the molar ratio of Tr-PE to DOPC in Fig. 5 (solid lines). In this series of measurements, the surface density of energy acceptor (Tr-PE) was varied by changing the amount of DOPC while keeping that of Re-PE to Tr-PE constant at 4.5/1. As the surface density of Tr-PE is increased, Re-PE emission at 540 nm decreases and the Tr-PE emission at 608 nm monotonically increases because of energy transfer. The extent of energy transfer can be seen clearly from the isolated emission spectra of Re-PE through the combined filters (Fig. 6). The efficiency of energy transfer is calculated by $E=1-(F/F_0)$, where F is the net integral of the emission of Re-PE in the presence of Tr-PE and F_0 is the integral in the absence of Tr-PE. These values are shown in

Fig. 7 (●) as a function of the acceptor surface density. At low surface concentration of Tr-PE, the transfer efficiency increases rapidly and then gradually reaches a plateau at high concentration of the acceptor.

There is a significant discrepancy between the transfer efficiencies obtained from the steady-state luminescence measurements and those predicted by the theory of Fung and Stryer (1978) which does not include lateral diffusion. In particular, the observed transfer efficiency from the steady state donor quenching (●) is substantially larger than the calculated values (Fig. 7, —). It seems clear that diffusion is contributing to the enhanced transfer efficiency.

We also measured the frequency-domain intensity decay of the Re-PE donor. The intensity decays of Re-PE in DOPC membranes are shown in Fig. 8 at various surface concentration of Tr-PE. These decays were fit to a three exponential decay law. The recovered lifetimes and amplitude averaged lifetimes are given in Table 1. As the surface concentration of Tr-PE is increased, the average lifetimes decrease because of energy transfer. The transfer efficiency was calculated using $E=1-(\langle\tau\rangle/\langle\tau_D\rangle)$ where τ_D is the average excited state lifetime of Re-PE in the absence of Tr-PE. These transfer efficiencies are plotted in Fig. 7 as a function of the acceptor surface density. It can be seen clearly that these transfer efficiencies are larger than those calculated using the theory of Fung and Stryer (1978). The high transfer efficiencies appear to be due to lateral diffusion of Re-PE and Tr-PE in the DOPC membranes.

5. Discussion

The use of Re-PE in the study of RET in lipid membranes is part of the continuing effort to develop the use of μ s MLCs in the study of biological macromolecules (Lakowicz, Terpetschnig, Murtaza and Szmazinski, 1997; Li, Szmazinski and Lakowicz, 1997). It is important to note that MLCs can display a variety of spectral properties depending upon the selection of the metal ion and ligands. For instance, one can extend the excited state lifetime to over 10 μ s using diphenyl-phenanthroline as a ligand (Demas, Harris and McBride, 1977). Because of such flexibility in the design of MLCs, we have been able to synthesize a long lifetime Re-PE probe for the present report. There are a few biophysical characteristics of Re-PE worthy of mention. In bilayer membranes of DOPC, Re-PE shows an excited state lifetime of $\sim 1 \mu$ s, a large Stokes' shift to an emission maximum of 540 nm, with a quantum yield of 0.075. The large Stokes' shift simplifies the experimental design because of the absence of an inner filter effect, and the lack of donor-donor interactions in the membranes. The long lifetime allows time for lateral diffusion to affect the intensity decay of the donor.

In calculating the Förster distance (R_0), we assumed that $\kappa^2=2/3$. It has been found that the anisotropy of Re-PE drops by more than 90% in the time interval of ~ 20 ns from frequency-domain anisotropy measurements of Re-PE in DPPG vesicles (Li, Castellano, Gryczynski and Lakowicz, 1999). This rapid depolarization indicates that the rhenium metal-ligand complex has a large degree of rotational freedom during its excited state lifetime ($\sim 1 \mu$ s). Also, it is known that the anisotropy of Texas Red chromophore is low with the lifetime of a few ns. Hence, the energy transfer is randomized by rotational diffusion resulting in a small uncertainty of R_0 .

In principle, the time-resolved donor intensity decays can be used to determine the mutual lateral diffusion coefficient of the donor- and acceptor-labeled lipids in the membranes. However, this requires theory for predicting the donor decays in two-dimensions in the presence of diffusion. To the best of our knowledge, such theoretical expressions have not been developed, and available theory for RET in membranes applies only to transfer in the absence of diffusion. We used the theory for RET in two dimensions to calculate the predicted intensity decays in the absence of diffusion. The simulated frequency response for the known R_0 of 35.3 is shown in Fig. 9 (---). These simulated data do not account for the observed shift in the frequency response at the known acceptor concentration.

In the absence of theory for diffusion in two dimensions we questioned how one could estimate the lateral diffusion coefficient from the data? This was accomplished by comparing the transfer efficiency calculated from the average lifetime with the transfer efficiency predicted for various values of R_0 . We found that the measured transfer efficiencies were approximated by the predicted transfer efficiencies calculated with $R_0=65$ Å. This result indicates that the effective distance between the donors and acceptors is decreased by 30 Å due to lateral diffusion.

The 30 Å difference between the known and apparent values of R_0 can be used to estimate the lateral diffusion coefficient. In a two-dimensional system the mean square displacement of a molecule is given by $\langle x^2 \rangle = 4D\tau$, where D is the diffusion coefficient and τ is the time for diffusion. Using $(\langle x^2 \rangle)^{1/2}=30$ Å and a 1.8 μs is donor lifetime for diffusion, one obtains an estimated diffusion coefficient of 2×10^{-8} cm²/s. This value is comparable to that found from fluorescence recovery after photobleaching (Gilmanshin, Creutz and Tamm, 1994; Ladha, Mackie, Harvey, Clark, Lea, Brullemans and Duclquier, 1996).

In summary, long lifetime lipid probes can provide information about the rates of lateral diffusion in membranes. Further development of theory and software is needed for a more quantitative interpretation of the donor decays in terms of the diffusion coefficients.

Acknowledgements

This work was supported by the NIH, National Center for Research Resources PN-08119, and by the National Institute for General Medical Sciences, GM-35154.

Abbreviations:

Re-PE	Conjugate of [Re(4,7-Me ₂ phen) (CO) ₃ (4-COOHPy)] with PE
PE	Dipalmitoyl-L-α-phosphatidylethanolamine
4,7-Me,phen	4,7-dimethyl-1,10-phenanthroline
TR	Texas Red
Tr-PE	N-(Texas Red Sulfonyl)-1,2-dihexadecanoyl- <i>sn</i> -glycero-3-phosphatidylethanolamine

DOPC	1,2-Dioleoyl- <i>sn</i> -glycero-3-phosphatidylcholine
FD	Frequency domain
RET	Resonance energy transfer
4-COOHPy	Isonicotinic acid

References

- Beitinger H, Vogel V, Möbius D, Rahmann H, 1989 Biochim. Biophys. Acta 984, 293. [PubMed: 2775779]
- Davenport L, Dale RE, Bisby RH, Cundall RB, 1985 Transverse location of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene in model lipid bilayer membrane systems by resonance excitation energy transfer. Biochemistry 24, 4097–4108. [PubMed: 3931673]
- Demas JN, Harris EW, McBride RP, 1977 J. Am. Chem. Soc 99, 3547–3551.
- Förster T, 1948 Intermolecular energy migration and fluorescence. Ann. Physik 2, 55–75.
- Fung BK-K, Stryer L, 1978 Surface density determination in membranes by fluorescence energy transfer. Biochemistry 17, 5241–5248. [PubMed: 728398]
- Gilmanshin R, Creutz C, Tamm L, 1994 Annexin IV reduces the rate of lateral lipid diffusion and changes the fluid phase structure of the lipid bilayer when it binds to negatively charged membranes in the presence of calcium. Biochemistry 33, 8225–8232. [PubMed: 8031756]
- Kleinfeld AM, Lukacovic MF, 1985 Energy-transfer study of cytochrome b5 using the anthroyloxy fatty acid membrane probes. Biochemistry 24, 1883–1890. [PubMed: 4016089]
- Ladha S, Mackie AR, Harvey LJ, Clark DC, Lea EJ, Brullemans M, Duclohier H, 1996 Lateral diffusion in planar lipid bilayers: a fluorescence recovery after photobleaching investigation of its modulation by lipid composition, cholesterol, or alamethicin content and divalent cations. Biophysical J. 71, 1364–1373.
- Lakowicz JR, 1999 Principles of fluorescence spectroscopy, 2nd Edition. Plenum Press, New York.
- Lakowicz JR, Terpetschnig E, Murtaza Z, Szmazinski H, 1997 Development of long-lifetime metal-ligand probes for biophysics and cellular imaging. J. Fluoresc 7, 17–25.
- Li L, Castellano FN, Gryczynski I, Lakowicz JR, 1999 Long-lifetime lipid rhenium metal-ligand complex for probing membrane dynamics on the microsecond timescale. Chem. Physics of Lipids, 99, 1–9.
- Li L, Szmazinski H, Lakowicz JR, 1997 Synthesis and luminescence spectral characterization of long-lifetime lipid metal-ligand probes. Anal. Biochem 244, 80–85. [PubMed: 9025912]
- Rehorek M, Dencher NA, Heyn MP, 1983 Fluorescence energy transfer from diphenylhexatriene to bacteriorhodopsin in lipid vesicles. Biophys. J 43, 39–45. [PubMed: 6882861]
- Sillen A, Engelborghs Y, 1998 The correct use of ‘average’ fluorescence parameters. Photochem. Photobiol 67, 475–486.
- Steinberg IZ, Katchalski E, 1968 Theoretical analysis of the role of diffusion in chemical reactions, fluorescence quenching, and nonradiative energy transfer. J. Chem. Phys 48 (6), 2404–2410.
- Stryer L, 1978 Fluorescence energy transfer as a spectroscopic ruler. Ann. Rev. Biochem 47, 819–846. [PubMed: 354506]
- Stryer L, Thomas DD, Meares CF, 1982 Diffusion-enhanced fluorescence energy transfer. Ann. Rev. Biophys. Bioeng 11, 203–222. [PubMed: 7049062]
- Thomas DD, Caslens WF, Stryer L, 1978 Fluorescence energy transfer in the rapid diffusion limit. Proc. Natl. Acad. Sci 75, 5746–5750. [PubMed: 16592590]
- Tolosa L, Szmazinski H, Rao G, Lakowicz JR, 1997 Lifetime-based sensing of glucose using energy transfer with a long lifetime donor. Anal. Biochem 250, 102–108. [PubMed: 9234903]
- Wallace L, Rillema DP, 1993 Photophysical properties of rhenium(I) tricarbonyl complexes containing alkyl- and aryl-substituted phenanthrolines as ligands. Inorg. Chem 32, 3836–3843.

- Wu PG, Brand L, 1994 Resonance energy transfer: methods and applications. *Anal. Biochem* 218, 1–13. [PubMed: 8053542]
- Youn HJ, Terpetschnig E, Szmecinski H, Lakowicz JR, 1995 Fluorescence energy transfer immunoassay based on a long-lifetime luminescent metal-ligand complex. *Anal. Biochem* 232, 24–30. [PubMed: 8600827]

Fig. 1.
Molecular structure of the energy donor (Re-PE) and acceptor (Tr-PE).

Fig. 2. Absorption spectra of Re-PE (---), Tr-PE (···), and both Re-PE and Tr-PE (—) in DOPC vesicles at room temperature.

Fig. 3. Emission spectrum of Re-PE (—) and absorption spectrum of Tr-PE (- - -) in DOPC vesicles. Also shown is the overlap of the two spectra as the shaded area.

Fig. 4. Normalized emission spectra of Re-PE (—) and Tr-PE (---) in DOPC vesicles. Also shown is the observation window for detecting emission of the donor (Re-PE) alone, via transmittance of the combined filters stated in the text, in the measurement of the frequency-domain intensity decays (···).

Fig. 5. Emission spectra showing energy transfer from Re-PE to Tr-PE in DOPC vesicles as a function of the molar ratio of Tr-PE to DOPC. The ratio of Re-PE to Tr-PE was kept constant at 4.5:1. For comparison, the emission spectrum of Tr-PE alone is also shown (···).

Fig. 6. Emission spectra, obtained by means of combined filters quoted in the text, show energy transfer from Re-Pe to Tr-PE in DOPC vesicles by the decrease of the fluorescence intensity of Re-PE as the ratio of Tr-PE to DOPC is increased.

Fig. 7. Energy transfer efficiencies obtained based on steady-state fluorescence intensity, SS, amplitude average lifetime ($\langle \tau \rangle$), and theoretical calculation using Eqs. (3), (4) and (5) (—) as a function of the molar ratio of Tr-PE to DOPC.

Fig. 8. Frequency-domain intensity decays of Re-Pe at various molar ratios of Tr-Pe to DOPC in bilayer membranes. The solid lines are the fitting curves by three exponential decay law.

Fig. 9.

Frequency-domain intensity decays of Re-PE in the absence of Tr-PE (●) and in the presence of Tr-PE at the ratio of Tr-PE to DOPC of 0.0101 (■). Also shown are the simulated data using Eqs. (3) and (4) in which diffusion process is not included (---). The solid lines are the fitting curves by three exponential decay law.

Fig. 10. Energy transfer efficiencies based on amplitude average lifetime (○), and theoretical calculations for R_0 of 35.3 Å (—) and 65 Å (---) using Eqs. (3)–(5) as a function of the acceptor surface density.

Table 1

The lifetimes recovered from frequency-domain intensity decays as a function of the surface density of the acceptor (Tr-PE)

Tr-PE/DOPC	a_I	τ_i (ns)	$\langle \tau \rangle$ (ns)	χ_R^{2a}
0	0.132	110.4	-	-
	0.534	377.5	986.3	3.11
	0.334	2303.4	-	-
	0.548	75.3	-	-
0.00202	0.247	547.2	581.8	1.09
	0.205	1977.2	-	-
	0.588	62.7	-	-
0.00303	0.265	480.7	428.4	2.84
	0.147	1791.6	-	-
	0.611	85.0	-	-
0.00450	0.303	598.7	383.9	1.95
	0.0860	1758.3	-	-
	0.617	60.1	-	-
0.00676	0.344	510.7	280.1	1.65
	0.0390	1723.4	-	-
	0.653	33.2	-	-
0.0101	0.319	409.7	191.6	1.72
	0.028	1415.5	-	-
	0.732	16.9	-	-
0.0145	0.243	341.3	122.7	3.65
	0.025	1083.7	-	-

^a χ_R^2 were obtained from the least-squares analysis under the condition that the uncertainties in the measured phase angle and modulation were 0.4° and 0.01, respectively.