Efficient Photoinduced Orthogonal Energy and Electron Transfer Reactions via Phospholipid Membrane-Bound Donors and Acceptors

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Abstract: A three component, liposome-bound photochemical molecular device (PMD) consisting of energy and electron transfer reactions is described. Bilayer membrane surface-associated dyes, 5,10,15,20-tetrakis[4-(trimethylammonio)-phenyl]-21*H*,23*H*-porphine tetra-*p*-tosylate salt and *N*,*N*'-bis[(3-trimethylammonio)propyl]thiadicarbocyanine tribromide, are the energy donor and acceptor, respectively, in a blue light stimulated energy transfer reaction along the vesicle surface. The electronically excited cyanine is quenched by electron transfer from the phospholipid membrane bound triphenylbenzyl borate anion, which is located in the lipid bilayer interior. The PMD exhibits sequential reactions following electronic excitation with the novel feature that the steps proceed with orthogonal orientation: energy transfer occurs parallel to the membrane surface, and electron transfer occurs perpendicular to the surface. Photobleaching and fluorescence quenching experiments verify the transfer reactions, and Stern–Volmer analysis was used to estimate the reaction rate constants. At the highest concentrations examined of energy and electron acceptor ca. 60% of the photoexcited porphyrins were quenched by energy transfer to the cyanine. The use of liposomes of well defined composition and dimensions in conjunction with molecular components that associate with the bilayer in a predictable manner permit the accurate estimation of molecular binding volumes and local concentrations for the analysis.

Introduction

Photochemical molecular devices (PMDs) have been defined by Balzani and Scandola[1] as "assemblies of molecules capable of performing light-induced functions". Examples of such systems range from simple donor–acceptor diad molecules[2] to the intricate reaction centers of photosynthetic organisms.[3-5] These systems are under intense investigation both for practical applications such as solar energy conversion as well as to understand the basic process involved in light energy transduction mechanisms in biological systems.

The individual components of PMDs most often function as donors or acceptors for energy and/or electron transfer reactions. Reasonable reaction efficiencies necessitate that the components be held in close proximity to one another. Conceptually, the simplest means of fulfilling this requirement is through covalent linkage of the various moieties. Such an approach offers fine control over intercomponent distances and orientations,[2,6-11] parameters which are critical to energy and electron transfer applications. One potential drawback to covalently linked systems is that the overall synthetic yields are often low. Recently, PMDs have been reported in which the individual components are assembled by noncovalent interactions such as complementary hydrogen bonding patterns[12] and simultaneous coordination to transition metals.[13,14]

Another strategy for PMD assembly involves sequestering the desired components within microheterogeneous media such as micelles, microemulsions, or liposomes. These supramolecular assemblies offer two appealing features: (1) Confining reactants to organized media effectively increases their local concentrations, often leading to enhanced or altered reactivity relative to that observed in homogeneous media.[15,16] (2) The anisotropic nature of organized media permits localization of reactants within different regions of the assembly, facilitating vectorial reactions.[17-20]

Within the regime of supramolecular assemblies, lipid bilayers represent an extremely flexible medium in which to confine chemical reactions. Judicious selection of constituent lipids as well as membrane-bound reactants can lead to reaction at the hydrophilic surface, in the hydrophobic interior or across the interface of the membrane in a reasonably predictable manner. In this report, we present a liposome-based PMD consisting of a cationic porphyrin (antenna pigment), a cationic cyanine dye (receiver pigment), and an anionic borate (electron donor). Previously, we demonstrated vectorial photoinduced electron transfer from the borate to the cyanine dye in phospholipid

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membranes.[21,22] The hydrophobic triphenylbenzylborate anion, bound in the interior of the bilayer, functioned as electron donor while the hydrophilic cationic cyanine dye acting as light absorber and electron acceptor was electrostatically bound to the membrane surface. Irradiation with red light initiated vectorial electron flow from the membrane interior to the surface. Also previously demonstrated was an energy transfer system that utilizes a surface-associated porphyrin as an energy donor to sensitize the aforementioned cyanine dye.[23] This blue light sensitive system performs an energy transfer reaction on the vesicle surface. Coupling the porphyrin-cyanine energy transfer with the cyanine-borate electron transfer results in the system described here, where the sensitivity of the simple cyanine-borate electron transfer PMD has been extended to the blue region of the visible spectrum, with the surface-associated porphyrin acting as an antenna pigment. Ideally, irradiation with blue light causes excitation of the porphyrin followed by energy transfer to the cyanine, triggering electron transfer from the borate to the cyanine. The PMD thus exhibits two sequential reactions following excitation with the novel feature that the two steps proceed with orthogonal orientation: energy transfer occurs parallel to the membrane surface, while the subsequent electron transfer step occurs perpendicular to the surface.

Experimental Section

Materials. *N,N'*-Bis[(3-trimethylammonio)propyl]thiadicarbocyanine tribromide (Cy^{3+}) was purchased from Molecular Probes (Eugene, OR) and used as received. 5,10,15,20-Tetrakis[4-(trimethylammonio)phenyl]-21*H*,23*H*-porphine, tetra-*p*-tosylate salt (P^{4+}), and sodium tetraphenylborate (Ph_4B^-) were purchased from Aldrich Chemical Company (Milwaukee, WI) and used as received. Methanol and acetonitrile (J. T. Baker Inc., Phillipsburg, NJ; Photrex grade) were used as received. Milli-Q water (Millipore Co., Bedford, MA) was used in all experiments. L-α-Dioleoylphosphatidylcholine (DOPC) and L-α-dioleoylphosphatidic acid (DOPA) were purchased from Avanti Polar Lipids (Pelham, AL) and used as received. Purity was checked by thin-layer chromatography (65:25:4 $CHCl_3$/MeOH/H_2O eluent). Lipids were stored as chloroform solutions at 5 °C.

Synthesis of the tetramethylammonium salt of triphenylbenzylborate (Ph_3BnB^-) is reported elsewhere.[24] The white crystalline solid gave satisfactory ^1H NMR and melting (decomposition) points.

Liposome Preparation. An appropriate amount of DOPC/DOPA (9:1) stock solution (in chloroform) was added to a 10-mL pear-shaped flask. The solvent was evaporated by passing a stream of argon gas over the sample, leaving a film of lipid on the wall of the flask. Residual solvent was removed by drying under vacuum for at least 8 h. The lipid film was hydrated with buffer (10 mM imidazole, pH = 7.0) and vortexed. The sample was then subjected to 10 freeze-thaw cycles, accomplished by immersion for 4 min in a dry ice/isopropyl alcohol bath followed by 10 min in a water bath. Liposomes were formed by extrusion 10 times through two 0.1 μm polycarbonate filters (Nuclepore Co.; Pleasanton, CA) at a pressure of 250 psi.[25] The extruder (Lipex Biomembranes, Inc.; Vancouver, B.C.) was warmed to a temperature of 37 °C.

Determination of Liposome Concentration. The resulting liposomes were assayed for phosphatidylcholine content (90% of the total lipid content).[26] This was done by removing 40 μL of the liposome suspension and adding this to approximately 0.5 mL spectral grade methanol in a test tube. The solvent was then removed by placing the test tube in a warm water bath and passing argon gas over the solution. Subsequently, 0.5 mL spectral grade chloroform was added and evaporated as above. The first step ensures that the liposomes are disrupted, while the second guarantees the complete removal of methanol. This is particularly important since methanol can cause inaccurate results in the assay.

Aftery drying, 2.0 mL spectral grade chloroform was added to the lipid film followed by 2.0 mL of the ammonium ferrothiocyanate test solution (prepared by dissolving 3.04 g of ammonium thiocyanate and 2.703 g of ferric chloride in 100 mL of water). The test tube was vortexed for 1 min and then allowed to stand for 10 min. The optical density of the chloroform layer at 488 nm was measured and compared to a calibration curve to determine the phosphatidylcholine content in the liposome suspension. (Phosphatidic acids are not detected in this assay, which requires a positive charge to be present in the lipid.) The entire procedure was done in triplicate. The calibration curve was generated by starting with a solution of DOPC in chloroform of known concentration (in which case the evaporation steps were unnecessary).

The liposomes were sized by dynamic laser light scattering (Brookhaven BI-80000AT correlator with a 5-mW He-Ne laser light source operating at 632.8 nm; Brookhaven Instruments Corp., Holtsville, NY) utilizing four separate analysis packages to extract the set of exponential functions which made up the autocorrelation functions. Measurements were made in triplicate at 60°, 90°, and 120° scattering angles.[27] Liposomes prepared by extrusion as outlined above were 105 ± 5 nm in diameter.

The diameter together with the lipid concentration were then used to determine the liposome concentration. The number of lipids in a single unilamellar liposome (N_L) was calculated using values for the bilayer width (50 Å),[28] the area per molecule (70 Å^2),[29] and the following equation

$$N_L = 4\pi(r_o^2 + r_i^2)/70 = 9.0 \times 10^4$$

where r_o = 525 Å and r_i = 475 Å correspond to the outer and inner radii of the liposome. The lipid concentration determined by the ammonium ferrothiocyanate assay was then divided by the number of lipids per liposome to give the liposome concentration.

Binding of Pigments to Liposomes. Cy^{3+} was previously found to bind to DOPC/DOPA liposomes using absorption and fluorescence spectroscopies.[21,22] The UV–vis absorption spectra of 3.85 μM solutions of P^{4+} in the absence and presence of DOPC/DOPA liposomes (0.5 mg/mL = 0.6 mM lipid) were acquired on a Varian DMS-200 Varian Techtron Pty. Limited, Victoria, Australia) double beam instrument. The absorption maximum shifted from 410 to 417 nm in the presence of the liposomes.

Fluorescence spectra of 10.0 μM solutions of P^{4+} in the absence and presence of 1.0 nM DOPC/DOPA liposomes were acquired on a Spex Fluorolog II spectrofluorometer (Spex Industries, Inc., Edison, NJ). Excitation was at 414 nm, which is an isosbestic point in the absorption spectra for the porphyrin in water and on DOPC/DOPA liposomes.

Additionally, P^{4+} was added to 5.0 nM DOPC/DOPA liposomes in 10 P^{4+} per liposome increments, up to 100 P^{4+}. The emission spectrum was recorded after each addition, and the integrated emission intensity was plotted versus number of porphyrins per liposome to investigate possible self-quenching. The procedure was repeated for Cy^{3+} using excitation at 650 nm and 40 cyanine per liposome increments up to 400 Cy^{3+}. The emission intensity increased linearly with added dye in both cases indicative of a lack of self-quenching.

Calculation of Concentration of Membrane-Bound Cy^{3+} and Ph_3BnB^-. In order to calculate the concentration of membrane-bound Cy^{3+} and Ph_3BnB^- the volume of their respective binding domains within the lipid bilayer must be estimated. For Cy^{3+}, we assume that the dye is bound only to the exterior surface of the membrane. This seems reasonable as human erythrocytes are impermeable to Cy^{3+}.[30] Our second assumption is that Cy^{3+} is bound within a 5 Å-wide domain within the hydrophilic head-group region of the membrane. This is consistent with our previous arguments favoring a well-defined binding site for liposome-bound Cy^{3+}.[22] For a 1050 Å-diameter liposome the volume of the outermost 5 Å of the membrane is 1.7×10^7 Å^3 = 1.7×10^{-20} L. Multiplying this number by the total number of liposomes in the sample (vide supra) gives the total volume available to Cy^{3+}. Binding of 200 Cy^{3+} per liposome gives a concentration of 19.5 mM. Alternatively, estimating the width of the Cy^{3+} binding domain at 3.5 Å and multiplying this by the surface area of a vesicle will provide a different binding volume (1.1×10^{-20} L/vesicle). The former calculation was used here; whereas the latter method was used in a previous publication.[23]

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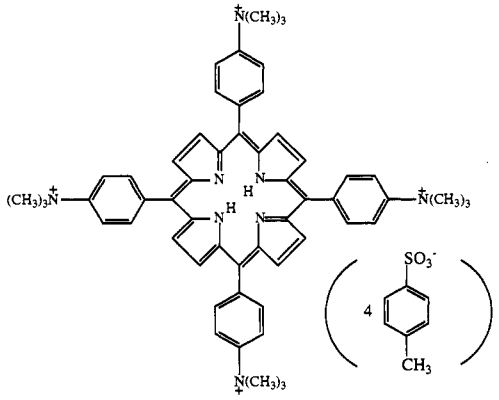
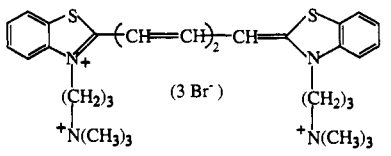
Chart 1



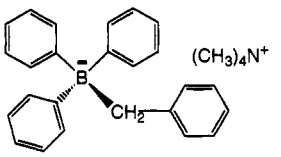
P^{4+}

Function: Light Absorber
Energy Donor



Cy^{3+}

Function: Energy Acceptor
Electron Acceptor



Ph_3BnB^-

Function: Electron Donor

For Ph_3BnB^-, the volume of the binding domain is estimated by analogy with Ph_4B^-, a close structural analogue. Flewelling and Hubbell have assigned Ph_4B^- to two binding sites within the membrane, each approximately 8 Å wide and 5 Å from the nearest surface.[31] While diffusion by Ph_3BnB^- between the two binding sites is permitted, the depth of the potential wells at these sites indicates that at any given time virtually all of the borate molecules will be in one of the two sites. Therefore, we take the total volume of these two binding domains, 5.0×10^7 Å^3 = 5.0×10^{-20} L per liposome, as the available volume. Binding of 200 Ph_3BnB^- per liposome gives a concentration of 6.6 mM.

Ph_4B^- partitions from water into lipid bilayers with an equilibrium constant of 10^5.[32-34] Ph_3BnB^- is expected to have similar partitioning behavior. The volume of bilayer available for borate binding is four orders of magnitude less than the bulk aqueous volume. This difference creates the possibility that as much as 10–15% of the borate molecules are solvated in the buffer. If borate molecules located in the bulk aqueous phase complexed with Cy^{3+}, they would induce changes in the cyanine absorption spectrum as previously reported.[21,22,24] If this occurs it should be more pronounced at lower vesicle concentrations due to the greater difference in the bilayer to water volume ratio. The Cy^{3+} absorption spectral shapes at 1 and 5 nM vesicle concentrations with 400 Cy^{3+} and 1000 Ph_3BnB^- per liposome were identical when normalized for the concentration difference. Therefore, only membrane bound borates appear to interact with the dye molecules.

Singlet Lifetimes of Membrane-Bound Cy^{3+} and P^{4+}. The singlet excited state lifetimes of membrane-bound Cy^{3+} and P^{4+} were estimated based on their respective lifetimes in water. For P^{4+}, Kalyanasundaram reported a value of 9.3 ns.[35] The lifetime of Cy^{3+} in water was measured at the University of Illinois Laser Laboratory using a picosecond timescale apparatus described elsewhere.[36] A value of 950 ps was obtained. Assuming that the radiative rate constants for the two pigments do not change upon binding to the liposome, the lifetimes of the membrane-bound dyes are given by the following equation

$$\tau^{membrane} = \frac{\Phi_f^{membrane}}{\Phi_f^{H_2O}} \tau^{H_2O}$$

The relative fluorescence yields were obtained by exciting liposomal and aqueous solutions of each dye at their respective isosbestic points in order to insure excitation of equal numbers of dye molecules in each case. The lifetimes ($\tau^{membrane}$) obtained by this procedure are 10.5 ns for P^{4+} and 1.7 ns for Cy^{3+}.

Energy Transfer Experiments. P^{4+} was added to a suspension of 5.0 nM DOPC/DOPA liposomes in a ratio of 10 porphyrins per liposome. The fluorescence emission spectrum was recorded with excitation at 417 nm, the P^{4+} absorption maximum. Subsequent spectra were recorded after addition of Cy^{3+} to the liposomes in 20 Cy^{3+} per liposome increments, up to 460 Cy^{3+} per liposome. The ratio of emission intensity at 650 nm without and with Cy^{3+} was calculated for each addition and plotted versus cyanine concentration. Additionally, a sample was prepared having 460 Cy^{3+} per liposome but no P^{4+}. The emission spectrum was then acquired under identical conditions to the porphyrin-containing sample.

Fluorescence Quenching Experiments. Electron-transfer quenching of P^{4+} and Cy^{3+} fluorescence by Ph_3BnB^- was studied using standard Stern–Volmer analysis.[37,38] P^{4+} (10 per liposome) was added to 5.0 nM DOPC/DOPA liposomes. The emission intensity at 696 nm was measured with excitation at 417 nm. Ph_3BnB^- was added in 200 borate-per-liposome increments up to 1000 Ph_3BnB^- per liposome. The emission intensity was recorded after each addition. Similar experiments were run for Cy^{3+} (various concentrations, excitation at 669 nm) and mixtures of P^{4+} and Cy^{3+} (excitation at 417 nm). The ratio of the emission intensity without and with Ph_3BnB^- (I_0/I) was calculated for each addition and plotted versus [Ph_3BnB^-].

Electron Transfer Experiments: Photobleaching. A sample containing 6.25 nM DOPC/DOPA liposomes with P^{4+}, Cy^{3+}, and Ph_3BnB^- (variable amounts of each per liposome) was purged in the dark with argon gas for 15 min (P^{4+} photobleaching occurred in the absence of Cy^{3+} and Ph_3BnB^- in air-equilibrated samples). The sample was then irradiated in the fluorometer sample compartment using 417 nm light with 1.0 mm (3.6 nm bandpass) slits on the excitation monochromator to filter the light from the excitation source, a 450W Xe lamp (Osram Corp.; Newburgh, NY). The sample was stirred during irradiation, and the shutter in the instrument was opened and closed from the computer keyboard. UV–vis spectra were recorded every 2 min, and the reaction was followed by monitoring the loss in optical density of the two pigments.

Results

Optical Properties of Pigments. Structures of P^{4+} and Cy^{3+} are shown in Chart 1. Addition of either pigment (or a combination of the two) to a suspension of DOPC/DOPA liposomes in low ionic strength buffer results in electrostatic association of the cationic dye(s) with the anionic membranes. Binding results in (a) bathochromic shifts of the absorption maxima, (b) enhanced fluorescence yields, and (c) increased singlet lifetimes of both dyes (Table 1). Similar spectral perturbations have been observed for binding of anionic cyanine

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Table 1. Photophysical Properties of Cy^{3+} and P^{4+}

dye	λ_{max} (H_2O) (nm)	λ_{max} (vesicle) (nm)	fluorescence yield enhancement	τ, ns
Cy^{3+}	650	669	90%	1.7
P^{4+}	410	417	13% (av)	10.5 (av)

dyes to cationic lipid bilayers.[39,40] As seen in Figure 1, the absorption spectra of P^{4+} and Cy^{3+} are completely resolved from one another, permitting selective excitation of either chromophore. However, the fluorescence emission spectrum of P^{4+} has extensive overlap with the absorption spectrum of Cy^{3+} (Figure 1), indicating that energy transfer from P^{4+} to Cy^{3+} should be very efficient. Thus photochemistry involving excited Cy^{3+} should result from either direct excitation of Cy^{3+} in the red or from excitation of P^{4+} in the blue followed by energy transfer to Cy^{3+}.

Energy Transfer on the Liposome Surface. Energy transfer reactions in liposome-based systems have been studied using (a) amphiphilic dyes which are integrated into the bilayer[41,42] (b) or charged, water-soluble dyes which are electrostatically associated with an oppositely charged membrane surface[43-46] and (c) a combination of amphiphilic and hydrophilic dyes.[47,48] Figure 2 illustrates the effect of increasing surface concentration of Cy^{3+} on the fluorescence emission spectrum of P^{4+}. As more Cy^{3+} is bound to the liposome surface, the characteristic doubly-peaked porphyrin fluorescence is quenched and replaced by the cyanine emission profile, evidence of singlet energy transfer from P^{4+} to Cy^{3+}. The clean isoemissive point at 660 nm indicates that quenching occurs solely by energy transfer. Excitation at 417 nm in the absence of P^{4+} leads to virtually no Cy^{3+} emission. Overall, the Cy^{3+} emission yield upon excitation at 417 nm is enhanced 60-fold by binding of just 10 porphyrins per liposome, proving that the cyanine emission evident in Figure 2 is sensitized by energy transfer from the porphyrin.

Emission intensity values from Figure 2 were used to determine the Stern–Volmer constant for energy transfer. Emission intensity ratios (I_o/I) at 650 nm without and with Cy^{3+} were plotted against cyanine concentration. The slope of the straight line obtained is the K_{sv} for energy transfer (plot not shown). K_{sv} for energy transfer is 29.1 M^{-1} s^{-1}; the corresponding rate constant (k_{ET}) is 2.7×10^9 M^{-1} s^{-1}.

R_o, the average distance of P^{4+}–Cy^{3+} separation when porphyrin emission is 50% quenched, was calculated from the experimental data. Thus 360 Cy^{3+} per liposome are required to quench the fluorescence of 10 P^{4+} per liposome by 50% (Figure 2). The R_o for 370 pigments randomly distributed on the surface of a 100 nm vesicle is 52 Å. Alternatively, R_o was calculated using the following equation[49]

$$R_o = (JK^2Qn^{-4})(9.79 \times 10^3)$$

where J is the overlap integral, K is the orientation factor (estimated to be 2/3), Q is P^{4+} quantum yield in the absence of Cy^{3+}, and n is the refracive index (1.5). The reported porphyrin quantum yield, 0.08,[35] was enhanced by 13% to 0.09 in the same manner that the lifetime was enhanced. J was calculated using the following equation

$$J = \frac{\int F_\lambda E_\lambda \lambda^4 d_\lambda}{\int F_\lambda d_\lambda}$$

where F_λ is the normalized P^{4+} emission spectrum, E_λ is the Cy^{3+} absorption spectrum in terms of extinction coefficient, and λ is the wavelength in cm. The calculated J value was 1.3×10^{-12} cm^3/M which yields a R_o of 46 Å.

Vectorial Photoinduced Electron Transfer in Liposomes: Fluorescence Quenching. Emission from surface-associated P^{4+} and Cy^{3+} is quenched by vectorial electron transfer from the hydrophobic donor Ph_3BnB^-, which resides in the membrane interior.[21,22] In initial experiments, quenching of P^{4+} in the absence of Cy^{3+} (using 417 nm excitation) and vice versa (using 669 nm excitation) by Ph_3BnB^- was studied. The resulting Stern–Volmer plots are linear (Figure 3) and yield quenching constants (K_{sv}) which, together with lifetimes (τ), permit calculation of the electron transfer rate constants

$$\frac{K_{sv}\ (M^{-1})}{\tau\ (s)} = k_{et}(M^{-1}\ s^{-1}) \qquad (1)$$

Stern–Volmer constants as well as rate constants for the energy and electron transfer reactions are collected in Table 2.

Equation 1 is derived from the classical Stern–Volmer equation.

$$\frac{\Phi_o}{\Phi} = 1 + K_{sv}[Q] = 1 + k_q\tau[Q] \qquad (Q = Ph_3BnB^-) \qquad (2)$$

This equation cannot be used for photoinduced electron transfer from Ph_3BnB^- to Cy^{3+} sensitized by energy transfer from P^{4+} to Cy^{3+}. As outlined below, a quadratic dependence of the relative fluorescence yield on borate concentration is predicted.

The quantum yield (Φ_o) for sensitized cyanine fluorescence is simply the product of the energy transfer quantum yield and the cyanine fluorescence quantum yield (eq 3)

$$\Phi_o = \frac{k_{ET}[C]}{k^p_f + k^p_{nr} + k_{ET}[C]} \cdot \frac{k^c_f}{k^c_f + k^c_{nr}} \qquad (3)$$

([C] is cyanine concentration.) In the presence of Ph_3BnB^-, the quantum yield (Φ) now includes an electron transfer quenching component:

$$\Phi = \frac{k_{ET}[C]}{k^p_f + k^p_{nr} + k_{ET}[C] + k^p_{et}[B]} \cdot \frac{k^c_f}{k^c_f + k^c_{nr} + k^c_{et}[B]} \qquad (4)$$

([B] is Ph_3BnB^- concentration.) The ratio of eq 3 to eq 4 provides an experimentally useful equation, since the quantum yield ratio is equivalent to the easily measured fluorescence intensity ratio

$$\frac{\Phi_o}{\Phi} = \left[1 + \frac{k^p_{et}[B]}{k^p_f + k^p_{nr} + k_{ET}[C]}\right]\left[1 + \frac{k^c_{et}[B]}{k^c_f + k^c_{nr}}\right] \qquad (5)$$

Equations 6 and 7 describe the lifetimes of P^{4+} and Cy^{3+}, respectively, in the absence of the electron donor.

$$\tau^p = \frac{1}{k^p_f + k^p_{nr} + k_{ET}[C]} \qquad (6)$$

$$\tau^c = \frac{1}{k^c_f + k^c_{nr}} \qquad (7)$$

(Note that this τ^p is not equal to the lifetime for P^{4+} given in

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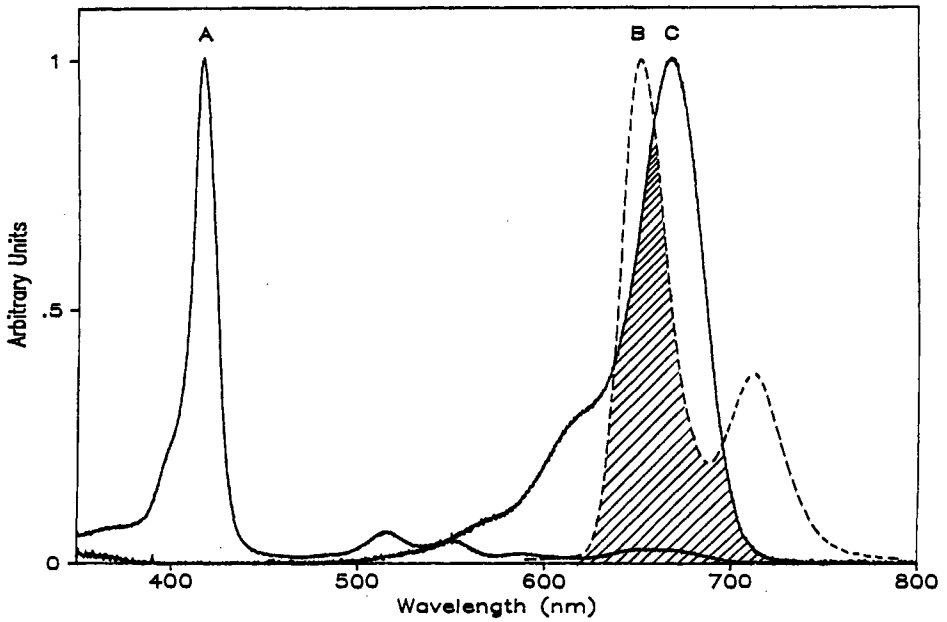


Figure 1. Absorption spectra of liposome bound P^{4+} (A) and Cy^{3+} (C) and emission spectrum of P^{4+} (B). Overlap of P^{4+} emission with Cy^{3+} absorption is shaded.

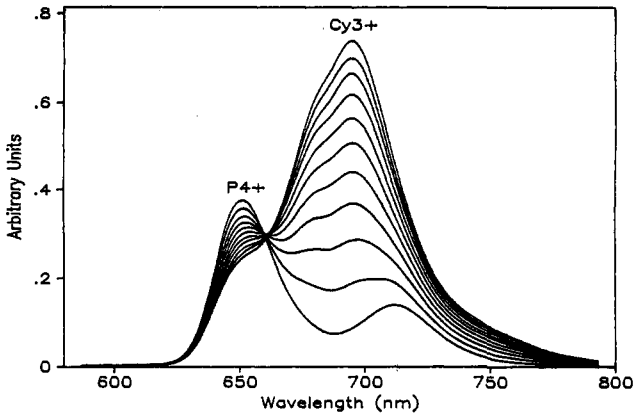


Figure 2. Emission spectra of liposome-bound P^{4+} (10 per liposome; 4.7 nM liposomes) in the presence of increasing amounts of Cy^{3+}. Each successive spectrum corresponds to addition of 20 Cy^{3+} per liposome. Excitation at 417 nm.

Table 1.) Substitution of eqs 6 and 7 into eq 5 provides a much simplified form of the quantum yield ratio.

$$\frac{\Phi_o}{\Phi} = (1 + k^{P}_{et}\tau^{P}[B])(1 + k^{C}_{et}\tau^{C}[B]) \qquad (8)$$

Replacing $k_{et}\tau$ with K_{sv} (eq 2) simplifies the expression even further

$$\frac{\Phi_0}{\Phi} = K^{P}_{sv}K^{C}_{sv}[B]^2 + (K^{P}_{sv} + K^{C}_{sv})[B] + 1 \qquad (9)$$

Quenching experiments were performed using P^{4+}, Ph_3BnB^-, and Cy^{3+}. Figure 4 shows the results for three different cyanine concentrations. Fitting to second-order polynomials yields correlation coefficients of at least 0.999. The K^{C}_{sv} in eq 9 is identical to that given in Table 2 for electron transfer from Ph_3BnB^- to Cy^{3+} since k_{et} and τ are the same for excited Cy^{3+} whether it is produced by absorption of red light or by energy transfer from P^{4+}. K^{P}_{sv}, on the other hand, should be dependent on the cyanine concentration. Since energy transfer from P^{4+} to Cy^{3+} decreases the porphyrin lifetime, K^{P}_{sv} should decrease with increasing cyanine concentration. Using $K^{C}_{sv} = 33.4\ M^{-1}$ (Table 2) and the results of the second-order fits in Figure 4, K^{P}_{sv} and

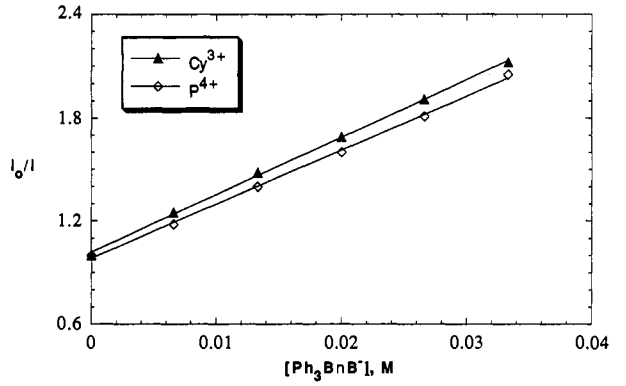


Figure 3. Fluorescence quenching as a function of Ph_3BnB^- concentration for two component electron transfer systems (10 P^{4+} per liposome; or 200–400 Cy^{3+} per liposome; 5.0 nM liposomes).

Table 2. Stern–Volmer, Energy and Electron Transfer Quenching Constants

reaction	K_{sv} (M^{-1})	k_q ($M^{-1}\ s^{-1}$)
$*P^{4+} + Cy^{3+} \rightarrow P^{4+} + *Cy^{3+}$	29.1 ± 0.4	2.7×10^9
$*P^{4+} + Ph_3BnB^- \rightarrow P^{\bullet 3+} + Ph_3BnB^{\bullet}$	31.5 ± 2.9	3.0×10^9
$*Cy^{3+} + Ph_3BnB^- \rightarrow Cy^{\bullet 2+}\ Ph_3BnB^{\bullet}$	33.4 ± 2.4	2.0×10^{10}

Table 3. Stern–Volmer Constants and Porphyrin Lifetimes at Various Cyanine Concentrations

# Cy^{3+} per liposome	K^{P}_{sv} (M^{-1})	τ^{P} (ns)
0	31.5 ± 2.9	10.5
200	24.2 ± 5.2	8.1
300	10.9 ± 1.0	3.6
400	8.7 ± 0.9	2.9

τ^{P} are calculated for a variety of cyanine concentrations (Table 3). (The minimal curvature evident in Figure 4 is due to the relatively small contribution the second-order term in eq 9 makes to the overall quenching for the borate concentrations used in these experiments).

Vectorial Photoinduced Electron Transfer in Liposomes: Photobleaching. For liposome systems involving direct excitation of the cyanine, back electron transfer from reduced cyanine to oxidized borate was not found to be a significant pathway,[21,22] presumably due to the known rapid decomposition to the incipient

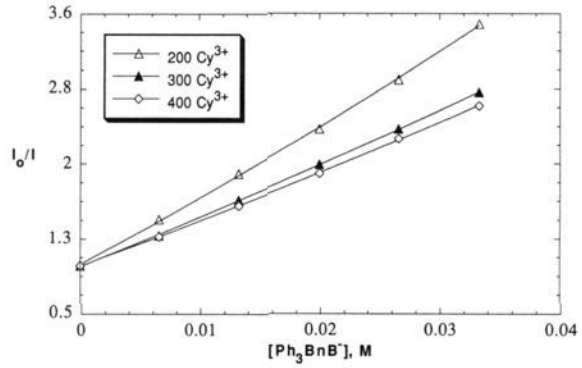


Figure 4. Fluorescence quenching as a function of Ph_3BnB^- concentration for the three component PMD (10 P^{4+} per liposome; and 200, 300, or 400 Cy^{3+} per liposome; 5.0 nM liposomes).

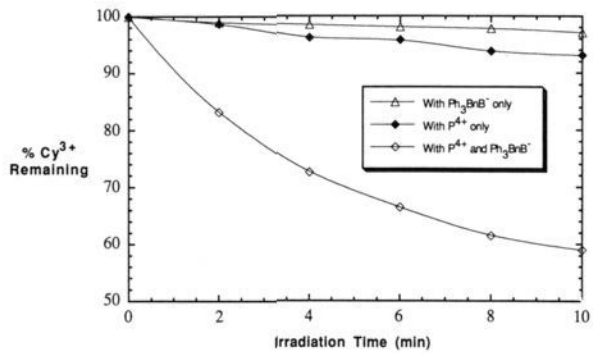


Figure 5. Photobleaching of Cy^{3+} (irradiation at 417 nm): 400 Cy^{3+} and P^{4+} (where appropriate), 2000 Ph_3BnB^- (where appropriate) bound per liposome (6.3 nM liposomes).

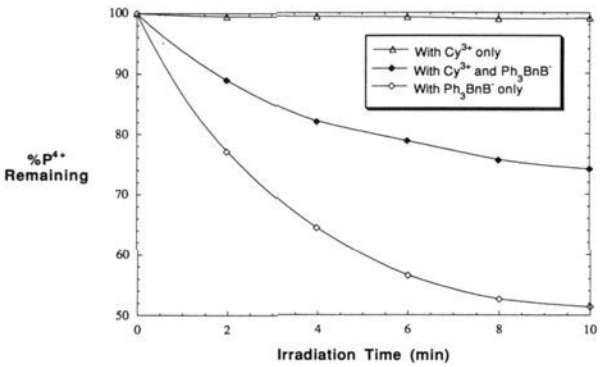
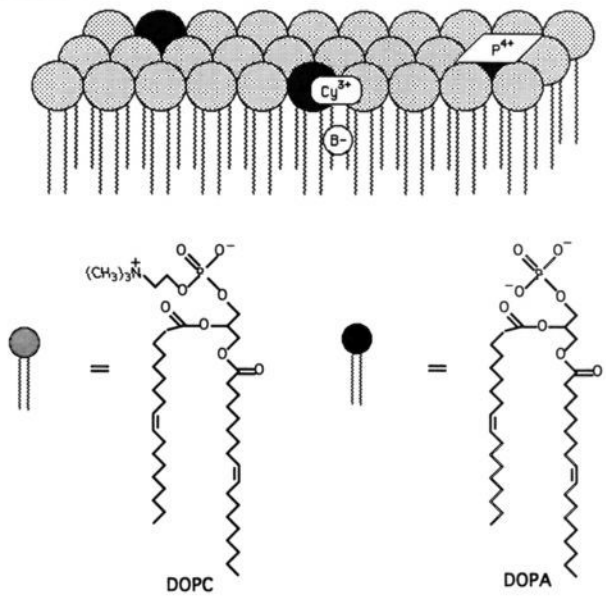


Figure 6. Photobleaching of P^{4+} (irradiation at 417 nm): 400 P^{4+} and Cy^{3+} (where appropriate), 2000 Ph_3BnB^- (where appropriate) bound per liposome (6.3 nM liposomes).

boranyl radical.[36,50-53] Rather, the reduced dye ultimately undergoes irreversible bleaching of its absorption band. Figure 5 illustrates photobleaching of Cy^{3+} under various conditions. In the absence of either the energy donor (P^{4+}) or the electron donor (Ph_3BnB^-), nominal bleaching occurs upon excitation at 417 nm. (In the presence of Ph_3BnB^-, excitation at 669 nm leads to rapid bleaching of the cyanine, regardless of whether or not the porphyrin is present.) In the presence of both P^{4+} and Ph_3BnB^-, the system exhibits greatly enhanced reactivity, illustrating that all three components are required to be present in order for the blue light activated PMD to function. Additionally, substitution of the weaker electron donor tetraphenylborate (Ph_4B^-)[36,50] for Ph_3BnB^- and irradiation under identical conditions leads to virtually no bleaching of the cyanine. Previously, we observed that liposome-bound Cy^{3+} is neither quenched nor photobleached in the presence of Ph_4B^-.[21,22]

Spectroscopically, P^{4+} suffers the same fate as Cy^{3+} upon photoinduced reduction by Ph_3BnB^-, that is, it photobleaches. Figure 6 shows the results for P^{4+} photobleaching in the same experiments used to generate Figure 5. In this case, maximal bleaching of P^{4+} occurs in the absence of the energy acceptor (Cy^{3+}), whereas minimal bleaching occurs in the absence of the electron donor (Ph_3BnB^-). Bleaching is inhibited approximately 50% when Cy^{3+} and Ph_3BnB^- are present.

Scheme 1



Discussion

Scheme 1 illustrates the liposome-based PMD described above. (Only the outer leaflet of the bilayer is shown for simplicity.) The cationic pigments are bound to the membrane surface by electrostatic attraction to the anionic DOPA lipids. Meanwhile, the hydrophobic borate burrows into the interior of the bilayer. This may seem counterintuitive due to the negative charge on the borate; however, the presence of the large aromatic groups tends to shield the charge from water. Indeed tetraphenylborate anion, the close structural analogue of Ph_3BnB^-, is known to partition from water into lipid bilayers with an equilibrium constant of approximately 10^5.[32-34] The system is thus configured for the following sequence:

$$P^{4+} \xrightarrow{h\nu\ (417\ nm)} *(P^{4+}) \qquad (10)$$

$$*(P^{4+}) + Cy^{3+} \xrightarrow{\text{energy transfer}} P^{4+} + *(Cy^{3+}) \qquad (11)$$

$$*(Cy^{3+}) + Ph_3BnB^- \xrightarrow{\text{electron transfer}} Cy^{\bullet 2+} + Ph_3BnB^{\bullet} \qquad (12)$$

The intense Soret band of the porphyrin ($\epsilon_{max} \simeq 4.0 \times 10^5\ M^{-1}\ cm^{-1}$) permits step 10 to proceed very efficiently. The large overlap between porphyrin emission and cyanine absorption (Figure 2) leads to facile energy transfer, step 11, along the membrane surface. In step 12, the excited cyanine is quenched by electron transfer from the borate bound in the interior of the bilayer, a vectorial reaction occurring perpendicular to the membrane surface.

Scheme 2 outlines the various reaction pathways available to the system (photophysical processes such as fluorescence are not

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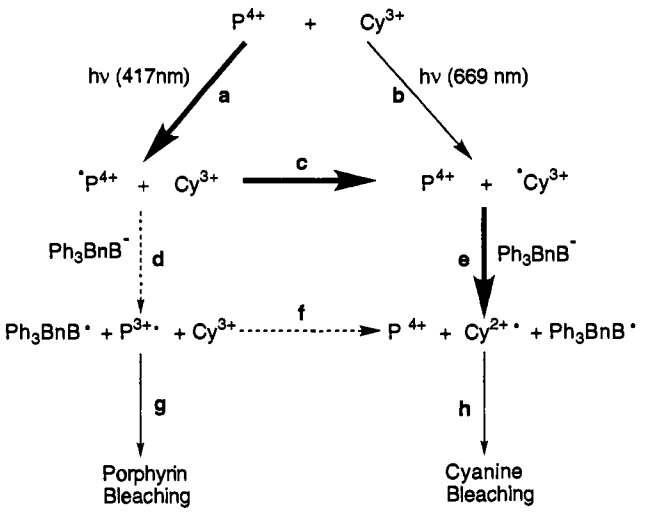
Scheme 2



shown for simplicity). Direct excitation of either pigment (417 nm for P^{4+}, 669 nm for Cy^{3+}) is facile due to their well-resolved absorption spectra. Red-light irradiation leads to vectorial electron transfer from Ph_3BnB^- to Cy^{3+}, resulting in quenching of Cy^{3+} fluorescence and irreversible bleaching of the absorption band of the dye. The electron transfer step is irreversible due to rapid decomposition of the oxidized borate. It is interesting to note that k_{et} for electron transfer from Ph_3BnB^- to Cy^{3+} is nearly an order of magnitude greater than for the analogous reaction involving P^{4+}. This is likely due to three factors: (1) The reduction potential for Cy^{3+} (ca. –0.88 V based on the literature value for the thiadicarbocyanine bearing ethyl rather than trimethylammoniumpropyl substituents[54]) is 270 mV higher than that of P^{4+} (–1.15 V[35]). Since the excitation energy of P^{4+} is ca. 100 mV greater than that of Cy^{3+}, there is ca. 170 mV difference in the excited states which favors electron transfer to the cyanine. (2) A smaller reorganization energy is expected for electron transfer to Cy^{3+} relative to P^{4+}. We have previously demonstrated that Cy^{3+} binds in a region of the membrane possessing an effective dielectric constant of 13–14. Because of its symmetrical structure, we envision binding of P^{4+} flat to the surface of the membrane, exposing the porphyrin to water ($\epsilon \approx 78$). The lower dielectric of the environment surrounding Cy^{3+} should favor a lower reorganization energy and thus a faster electron transfer reaction.[38] (3) The deeper penetration of Cy^{3+} into the bilayer suggests a shorter donor–acceptor separation relative to P^{4+}, leading to faster electron transfer,[55] although orientation differences could offset this effect.

Electron transfer from P^{4+} to Cy^{3+} is energetically favorable, and photoexcitation of the dyes enhances their redox properties. However, this process is not considered competitive with energy transfer for the following reasons. Electron transfer between excited cyanine and P^{4+} evidently does not occur because the Cy^{3+} fluorescence is not quenched by the porphyrin. The possibility of electron transfer from the excited porphyrin to Cy^{3+} is also unlikely because of the average distance of separation. The rates of both electron and energy transfer depend on the cyanine concentration since each process is distance dependent. The rate of energy transfer is proportional to r^{-6}, while the rate of electron transfer is proportional to e^{-2r}. Using the value of $r = R_0 = 46$ Å (see Results section) gives $r^{-6} \sim 1 \times 10^{-10}$ and $e^{-2r} \sim 1 \times 10^{-40}$. Since the rate constants for the two processes depend differently on distance, the isoemissive point in Figure 2 would not be present if electron transfer does in fact compete with energy transfer. Therefore, at the cyanine concentrations and P^{4+}–Cy^{3+} distances used in this PMD, electron transfer between the dyes is not competitive with energy transfer.

Irradiation at 417 nm results in exclusive excitation of P^{4+}. The excited porphyrin can either transfer energy to Cy^{3+} (c) or accept an electron from Ph_3BnB^- (d). The excited cyanine produced by the former can radiatively return to the ground state. The quenching of this emission illustrated in Figure 4 could result from electron transfer from Ph_3BnB^- (e). Alternatively, step d, which quenches P^{4+} in competition with energy transfer, also could "quench" the sensitized cyanine fluorescence by preventing production of excited Cy^{3+}. The observation that the porphyrin lifetime decreases with increasing Cy^{3+} concentration (Table 3) provides unequivocal proof that in the presence of the borate energy transfer occurs from $*P^{4+}$ to Cy^{3+} although it does not eliminate the possibility that step d competes with c. The rate constants for energy and electron transfer from $*P^{4+}$ (Table 3) allow the use of eq 13 to calculate the efficiency of energy transfer. Indeed, at the highest concentrations used—400 Cy^{3+} per liposome = 39.1 mM and 2000 Ph_3BnB^- per liposome = 66.6 mM—ca. 60% of the excited porphyrins are quenched by energy transfer

$$\frac{\Phi_{ET}}{\Phi_{et}^{p}} = \frac{k_{ET}[Cy^{3+}]}{k_{et}^{p}[Ph_3BnB^-]} \approx \frac{[Cy^{3+}]}{[Ph_3BnB^-]} = 0.59 \qquad (13)$$

One of the principal goals for a PMD based on photoinduced electron transfer is the storage of energy as chemical potential in the redox products. For the system described here, these products are $Cy^{\bullet 2+}$ and $Ph_3BnB^{\bullet}$. The sequences a, c, e and a, d, f both yield these products. Since Cy^{3+} is more easily reduced than P^{4+}, step f is thermodynamically possible.[56] Thus step d is not necessarily detrimental to the system. The real source of inefficiency arises from the competition between steps f and g. Bleaching of P^{4+} is irreversible, effectively removing the antenna pigment from the PMD. Based on the porphyrin photobleaching results (Figure 6), we can estimate the partitioning of $P^{\bullet 3+}$ between steps f and g. In the photobleaching experiments, 400 Cy^{3+} per liposome was found to inhibit P^{4+} bleaching by approximately 50%. Thus,

$$\frac{\Phi_{bleach}^{Cy^{3+}}}{\Phi_{bleach}} = 0.50 \qquad (14)$$

where $\Phi_{bleach}^{Cy^{3+}}$ and Φ_{bleach} are the quantum yields for bleaching of P^{4+} in the presence and absence, respectively, of Cy^{3+}.

$$\Phi_{bleach} = \Phi_d\Phi_g, \qquad \Phi_{bleach}^{Cy^{3+}} = \Phi_d{}^{Cy^{3+}}\Phi_g{}^{Cy^{3+}} \qquad (15)$$

$$\Phi_d{}^{Cy^{3+}} = 0.85\Phi_d \text{ (for 2000 } Ph_3BnB^- \text{ per liposome)} \qquad (16)$$

$$\frac{\Phi_{bleach}^{Cy^{3+}}}{\Phi_{bleach}} = \frac{0.85\Phi_d\Phi_g{}^{Cy^{3+}}}{\Phi_d\Phi_g} = 0.50 \qquad (17)$$

$$\frac{\Phi_g{}^{Cy^{3+}}}{\Phi_g} = \frac{0.50}{0.85} = 0.59 \qquad (18)$$

Therefore, in the presence of Cy^{3+}, 41% of the reduced porphyrins ($P^{\bullet 3+}$) react by step f, while the remaining 59% ultimately bleach. These calculations indicate that for 400 Cy^{3+} and 2000 Ph_3BnB^- per liposome, only ca. 24% of the porphyrin molecules react by

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(56) Although electron transfer to Cy^{3+} is thermodynamically possible from both the excited and the reduced states of P^{4+}, it is kinetically probable only from the latter. The relatively short excited state lifetime of $*P^{4+}$ and the large average distance of separation between the cyanines and porphyrins diminish the probability of diffusive encounter between $*P^{4+}$ and Cy^{3+}. In contrast the $P^{3\bullet+}$ is likely to be stable over a much longer period than $*P^{4+}$, thereby increasing the probability of electron transfer from the reduced porphyrin to the cyanine.

the undesired sequence a, d, g. This feature imposes some restrictions on the system. Step c would be enhanced relative to step d by either decreasing the Ph_3BnB^- concentration or increasing the Cy^{3+} concentration. The former option would lead to a lower efficiency for step e, electron transfer from Ph_3BnB^- to Cy^{3+}, while the latter runs the risk of saturating the liposome surface with Cy^{3+}, preventing the binding of P^{4+}. Without the antenna pigment bound to the liposome, the PMD will not be sensitive to blue light.

The PMD described above takes advantage of the many degrees of freedom available in the design of lipid bilayers as media for chemical reactions. Properties such as fluidity, surface charge, and membrane dipole potential are easily varied simply by selecting different lipids from which to construct the membrane. The liposomes used here as the hosts for the P^{4+}-Cy^{3+}-Ph_3BnB^- PMD play two very important roles. First, concentration of the three components of the system upon binding to the liposome removes the need for covalent linkage of the respective parts. Second, the anisotropic medium afforded by the membrane results in a nonrandom yet predictable distribution of the three components. In addition, it should be noted that the use of liposomes of uniform and well-defined size permit reasonable calculation of the concentration of bound reactants. This is critical to the quantitative analysis described above, since these concentrations are used to generate the Stern–Volmer plots from which the reaction rate constants are extracted. The cationic pigments are bound electrostatically to the anionic surface of the membrane leading to energy transfer parallel to the liposome surface, whereas the hydrophobic borate anion is bound within the bilayer interior, resulting in vectorial electron flow perpendicular to the surface. Thus energy transfer and electron transfer operate not only sequentially but also orthogonally.

The facility with which different physical properties can be endowed upon lipid bilayers coupled with the vast array of energy/electron donors and acceptors available suggest myriad possible configurations for liposome-based PMDs. (An additional appealing feature of liposomes as media for PMDs is the possibility of incorporating transmembrane processes into these systems.) The system described above is composed of very simple components and features "easy assembly". Future efforts will be directed toward optimizing the efficiencies of various liposome-based PMDs by modification of the membrane[23] as well as through variation of the actual PMD components.

Acknowledgment. The authors thank Professor Gary B. Schuster and Dr. Chelladurai Devadoss, University of Illinois, for measurement of the singlet lifetime of Cy^{3+} in water and for helpful discussions. Partial support of this research by the National Science Foundation is greatly appreciated.