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ENERGY INTERACTIONS AND PHOSPHOLIPID VESICLES

David Edmund Baker
(M.S. thesis)

June 1982

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ENERGY INTERACTIONS AND PHOSPHOLIPID VESICLES

David Edmund Baker

M.S. Thesis

June 1982

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Energy Interactions and Phospholipid Vesicles

by

David Edmund Baker

ABSTRACT

Luminescence quenching studies of unilamellar phosphatidylcholine vesicles containing a surfactant sensitizer derived from tris(2,2'-bipyridine)ruthenium(II) have shown that only a fraction of these sensitizers is quenchable when ferricyanide is added to the continuous aqueous phase, and that this fraction is made up exclusively of the sensitizers located in the outer monolayer. The size of this fraction is independent of the sensitizer concentration. In contrast to this, the quenching rate constant increases with sensitizer concentration. From these observations, one can conclude that energy transfer occurs among sensitizers located on the same surface of a vesicle, but energy transfer between these sensitizers is not long range enough to reach efficiently across the membrane from inside to outside (and vice versa). The existence of this energy transfer in the first case has important implications on the kinetic scheme for photosensitized electron transport reactions through vesicle walls. The size of the vesicles studied was determined to be ≈ 500 angstroms in diameter by ^{14}C -sucrose entrapment. This experimental finding in addition to the fixed fraction of quenchable fluorescence of 0.67 ± 0.02 implies that the surfactant ruthenium molecules extend a considerable distance out of the vesicle into the aqueous phase, and/or they are asymmetrically distributed between inner and outer vesicle surfaces.

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Many people have been involved in the production of this work, and as a result, they have contributed to who I am and who I will become. Most will go unnamed here, but not unappreciated. I want to thank Jer-Ming Yang and Bill Ford for unselfishly, untiringly, and unforgettably sharing their experience and knowledge (and reprints). I also want to thank John Otvos for his encouragement, his willingness to help, and the countless hours he spent reading drafts of this paper and making suggestions for its improvement. To Professor Melvin Calvin, I will always be grateful and indebted for the opportunity to work with him in his endeavor to discover, understand, and recreate this biophysical chemical creation. And with respect to the Creator, I stand in awe.

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INTRODUCTION

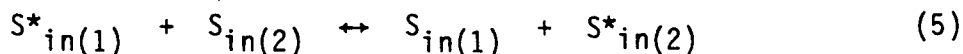
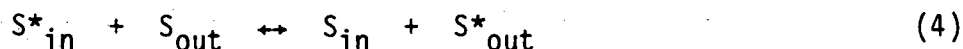
Unilamellar vesicles are often used to separate reactants in photosynthetic mimetic processes.¹⁻⁶ As in Figure 1, surfactant photosensitizers can be incorporated into the bilayer membrane and electron donors and acceptors can be arranged on opposite sides of the membrane wall. Ford, et al., have demonstrated that a vesicle system such as this can allow the transport of electrons through the membrane and also inhibit the back reaction of the products formed.⁴

Though electron transport has been investigated, the question of energy transfer among sensitizer molecules either on the surface of the vesicle or between sensitizer molecules on opposite sides of the vesicle wall has not been previously explored. Use of the term "energy transfer" in this paper is meant to include delocalization, as well as migration, of the excitation energy.

For an electron transport system in which the sequence of reactions is



it is conceivable that the excitation energy of the excited sensitizer, S^* , is transferred to other sensitizers before there is a reaction with an acceptor molecule, A:



Although it was not considered in the kinetic studies of electron transport through the vesicle membrane,^{5,7} this energy transfer, if it occurs, can affect the rate of the electron transport reaction. The anticipated effect would be to increase the reaction rate if, on the average, more than one sensitizer is excited as a result of a single excitation during the collision period of an acceptor with a sensitizer. In effect, energy transfer, whether it occurs by fast migration or delocalization, can increase the reaction rate by increasing the cross-sectional area for reaction with electron acceptors.

A naturally occurring example of how energy transfer can lead to an increase in a redox reaction rate can be found in photosynthesis. Most of the chlorophyll in chloroplasts is light-harvesting antenna chlorophyll (>99%).⁸ These antenna molecules transfer their energy to other chlorophyll molecules until a "reaction center" chlorophyll is found and a reaction occurs, or until the energy is lost through fluorescence or degradative mechanisms. If antenna chlorophylls were unable to transfer excitation energy, the reaction rate would be at least two orders of magnitude smaller than it actually is.

The sensitizer molecule used in the vesicle studies cited above is an amphiphilic derivative of tris(2,2'-bipyridine)ruthenium(II). See Figure 2. Although its spectral and physical properties are somewhat different from its parent, its chemical properties are largely the same. Upon illumination ($\lambda_{\max}=481$ nm), a relatively long-lived charge transfer complex is formed (lifetime = 0.6 μ sec in deoxygenated water).¹⁸ In the absence of suitable electron donors or acceptors, in deoxygenated water at room temperature, the excited state complex has a luminescence quantum yield of ≈ 0.04 ($\lambda_{\max}=658$ nm).⁹

In the presence of suitable redox reactants, this excited ruthenium complex undergoes facile electron transfer reactions while in the charge transfer state. Upon transfer of an electron, the excited state is said to be quenched since it is no longer capable of emitting light. If the redox reaction is the result of a close encounter of the excited molecule with the quencher, and is influenced by the local charge and the viscosity of the solution, the quenching mechanism is termed "collisional" and the quenching rate is proportional to the product of the effective concentrations.

$$\text{Quenching rate} = K_Q[S^*][Q] \quad (7)$$

As described above, the existence of energy transfer among sensitizers could increase the quenching rate by increasing the effective cross-sectional area of encounter with quencher. Given $[S^*]$ and $[Q]$, the quenching rate constant K_Q reflects the influence of energy transfer and can be used to test for its existence. Luminescence quenching, therefore, is a suitable tool to study the energy transfer properties of this ruthenium compound, and is the source of most of the conclusions reached in this paper.

The Stern-Volmer treatment is the traditional way to analyze fluorescence quenching studies.¹⁰ In the ideal case, one obtains a straight line plot passing through 1.0 on the ordinate axis when the original fluorescence intensity divided by the fluorescence intensity at a given quencher concentration, F_0/F , is plotted against the quencher concentration, $[Q]$. The slope of this line is the quenching rate constant, K_Q , which is the product of the bimolecular rate constant and the lifetime of the excited molecule (see Figure 3).

However, in cases where some of the fluorophors are inaccessible to the quencher, a curve is obtained that approaches a maximum value equal to the inverse of the fraction not quenchable (see Figure 4). K_Q for the quenchable fraction is not easily extracted from this curve. This is the type of plot expected to result from quenching studies where a non-permeating quencher is added to a solution of vesicles having the fluorescent species on both surfaces of the vesicle membrane. If, for example, half of the fluorophors are on the inside of the vesicles, and therefore inaccessible to the quencher, a maximum ordinate value of 2.0 would be approached and not exceeded, no matter how high the quencher concentration is.

To analyze cases such as these, where a fraction of the fluorescence is not quenchable, Lehrer modified the Stern-Volmer treatment in a way that makes it easier to determine the fraction of quenchable fluorescence and the quenching rate constant for that fraction.¹¹ His method will be used here.

Vesicle size can be determined by entrapment of ^{14}C -sucrose. If the vesicles are formed in a solution containing a trace quantity of radioactive sucrose and subsequently gel-filtered to remove the radioactive sucrose from the continuous aqueous phase, then the amount of radioactivity remaining in the vesicle solution is proportional to the internal volume of the vesicles. Knowing also the amount of phospholipid used, the average surface area occupied by a each phospholipid molecule, and the bilayer thickness is sufficient information for the calculation of the vesicle diameter.

This method for determining vesicle size has been used here, and the results are compared with vesicle sizes determined by fluorescence

correlation spectroscopy, negative-stain electron microscopy, and a theoretical calculation based on existing literature.

Taken together, the vesicle size and the fraction of quenchable fluorescence have implications concerning the position of ruthenium head groups in the vesicle wall and the relative distribution of these amphiphilic sensitizers on the inner and outer vesicle surfaces. Depending on the assumptions made, it can be concluded either that the sensitizers extend out of the vesicles and are symmetrically distributed (i.e., the average number of ruthenium molecules per unit area is the same for both inner and outer surfaces), or that the sensitizers do not extend out of the vesicles and are distributed asymmetrically, preferentially locating on the outer surface. If no assumptions are made, a range of possibilities exists for these two aspects of vesicle geometry, but they remain interdependent.

MATERIALS

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification.

Vesicles were prepared from an ethanolic solution of phosphatidylcholine (abbreviated to "PC"), which was purified from chicken egg yolks by Dr. S. Kohler. The purification procedure was that described by Singleton et al.¹² To prevent decomposition of the PC, the solution was stored under nitrogen at -20 °C.

The amphiphilic ruthenium compound (abbreviated to "Ru-surf") was derived from tris(2,2'-bipyridine)ruthenium(II) (abbreviated to "Ru(bpy)₃²⁺") by W. Ford.¹³ Two C₁₆-hydrocarbon chains are attached to one of the bipyridine ligands by amide linkages at the 4 and 4' positions. See Figure 2. A stock solution of this compound was made by dissolving it in dimethylformamide. Subsequently, the solution was stored in the dark at room temperature.

Aqueous ferricyanide ion, Fe(CN)₆³⁻, was used as the quencher in the luminescence quenching experiments.

All aqueous solutions were prepared with carbon-purified, deionized water (Millipore's Milli-Q Water Purification System).

Gel filtration of the vesicles for the ¹⁴C-sucrose entrapment experiments was done on an 18 x 1 cm column of Sephadex G-25 Medium dextran beads.

METHODS

All measurements were carried out at room temperature (22.5 ± 1.0 °C) and vesicle solutions were air-saturated.

In all of the vesicle preparations used for luminescence quenching, the bulk Ru-surf concentration was held constant ($\approx 6 \times 10^{-6}$ M), as determined by absorption (Cary model 118 spectrophotometer) at 481 nm ($\epsilon = 12,000 \text{ M}^{-1} \text{ cm}^{-1}$).¹⁴ The amount of PC was varied to achieve PC:Ru-surf ratios of approximately 10, 15, 20, 30, 50, and 100 to 1. Typically, 1 to 2 μl of Ru-surf stock solution (≈ 0.025 M) was mixed with 10 to 80 μl of PC stock solution (≈ 0.037 M), depending on the ratio desired. The mixing was done in a small conical testtube to minimize material loss. Uniform mixing was ensured by pumping the solution in and out of a Hamilton syringe (25, 50, or 100 μl) several times. The Ru-surf stock solution was sealed and returned to the dark immediately after use. The air space above the PC stock solution was flushed briefly with nitrogen before sealing and returning it to cold storage.

To form the vesicles, a measured volume of the PC/Ru-surf solution was injected (Hamilton syringe) into 4.0 ml of 1.0 M ammonium acetate buffer while agitating the solution as vigorously as possible on a vortex mixer.¹⁵ The tip of the syringe needle was not submerged, but care was taken that the organic solution struck the aqueous phase without first contacting the glass tube wall. Vortexing of the mixture was continued for about 15 seconds. It has been shown that this injection method produces fairly monodisperse vesicles.¹⁶

Three milliliters (volumetric pipette) of the resulting solution was dispensed to a 1.00 x 1.00 cm quartz cuvette with four polished

sides. The solution's absorption at 481 nm was determined before adding quencher.

Luminescence quenching was measured on a Perkin-Elmer model MPF-2A fluorescence spectrophotometer equipped with a red-sensitive, type R-136, photomultiplier (Hamamatsu TV Co.). Luminescence was detected at a right angle to the excitation. Excitation was at 481 nm (slitwidth = 3 nm) and emission was detected at 658 nm (slitwidth = 8 nm). The reference adjust and sensitivity settings were adjusted to give an initial luminescence signal height that was at least 80% of the chart paper width. Care was taken to use the same cuvette holder position and to use the same cuvette faces for excitation and emission, as these factors were observed to influence the signal significantly.

Additions of the quencher, $0.05 \text{ M K}_3\text{Fe}(\text{CN})_6^{3-}$, were made by lambda pipette in some experiments and Hamilton syringe in others. Mixing was achieved by pumping the solution in and out of a disposable-tipped "Pipetteman". The luminescence intensity of the resulting solution was recorded for at least 30 seconds to obtain a value less sensitive to momentary fluctuations. The absorption at 481 nm was recorded after each addition of quencher to note the effect of $\text{Fe}(\text{CN})_6^{3-}$ on the light available for Ru-surf absorption. Over the range of $\text{Fe}(\text{CN})_6^{3-}$ concentration used ($<5 \times 10^{-4} \text{ M}$), $\text{Fe}(\text{CN})_6^{3-}$ absorption at 481 nm was calculated to be insignificant.

In one experiment, $\text{Fe}(\text{CN})_6^{3-}$ was present in the buffer before the vesicle material was injected. This allowed quencher to be present inside as well as outside of the vesicles. Eight vesicle solutions with different $\text{Fe}(\text{CN})_6^{3-}$ concentrations (0 to $4 \times 10^{-4} \text{ M}$) were prepared using

identical volumes of the same PC/Ru-surf mixture. The absorption and luminescence of each was then measured as in other experiments.

With the instrument used, the reproducibility of the luminescence signal height for a given sample is not guaranteed. In one experiment, to correct for this, a reference cuvette containing an aliquot of the same vesicle solution used for the experiment was used to obtain F_0 for each F determined at a given quencher concentration. Another cuvette containing a vesicle solution without Ru-surf was used to obtain a baseline measurement for each F . Corrected F values were different but not significantly different to alter the qualitative conclusions of these experiments ($\Delta K_Q < 3\%$).

Vesicles used in the ^{14}C -sucrose entrapment experiments had a PC:Ru-surf ratio of 20:1. They were formed in the same manner as the vesicles used for luminescence quenching except for the presence of "cold" and "hot" sucrose ($\approx 1 \times 10^{-3}$ M), and that the volume of PC:Ru-surf solution injected was about double to compensate for dilution during gel filtration. These differences should not affect the vesicle size.¹⁶

Two of these experiments were performed using different amounts of labeled and unlabeled sucrose. Although the second run used about ten times the amount of ^{14}C to improve the counting reliability, the vesicle dimensions calculated from each were nearly the same. The procedure for this latter experiment will be described here.

All sucrose solutions were freshly prepared for the experiment to eliminate problems with bacterial growth.

0.56 ml of labeled sucrose solution (50 $\mu\text{Ci}/967 \mu\text{l}$) was syringed into the testtube in which the vesicles were to be prepared. 3.044 ml of a stock solution of ammonium acetate and unlabeled sucrose was added

to give a solution that was ≈ 1.0 M $\text{CH}_3\text{COONH}_4$ and $\approx 2.5 \times 10^{-3}$ M total sucrose. $2.4 \mu\text{l}$ of Ru-surf solution (≈ 0.02 M) was mixed with $25.6 \mu\text{l}$ of PC solution (≈ 0.04 M), and $24.0 \mu\text{l}$ of the resulting solution was injected, as described previously, into the radioactive buffer to form vesicles. $10 \mu\text{l}$ of this solution was analyzed by liquid scintillation counting (Packard model 460) to obtain the concentration of radioactivity.

After an absorption spectrum was taken, 3.0 ml of the vesicle solution was passed through a gel filtration column (see materials section) to replace the "hot" continuous aqueous phase with "cold" buffer which was equivalent in ammonium acetate and total sucrose concentrations. The fractions collected off the column containing Ru-surf (yellow fractions) were reapplied to the column three more times to ensure that all of the ^{14}C -sucrose not contained in the vesicles was removed. The test for this was as follows.

After the third pass, the two 0.5 ml fractions with the most Ru-surf were combined for an absorption spectrum to determine the amount of Ru-surf present. Subsequently, 0.5 ml of this solution was analyzed for its radioactivity. The remainder was used with the other Ru-surf fractions for the fourth pass. In the same manner, the two 0.5 ml fractions containing the most Ru-surf after the fourth filtration was analyzed for Ru-surf and radioactivity. The ratio of these quantities was compared with the ratio obtained after the third filtration. The ratios were the same within experimental error and therefore, no additional filtration was needed.

Of importance for the calculation of vesicle size is the concentration of PC used. This was determined by evaporating the solvent from

a measured volume of the solution under a stream of nitrogen and also by evaporation under high vacuum. A 50 μ l aliquot of PC stock solution was dried in each case and both methods gave the same concentration of 31.8 mg/ml. A Mettler single-pan micro balance was used for these mass measurements with which a precision of ± 2 μ g can be achieved.[†]

[†] Upon full release of the weighing pan, the reading given by this Mettler balance drifts for up to fifteen minutes. It may be a positive drift or a negative drift. Obtaining reproducible results (± 2 μ g) requires that the reading be stable for a least one minute.

RESULTS AND DISCUSSION

Energy Transfer in Vesicles

Lehrer's modified Stern-Volmer treatment allows one to calculate the fraction of quenchable fluorescence and the quenching rate constant for that fraction in collisional quenching studies where not all of the emitter is accessible to quencher. The relation he derives is^{11a}

$$\frac{F_0}{\Delta F} = \frac{1}{[Q]f_a K_Q} + \frac{1}{f_a} \quad (8)$$

where F_0 is the fluorescence intensity in the absence of quencher, ΔF is the amount the fluorescence intensity decreases for a given quencher concentration $[Q]$, f_a is the fraction of quenchable fluorescence, and K_Q is the quenching rate constant for that fraction. A plot of $F_0/\Delta F$ versus $1/[Q]$ should yield a straight line of slope $(f_a K_Q)^{-1}$ and intercept $1/f_a$, with K_Q equal to the intercept divided by the slope.

In the special case where all of the fluorophors are accessible ($f_a = 1$), the straight line will pass through the ordinate value 1.0 as in the traditional Stern-Volmer treatment, but the slope will be the inverse of K_Q .

Quenching experiments were performed on several vesicle preparations differing in their PC:Ru-surf ratios as described above. Plots of $F_0/\Delta F$ versus $1/[Q]$ were drawn for each, e.g. see Figure 5. Error bars were constructed from the maximum and minimum luminescence values that may be interpreted from the raw data. Since the error limits differ greatly in magnitude from point to point, the line used to extract K_Q and f_a is not a simple least squares line. Instead, they were extracted from the highest sloping line that could be drawn through all or all but

one of the error bars, and averaged with those extracted from the lowest sloping line that met the same criteria. The values obtained are tabulated in Table 1. A graphic comparison of the raw data from which some of these tabulated values were derived, is presented in Figure 6.

For any given experiment, it is observed that a fraction of the excited Ru-surf is not quenchable. Since $\text{Fe}(\text{CN})_6^{3-}$ is added only after the vesicles are formed, it is likely that this unquenchable fraction is made up of Ru-surf located on the inner surface of the vesicle wall. $\text{Fe}(\text{CN})_6^{3-}$ is not expected to permeate the membrane because it is bulky and has a high negative charge.

To verify that it is inner Ru-surf that was not being quenched, an experiment was performed in which vesicles were formed in the presence of quencher. Eight vesicle solutions were prepared with differing amounts of $\text{Fe}(\text{CN})_6^{3-}$ present. In this case, $\text{Fe}(\text{CN})_6^{3-}$ would be expected to be found on the inside as well as the outside, and to have access to all of the excited Ru-surf. The straight-lined Stern-Volmer plot passing through 1.0 verifies this (see Figure 7).

There are two important observations to be made from the data in Table 1: (1) f_a is independent of PC:Ru-surf with an average value of 0.67 (std. dev. = .02), and (2) above a critical amount of Ru-surf (PC:Ru-surf = 30:1), K_Q increases with the Ru-surf concentration in the vesicle wall.

The first observation allows one to conclude that energy transfer between sensitizers on opposite sides of the vesicle wall cannot take place, or that the rate is so slow that it is not competitive with luminescence or internal conversion. This follows from the argument presented in the succeeding paragraph.

Considering measurements done at only one PC:Ru-surf ratio, one cannot conclude that energy transfer does or does not take place between sensitizers on opposite surfaces of a vesicle. The f_a determined may represent only the fraction of sensitizers that are on the outside vesicle surface, or it may represent the fraction on the outside plus a fraction of the sensitizers on the inside that are "close enough" to outside sensitizers to transfer energy. If the latter case were true, then increasing the Ru-surf concentration would increase the fraction of inside sensitizers that are able to transfer their energy to outside sensitizers. Consequently, the f_a would be expected to increase with an increase in Ru-surf concentration. In the former case, f_a would be constant and independent of Ru-surf concentration. As Figure 8 shows, the f_a 's obtained for different PC/Ru-surf vesicles are scattered around an average value of approximately 0.67 and are independent of Ru-surf concentration. Therefore, we conclude that energy transfer across the membrane does not take place on the time scale of the luminescence.

The question arises that if energy transfer is not observed between sensitizers on opposite sides of the membrane, how can electron transport across the membrane be explained, which was reported previously;⁴ electron transport is generally thought of as a short-range process when compared to energy transfer. This paradox can be understood by considering the lifetimes of the species that are involved in these processes. The excited state lifetime of Ru-surf at room temperature in air-saturated aqueous solution is approximately 0.4 μsec .¹⁸ In contrast, oxidized Ru-surf will have a much longer lifetime. It will exist until an electron is transported to it from a Ru-surf on the inside of the vesicle, or until it back reacts with a reduced acceptor.

Therefore, electron transport is observable because of the comparatively long lifetime of oxidized Ru-surf.

The second observation, above, is evidence that energy transfer does occur among Ru-surf on the same side of the vesicle wall.

As described in the introduction, the presence of excitation energy transfer can affect the quenching rate constant K_Q by increasing the sensitizer's effective cross-sectional area for reaction with an electron acceptor. The rate of energy transfer is a function of the distance between sensitizers.¹⁹ This distance can be controlled by varying the concentration of the sensitizer in the vesicle wall. Therefore, K_Q will be dependent on the sensitizer concentration if a significant rate of energy transfer is present.

If the energy transfer rate is significant between Ru-surfs, a plot of K_Q versus Ru-surf concentration is expected to have lower and upper limits, and as a consequence of the two limits, a smooth S-shaped curve connecting them. This expectation derives from the relation that the average "nearest neighbor" distance between sensitizers has to the concentration of sensitizer.

To obtain this relation, a computer program was written to simulate randomly distributed Ru-surfs on a portion of the vesicle surface. The program generates pseudo-random numbers that are used as coordinates for points representing Ru-surfs on a 100 x 100 Cartesian coordinate system. After a specified number of points are generated, which determines the simulated concentration of Ru-surf, the distance of the nearest neighboring point to each point is calculated. An average nearest neighbor distance is then calculated for that concentration. This procedure was repeated for several different concentrations of Ru-surf

(see Figure 9). For mole fractions (moles Ru-surf/moles PC + Ru-surf) less than 0.1, the average nearest neighbor distances demonstrate an inverse square root dependence on concentration, and were calculated to be 0.52 ± 0.02 of the square lattice distances.

With regard to the anticipated plot of K_Q versus Ru-surf concentration, the lower limit of K_Q will be obtained at low Ru-surf concentrations where nearest neighbor distances are greatest. Here, "low" concentration is meant to imply that the distances between sensitizers are great enough that energy transfer is not likely to occur during a collision period. Therefore, even though the first derivative of the nearest neighbor distance with respect to concentration is large, K_Q will be independent of concentration.

The upper limit of K_Q will be approached at high concentrations of Ru-surf. Here, "high" concentration corresponds to the nearly flat region in the nearest neighbor distance versus concentration plot. In this region, the nearest neighbor distance is almost independent of concentration, and hence, so is the rate of energy transfer. Consequently, the slope of K_Q versus Ru-surf concentration will be nearly zero at high Ru-surf concentration.

Connecting the high and low concentration ends of this plot with a smooth curve will give the curve an S-shaped appearance.

The experimental plot of K_Q versus the mole fraction of Ru-surf is in agreement with our simple model (see Figure 10). In experiments using lower Ru-surf concentrations (PC:Ru-surf = 100, 50, and 30), the value of K_Q is the same within experimental error. For larger Ru-surf concentrations (PC:Ru-surf = 20, 15, and 10), K_Q increases and appears to be approaching a maximum value. Therefore, we conclude that energy

transfer does occur among Ru-surf on the same side of a vesicle membrane.

If one considers the magnitudes of the Ru-surf nearest neighbor distances in the vesicles where K_0 was observed to increase, the existence of energy transfer becomes reasonable. For the PC:Ru-surf ratios of approximately 30, 20, 15, and 10, the average nearest neighbor distances are only 2.9, 2.4, 2.1, and 1.8 molecular units, respectively. As will be discussed in a subsequent section, the area occupied by a PC molecule is approximately 70 \AA^2 . Making the rough approximation that Ru-surf and PC occupy the same amount of area, the above distances correspond to 24.5, 20, 17.5, and 15 \AA , respectively. The lowest possible nearest neighbor distance is 1.0 molecular unit (8.5 \AA), which corresponds to two Ru-surfs located side by side.

Claiming energy transfer is present on the surfaces of vesicles and yet absent across vesicle walls would not seem so reasonable if a square lattice arrangement had been assumed (incorrectly) for Ru-surfs. As specified above, the average nearest neighbor spacing is about half of the lattice spacing. Therefore, the lattice distances for PC:Ru-surf ratios of 30, 20, 15, and 10, would be approximately 49, 40, 35, and 30 \AA , respectively. As will be discussed later, the thickness of the vesicle wall is expected to be 45 \AA . Since this thickness is comparable to the lattice distance, one might have concluded that transmembrane energy transfer is possible if lateral energy transfer exists.

Electron Transport Kinetics and Energy Transfer

The existence of energy transfer among Ru-surfs on the surface of vesicles has a direct impact on proposed kinetic schemes for photosensitized electron transport through vesicle membranes.^{5,7}

This process involves dye molecules (Ru-surf) located on both surfaces of a vesicle bilayer membrane (PC), acceptor molecules in the continuous aqueous phase (heptyl viologen, C_7V^{2+}), and donor molecules in the aqueous phase inside the vesicle (EDTA). The sequence of reactions begins with an outer excited sensitizer ($*Ru-surf$) reducing C_7V^{2+} . Subsequently, the electron-deficient Ru-surf is restored to its original state by an inner Ru-surf transferring an electron to it through the membrane. Finally, EDTA reduces the oxidized inner Ru-surf.

The kinetic scheme proposed by Ford (see Figure 11) involves five states. The subscripted k's denote rate constants for the reactions. State A is the unperturbed ground state. State B results upon absorption of a photon (rate = I) by an outer Ru-surf. At this point, $*Ru-surf$ either decays to the ground state A by luminescence or internal conversion (k_0), or it is oxidatively quenched by C_7V^{2+} (k_1) resulting in state C. Back transfer of electrons (k_2) from C_7V^{2+} to the oxidized Ru-surf (k_2) competes with electron transport (k_3) across the membrane, state D, which is assumed to be reversible to state C (k_{-3}). Oxidation of EDTA by Ru-surf (k_4) is irreversible because of fragmentation and addition of water.

"With the steady-state approximation that the concentrations of $*Ru-surf$ and its oxidized form are very small and the assumption that k_3 equals (k_{-3}), the overall quantum yield of photochemistry (sequence A \rightarrow E) is given by:

$$\phi_{AE} = \phi_{AC} \cdot \phi_{CE} \quad (9)$$

$$= \frac{k_1}{k_0 + k_1} \cdot \frac{k_3 k_4}{k_3 k_2 + k_2 k_4 + k_3 k_4} \quad (10)$$

in which ϕ_{AC} and ϕ_{CE} are the yields for sequences $A \rightarrow C$ and $C \rightarrow E$."⁵

A major point of Ford's work is that, based on quantum yields and rate laws derived from the proposed kinetic scheme, two possible mechanisms for electron transport across the membrane can be distinguished. The two mechanisms are: (1) Diffusion of oxidized Ru-surf from the outside of the membrane to the inside, and (2) transfer of electrons from Ru-surf inside to oxidized Ru-surf on the outside (electron exchange).

Kinetically the two mechanisms are different; diffusion is unimolecular and exchange is bimolecular. Therefore, k_3 and k_{-3} are either unimolecular rate constants, or pseudo-unimolecular rate constants containing Ru-surf concentration factors. This difference affects the predicted effect on the quantum yield of photochemistry upon altering the Ru-surf concentration. For case (1), one would predict that the quantum yield would remain constant since k_3 and ostensibly no other rate constants are functions of [Ru-surf]. On the other hand, if case (2) were the actual mechanism, one would predict the quantum yield would be affected. Ford's experimental results show that increasing [Ru-surf] by a factor of 2.8 ± 0.2 , from PC:Ru-surf = 20:1 to 7:1, increased the quantum yield by a factor of 2.2 ± 0.3 . Therefore, he concludes that electron exchange, is the actual mechanism.

However, the validity of this conclusion has to be questioned in light of the energy transfer study presented here. Energy transfer increases the quenching rate constant, $k_1/(k_1 + k_0)$. Though not recognized by others describing the kinetics of this process, k_1 must also be a function of [Ru-surf]. Therefore, simply comparing overall

quantum yields from experiments differing in [Ru-surf] will not assess the relative magnitudes of k_1 's and k_3 's dependence on [Ru-surf].

If one compares the increase in quenching rate constants in the $\text{Fe}(\text{CN})_6^{3-}$ quenching experiments with the increase in the quantum yield found in Ford's electron transport study, a remarkable similarity is found. In going from a PC:Ru-surf ratio of 32 ± 1 to a ratio of 11 ± 1 , which is a factor of 2.9 ± 0.3 increase in [Ru-surf], the quenching rate constant increases by 2.1 ± 0.3 . This is nearly identical with Ford's increase in the overall quantum yield for a similar change in [Ru-surf].

Based on the similarity of these values, one should not conclude that energy transfer is totally responsible for the increase, since the studies involve two different quenchers and two PC/Ru-surf concentration ranges that are not identical. However, one can make the qualitative conclusion that the increase in energy transfer does contribute to the increase in quantum yield, since in both cases the quenching is collisional via electron transfer, and the quenching rate constants, both being diffusion limited, are comparable under similar conditions.²¹

It is likely that both k_1 and k_3 depend on [Ru-surf] and affect the quantum yield. The magnitude of k_1 's influence could be determined by performing a luminescence quenching study using C_7V^{2+} as quencher. k_3 's influence could then be deduced from the difference between the total change in quantum yield and k_1 's contribution to that change. The $\text{Fe}(\text{CN})_6^{3-}$ quenching study suggests that when [Ru-surf] is altered, energy transfer affects the quantum yield more than electron transport.

As a result of this additional experimentation, if the quantum yield increase can be totally accounted for by the increase in quenching rate constant, then k_3 's presumed dependence on [Ru-surf], and therefore

the electron exchange mechanism, could no longer be justified by this kinetics rationale.

Ford's work contains two arguments supporting electron exchange through the vesicle membranes: (1) the quantum yield's dependence on [Ru-surf] discussed above, and (2) an approximate rate constant determination for electron transport across the vesicle walls. The second argument shows that the observed rate of electron transport is too fast to be a result of Ru-surf diffusion, and is consistent with electron exchange. The energy transfer study presented here gives an alternative interpretation for the evidence cited in the first argument that makes the argument inconclusive. However, Ford's second argument remains intact, and therefore, so does his conclusion that electron transport occurs by electron exchange rather than Ru-surf diffusion.

Wamser's kinetic scheme differs from Ford's primarily in the addition of a "correlated radical pair" state where the redox products, C_7V^+ and oxidized Ru-surf, are held in proximity of each other.²² From this state, separation, back reaction, or reduction by another Ru-surf can occur (see Figure 12). The pseudo first-order rate constant for reduction, k_d is dependent on [Ru-surf].

Inclusion of this "correlated radical pair" state seems to remedy an apparent discrepancy in Ford's kinetic equations and his experimental results. Ford's overall quantum yield equation (Eqn. 10, above) indicates that the quantum yield is dependent on [Ru-surf], since k_3 is a pseudo first-order rate constant dependent on [Ru-surf]. The initial quantum yield equation, in contrast, is not dependent on [Ru-surf] since the factor in the equation containing k_3 drops out of the initial rate law leaving

$$\text{Initial } \phi_{AE} = \frac{k_1}{k_0 + k_1} \quad (11)$$

This occurs because k_2 in Eqn. 10 is another pseudo first-order rate constant and is dependent on the steady-state concentration of oxidized Ru-surf, which is initially zero. One would conclude, therefore, that the initial rate at which C_7V^+ is produced is independent of [Ru-surf]. This, however, was not the result found by Ford; instead, the vesicle solution with the higher [Ru-surf] had a higher initial rate.^{5a}

In Wamser's kinetic model, this discrepancy is not found. In his initial rate law, k_3 drops out as it does in Ford's, but k_d does not; therefore, the initial rate law retains [Ru-surf] dependency.

From this fact and citing Ford's experimental finding as support, Wamser concludes: "In cases in which increased sensitizer produces an increase in initial rate,....electron transport to the [correlated radical pair] occurs as well as the normal electron transport [from inner Ru-surf to uncorrelated oxidized Ru-surf]."

This conclusion, however, is not adequately supported, because here again, the effect of energy transfer on the initial rate was not considered. Therefore, the rates of electron transport either to a correlated or an uncorrelated oxidized Ru-surf must remain in question.

Vesicle Size by Radioactive Isotope Trapping

An attempt to determine the size of vesicles regularly prepared in this laboratory by the injection method has been carried out using a radioactive isotope trapping technique.^{15a} Other attempts have been made using negative stain electron microscopy²³ and fluorescence correlation spectroscopy.²⁴ In addition, the literature gives some idea

of the size to expect based on the phospholipid concentration in the ethanol solution injected.¹⁶ However, all three of the latter values differ for reasons that will be discussed.

As mentioned in the introduction, determining the amount of radioactivity contained inside vesicles formed in a radioactive buffer solution can lead to a value of the vesicle size. The total internal volume of all the vesicles in a preparation is given by the expression

$$\text{total internal aqueous volume} = \frac{4}{3} \pi r_i^3 n_v \quad (12)$$

where r_i is the average inner radius and n_v is the total number of vesicles. This total internal volume is also related to the disintegrations per minute observed, $[\text{dpm}]_{\text{obs}}$, in an aliquot of the gel filtered vesicles.

$$\text{total internal aqueous volume} = \frac{[\text{dpm}]_{\text{obs}} f_{\text{dil}}}{[\text{dpm}]_{\text{stock}}} \quad (13)$$

where $[\text{dpm}]_{\text{stock}}$ is the radioactivity of the stock solution, and f_{dil} is a filtration dilution factor. The total number of vesicles is given by

$$n_v = \frac{n_s}{n_{s/v}} \quad (14)$$

where n_s is the total number of surfactant molecules (PC and Ru-surf) injected to form vesicles, and $n_{s/v}$ is the average number of surfactant molecules per vesicle. $n_{s/v}$ can be determined by considering the combined areas of the inner and outer vesicle surfaces, $(a_i + a_o)$, and the average area per surfactant molecule, a_s .

$$n_{s/v} = \frac{a_i + a_o}{a_s} \quad (15)$$

An assumption has to be made as to the thickness of the phospholipid bilayer to determine the total surface area.

$$a_i + a_o = 4\pi r_i^2 + 4\pi r_o^2 \quad (16)$$

$$= 4\pi[r_i^2 + (r_i + t)^2] \quad (17)$$

where t is the thickness of the bilayer. Combining the above equations yields

$$\frac{[\text{dpm}]_{\text{obs}} f_{\text{dil}}}{[\text{dpm}]_{\text{stock}}} = \frac{r_i^3 n_s a_s}{3[r_i^2 + (r_i + t)^2]} \quad (18)$$

from which r_i can be calculated.

Sucrose was chosen as the radioactive probe because the membrane is relatively impermeable to it. Sucrose leakage out of the vesicle after gel filtration would render the experiment useless. However, the leakage is expected to be negligible during the time course of the experiment in light of experiments by Katz and Diamond.²⁵ The test for the completeness of outer ¹⁴C-sucrose removal described in "Methods" supports this expectation. That is, if ¹⁴C-sucrose would have leaked out of the vesicles at an appreciable rate, a constant ratio of Ru-surf to radioactivity would not have been obtained after successive gel filtrations.

Assumptions as to the average surfactant molecular area and the thickness of the bilayer have to be made to calculate the vesicle size. From x-ray diffraction, Small obtained 71.7 Å² as the area per molecule and 45.6 Å as the thickness of the bilayer for unsonicated phosphatidylcholine (PC) in water.²⁶ From electron micrographs, Bangham and Horne measured the thickness of a single bilayer and found it to be 44.2 Å

across.²⁷ Johnson et al. reported an area of 66 Å² for PC molecules in a vesicle surface using an indirect surface potential method.²⁸ More recently, Huang and Mason described PC vesicles that are 210 Å in diameter as being asymmetrical structures with molecules in the outer monolayer occupying an average surface area of 74 Å² while molecules in the inner monolayer occupy 61 Å².²⁹ They attribute this asymmetry to the constraints imposed by the high curvature in these smallest of PC vesicles. Their conclusions are based on a combination of ³¹P NMR experiments employing a shift reagent, which gives a ratio of the number of outer to inner PC molecules, and hydrodynamic data, which gives the hydrated vesicle radius, the vesicle weight, and the partial specific volume of the lipid. Subsequently, Cornell et al. reexamined Huang and Mason's physical data in terms of conventions used by Small and found them to be consistent.³⁰ They conclude that the area occupied per phospholipid molecule and the thickness of the bilayer are the same in vesicles as in a planar bilayer. The area they determined per phospholipid at the hydrocarbon/water boundary is 72.5 ± 1.5 Å² and the overall bilayer thickness is in the range 45.7 ± 0.1 Å. The phosphorylcholine group, they suggest, may occupy a greater or lesser surface area as it conforms to the geometry of the environment it is in. If it is located on the inside surface of a vesicle it adopts a more extended configuration with the N(CH)₃ nitrogen directly above the phosphorus. If located on the outside surface, the phosphorylcholine group lies roughly parallel to the membrane surface.

For the numerous studies performed to determine the surface area of PC molecules, it appears that 68 ± 4 Å² is a range that includes most of

them. Even in Huang and Mason's asymmetrical case, an average PC area of 69 \AA^2 is found.²⁹

$45 \pm 1 \text{ \AA}$ is a thickness range that seems to be reliable. Literature values of under 40 \AA can be found,³¹ but these values were obtained from electron density profiles which measure the distance between phosphate groups in opposing monolayers. It is assumed in these cases that the choline groups are in the same spherical shell as the phosphate groups, but this contradicts the conclusions of Cornell et al. mentioned above.

Using $68 \pm 4 \text{ \AA}^2$ as the area and $45 \pm 1 \text{ \AA}$ as the thickness, the ^{14}C -sucrose trapping experiment gave an average overall vesicle diameter of $505 \pm 27 \text{ \AA}$. This result was obtained from the experiment described in Methods. An earlier experiment using only a tenth as much ^{14}C -sucrose yielded a similar value of 546 \AA . Although the total counts were very low and therefore less reliable, the agreement of the values is seen as an indication that the experimental procedure is reproducible.

This vesicle diameter of $\approx 500 \text{ \AA}$ falls within the range determined by laser fluorescence correlation spectroscopy (LFCS) in this laboratory.²⁴ The comparatively small uncertainty range of $\pm 27 \text{ \AA}$ obtained in the ^{14}C -sucrose entrapment experiments serves as a refinement to the rather large range, $720 \pm 300 \text{ \AA}$, that resulted from the LFCS experiments. $505 \pm 27 \text{ \AA}$ is now the most reliable value for the average diameter of vesicles that are regularly prepared in this laboratory by the injection method.

An earlier attempt by this investigator to determine vesicle size with electron microscopy was largely unsuccessful. Vesicle samples were

negatively stained with uranyl acetate. Diameter sizes were obtained on the order of 150-200 A, which seemed too small in light of the literature on PC vesicles.^{28,32} Questions concerning vesicle dehydration resulting in size-distorting effects went unresolved, and therefore, the validity of the results remained in question. With the results of the ¹⁴C-sucrose experiments, it can be concluded that dehydration effects are important.

Kremer et al. provided a theoretical basis for determining vesicle size based on the phospholipid concentration in the ethanolic solution that is injected into rapidly stirred buffer.¹⁶ Before experimentation, they speculated that "the molecular weight and the radius of the vesicles produced by the injection method may depend on the injection velocity, the final alcohol concentration in the buffer, and the lipid concentration in the alcohol." They also considered the rate of stirring during injection and the size of the vessel. They concluded that "only the lipid concentration in the injected ethanol influenced the molecular weight and radius of the lipid particles markedly." From their experimental results using dipalmitoylphosphatidylcholine, and dimyristoylphosphatidylcholine, one could predict that injecting 0.04 M PC (i.e., the PC concentration used by this investigator) would result in vesicles on the order of 1000-1200 A in diameter. However, to do this assumes that the double bonds found in egg PC hydrocarbon chains will not have an effect on size. Also, though Kremer investigated the effect of injection rate, the range of rates was limited to 10^{-4} to 5×10^{-2} ml/min. This is well below the 1.5 ml/min rate estimated for vesicle preparations discussed in this paper. Here again, the results

of the ^{14}C -sucrose experiments suggest that these experimental differences cannot be ignored.

Luminescence Quenching and Vesicle Geometry

In addition to the radioactive isotope trapping results, the luminescence quenching data provide information as to the size of the vesicles. As described previously, luminescence quenching of Ru-surf in the membrane by $\text{Fe}(\text{CN})_6^{3-}$ in the continuous aqueous phase allows the calculation of the fraction of luminescence that is quenchable, i.e. the fraction of the Ru-surf located in the outer phospholipid monolayer. If the number of Ru-surfs per unit area is the same on both sides of the vesicle then the distribution is termed "symmetric". Hence for symmetric vesicles, the fraction of quenchable luminescence f_a , is equal to the fraction of the vesicle surface area making up the outer surface.

$$f_a = \frac{4\pi r_o^2}{4\pi(r_i^2 + r_o^2)} \quad (19)$$

where r_o and r_i are the outer and inner radii. This fraction is completely determined by two variables: the thickness of the vesicle wall t , defined by the average distance separating the ruthenium head groups of inner and outer Ru-surfs that are directly across the wall from each other, and either the inner radius or the outer radius.

$$f_a = \frac{r_o^2}{(r_o - t)^2 + r_o^2} \quad (20)$$

Therefore, if an assumption is made about the thickness, then the radius can be experimentally determined.

$$r_o = \frac{t (1 + \sqrt{f_a^{-1} - 1})}{2 - f_a^{-1}} \quad (21)$$

Two questions arise in considering this equation. First, is it a valid assumption that the vesicles are symmetric with respect to Ru-surf? Second, is the thickness of the wall as defined above, the same as the value determined for the thickness of a PC bilayer, i.e. could the ruthenium head groups on the average be "buried in" or "sticking out of" the PC bilayer? Neither of these questions can be definitively answered using the data presented here, but the range of possibilities can be considered for future verification.

As to the symmetry of the bilayer, it would seem that the randomization of the injection process and the repelling force of the 2+ charges on each Ru-surf would impose symmetry on the vesicle. However, the electrostatic repulsion may be neutralized by the electrolyte concentration in the buffer (1 M $\text{CH}_3\text{COONH}_4$), and the different configurational constraints of the inner and outer monolayers may offset the entropic effects of the vigorously stirred solution.

If one assumes that the vesicles, 505 Å in diameter with 45 Å thick PC bilayers, are symmetric with respect to Ru-surf, one can calculate the distance separating the head groups of inner and outer Ru-surf with the above equation. Knowing that f_a is 0.67 ± 0.02 , and substituting $(252.5 + x)$ for r_o and $(45 + 2x)$ for t , leads to an additional thickness of 16.7 ± 5.0 Å for each monolayer, i.e. the ruthenium head groups stick out of the bilayer on the average between 12 and 22 angstroms.

Support for this configuration comes from comparing the quenching rate constant of *Ru-surf in vesicles with the quenching rate constant

of $*\text{Ru}(\text{bpy})_3^{2+}$ in homogeneous aqueous solution. With $\text{Fe}(\text{CN})_6^{3-}$ as quencher in 1 M $\text{CH}_3\text{COONH}_4$, the rate constants are very similar at $\approx 3.5 \times 10^3 \text{ M}^{-1}$ and $\approx 2.4 \times 10^3 \text{ M}^{-1}$, respectively. If the ruthenium head groups were at all buried in the PC bilayer, one would expect the quenching rate constant to be lower since they would be less accessible to the $\text{Fe}(\text{CN})_6^{3-}$; but the quenching rate constant is not lower.

Support also comes from the absorption spectra of Ru-surf. As noted by Ford^{13b}, absorption by Ru-surf is solvent dependent. The Ru-surf absorption spectrum in vesicles is like its spectrum when it is dissolved in water without vesicles, and unlike its spectra in apolar solvents. This is consistent with the idea of Ru-surf protruding into the aqueous phase.

Alternatively, if one assumes that opposing ruthenium head groups are separated by the same distance as the thickness of a PC bilayer, i.e. neither buried nor protruding, then one must conclude that there is an asymmetry in Ru-surf distribution. A symmetric distribution for 505 Å diameter vesicles with 45 Å thick bilayers would result in a fraction of quenchable luminescence of 0.60. Therefore, given the above assumption, Ru-surf must preferentially locate on the outer surface to achieve a f_a of 0.67. It could be postulated that this is because the packing is not as strained at the outer surface, where the curvature spreads out the phosphorylcholines, as it is at the inner surface, where the curvature packs the phosphorylcholines closer together.

Either of the configurations postulated above may describe the actual structure of the vesicle. A third alternative is also possible, which is some combination of the first two configurations. That is, the

ruthenium head groups may stick out of the vesicles something less than 16.7 ± 5.0 A on the average and have a concomitant asymmetric distribution that is less pronounced than described above. The two features are interdependent; one must be balanced by the other to give a f_a of 0.67.

SUMMARY

A luminescence quenching study was performed with an amphiphilic derivative of tris(2,2'-bipyridine)ruthenium(II) situated at the surface of vesicle membranes. The average value of the fraction of quenchable fluorescence is 0.67 (std. dev. = 0.02), and is independent of the ruthenium compound's concentration. The quenching rate constant, on the other hand, increases with ruthenium concentration. These observations lead to two conclusions: 1) Energy transfer between ruthenium sensitizers on opposite sides of the vesicle wall does not take place on the time scale of luminescence, and 2) Energy transfer does occur among ruthenium sensitizers on the same side of the vesicle wall.

Energy transfer among ruthenium sensitizers on the surfaces of vesicles increases the quantum yield in experiments that use this sensitizer to demonstrate photosensitized electron transport through vesicle walls. However, investigators who have studied this transport process failed to consider the impact of energy transfer in their kinetic schemes. As a result, some arguments they present are inadequately supported.

Vesicle size can be determined by measuring the amount of radioactivity trapped inside vesicles that are formed in a buffer containing a known concentration of radioactive sucrose. Phosphatidylcholine vesicles regularly made in this laboratory by the "injection" method were determined to have an average diameter of 505 ± 27 Å.

If it is assumed that the surface concentration of ruthenium sensitizer is the same on both sides of the vesicle wall, then from the fraction of quenchable fluorescence and the vesicle size determined by radioactive sucrose entrapment, it can be calculated that the ruthenium

head groups extend out of the vesicle wall an average distance of 16.7 ± 5.0 Å. Conversely, if it is assumed that the ruthenium head groups neither "stick out of" nor are "buried in" the vesicle walls, then it can be concluded that the surface concentration of ruthenium surfactant is greater on the outside than on the inside. In the absence of either assumption, the degree to which ruthenium head groups extend out of the vesicle is balanced by the asymmetry of their distribution to yield a fraction of quenchable luminescence of 0.67.

Table 1LUMINESCENCE QUENCHING DATA

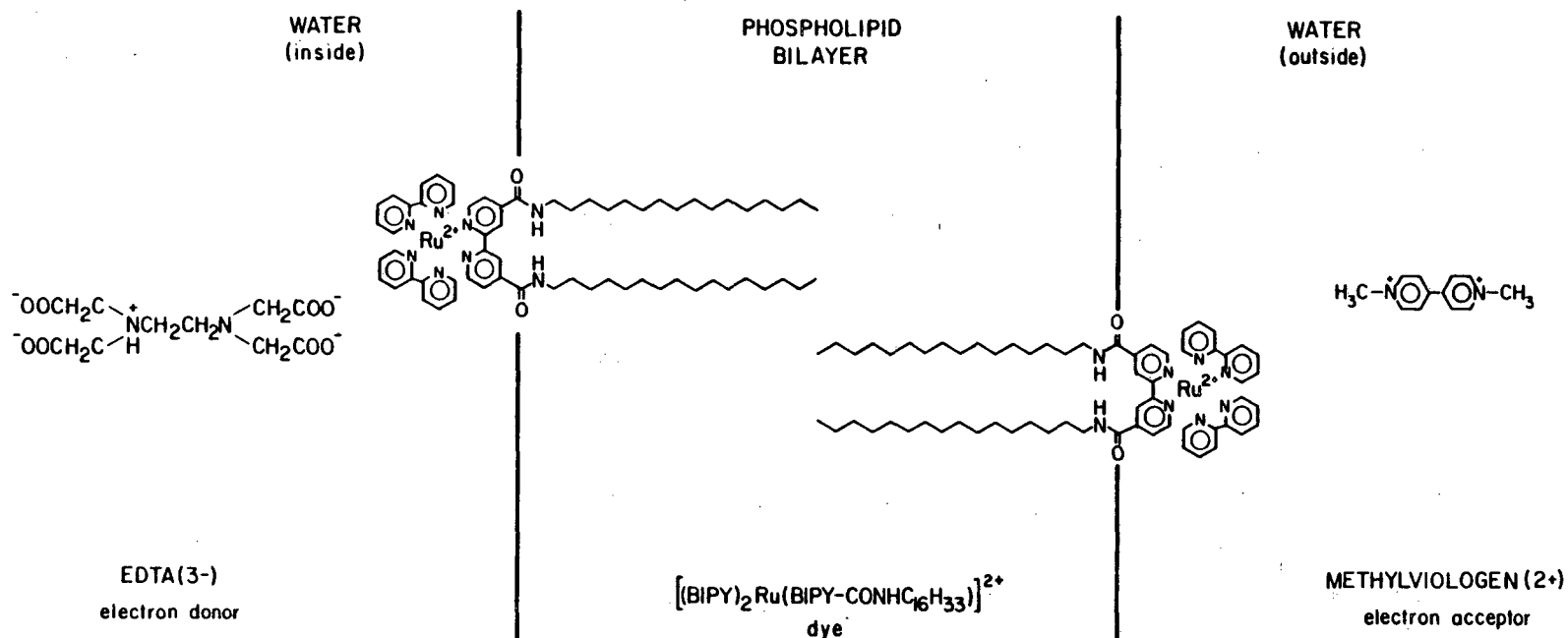
| <u>PC/Ru-surf</u> | <u>Slope x 10⁴</u> | <u>Intercept</u> | <u>f_a</u> | <u>K_Q x 10⁻³</u> |
|-------------------|-------------------------------|------------------|----------------------|--|
| 11 | 2.11 ± 0.05 M | 1.47 ± 0.02 | 0.68 ± 0.01 | 7.0 ± 0.3 M ⁻¹ |
| 15 | 2.47 ± 0.12 | 1.54 ± 0.03 | 0.65 ± 0.02 | 6.3 ± 0.5 |
| 21 | 2.97 ± 0.03 | 1.55 ± 0.01 | 0.65 ± 0.01 | 5.2 ± 0.1 |
| 31* | | 0.99 | 0.99 | 3.6 |
| 32 | 4.41 ± 0.22 | 1.46 ± 0.08 | 0.69 ± 0.04 | 3.3 ± 0.4 |
| 47 | 4.69 ± 0.15 | 1.46 ± 0.05 | 0.68 ± 0.03 | 3.1 ± 0.2 |
| 104 | 4.36 ± 0.20 | 1.55 ± 0.06 | 0.65 ± 0.03 | 3.6 ± 0.3 |
| 104 | 4.26 ± 0.06 | 1.50 ± 0.02 | 0.67 ± 0.01 | 3.6 ± 0.1 |

* This experiment differs from the others in that the quencher was present inside the vesicles as well as in the continuous aqueous phase. These data were extracted from the least square line of a regular Stern-Volmer plot (see Figure 7), thus, no uncertainties are given.

Table 2VESICLE DIMENSIONS

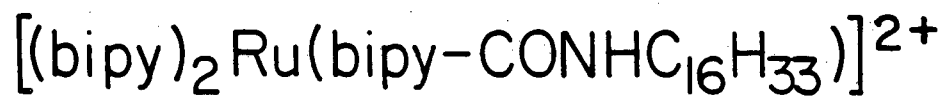
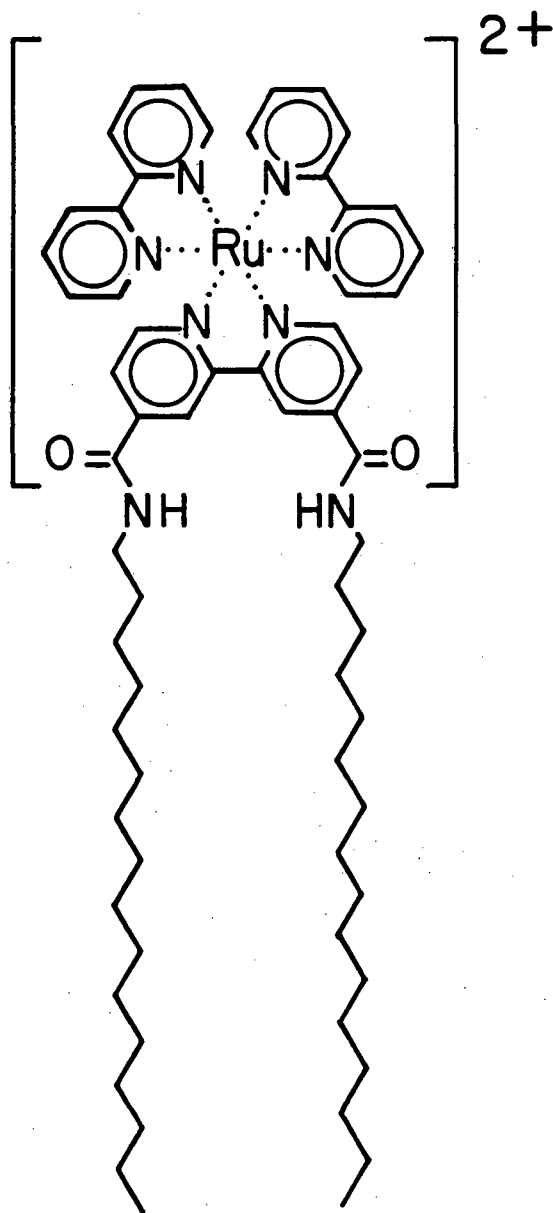
| | | |
|---|---|-----------------------|
| Vesicle diameter [§] | = | 505 ± 27 A |
| Area of PC molecule [†] | = | 68 ± 4 A ² |
| Thickness of PC bilayer [†] | = | 45 ± 1 A |
| Maximum additional monolayer thickness due to Ruthenium head group (assumes symmetrical distribution of ruthenium complex)* | = | 16.7 ± 5.0 A |

-
- § Experimental value (see text: "Vesicle Size by Radioactive Isotope Trapping").
- * Experimental value (see text: "Luminescence Quenching and Vesicle Geometry").
- † Summary of literature analysis.



XBL 807-4267

FIGURE 1. An example of a photosynthetic mimetic system using unilamellar vesicles (Ford, ref. 4). Upon excitation, the dye reduces methyl viologen outside the vesicle. Subsequently, electron transfer occurs between inner and outer dye molecules, and EDTA inside the vesicle donates an electron to an electron-deficient dye molecule. For each methyl viologen reduced and each EDTA oxidized, there is a net transport of one electron across the membrane.



XBL 806-4226

FIGURE 2.
An amphiphilic derivative of tris(2,2'-bipyridine)ruthenium(II)
(abbreviated to "Ru-surf" in this paper) synthesized by Ford (ref. 13a).

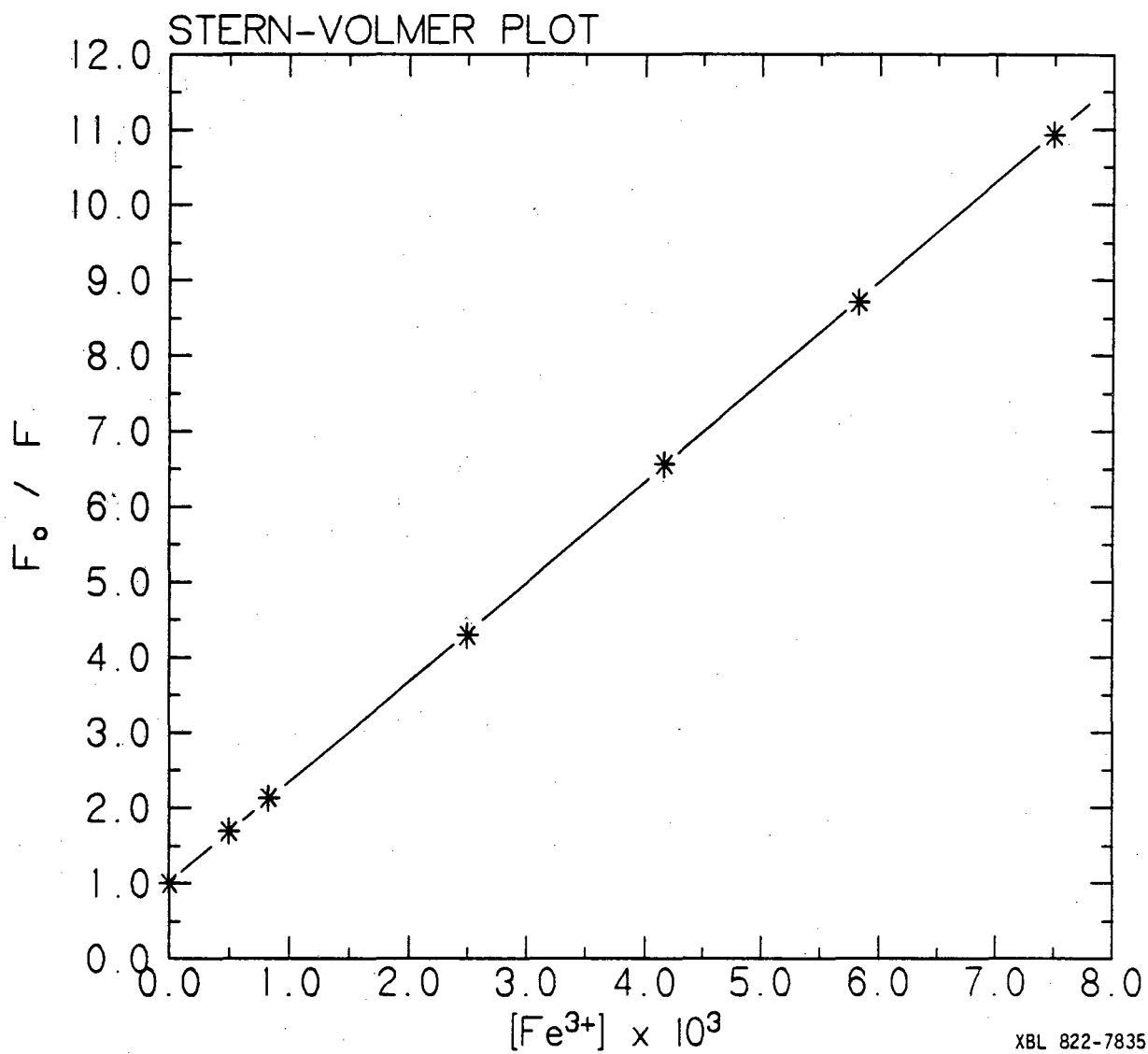


FIGURE 3.
Stern-Volmer plot: Luminescence quenching of $*Ru(bpy)_3^{2+}$ by Fe^{3+} in homogeneous aqueous solution. Excitation wavelength = 450 nm; emission wavelength = 602 nm.
Least square line data: slope = $1.322 \times 10^3 \text{ M}^{-1}$; intercept = 1.026.

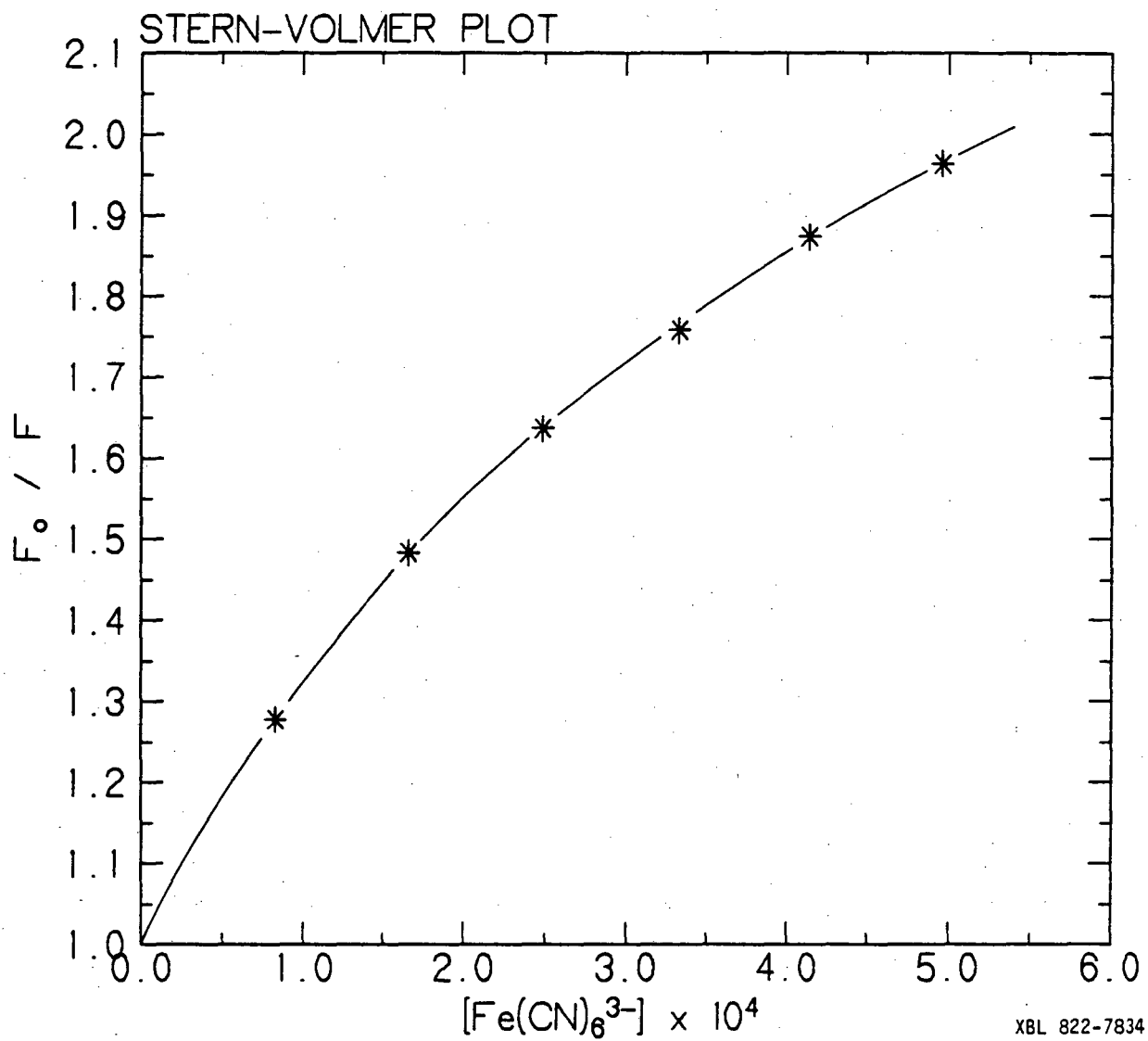


FIGURE 4. Stern-Volmer plot: Luminescence quenching of *Ru-surf in vesicle walls by $\text{Fe}(\text{CN})_6^{3-}$ in the continuous aqueous phase. Excitation wavelength = 481 nm; emission wavelength = 658 nm. PC/Ru-surf = 15.

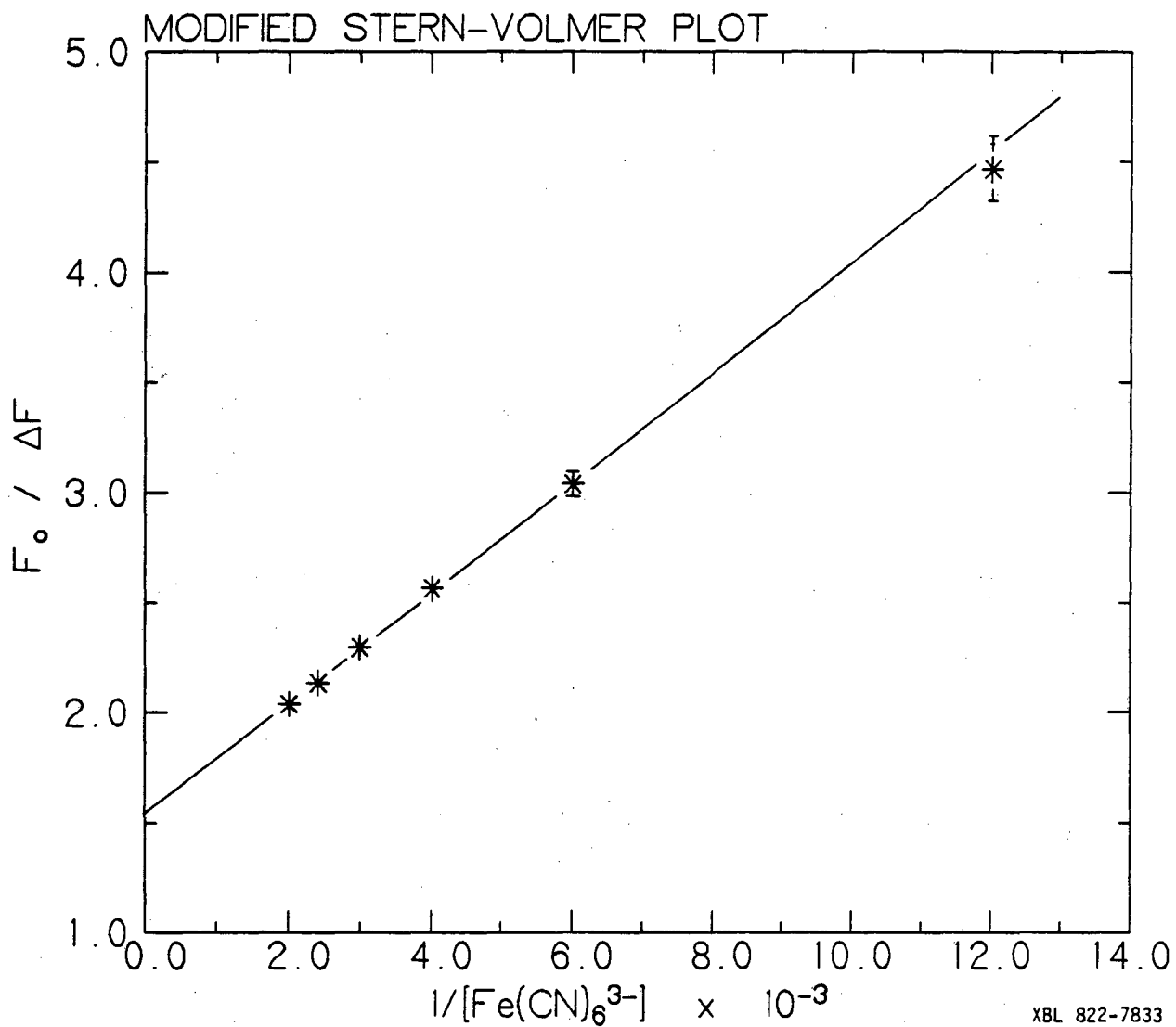


FIGURE 5. Modified Stern-Volmer plot: Same experiment as in Figure 4, but replotted according to Lehrer's theory. PC/Ru-surf = 15. Error bars of the first four data points are smaller than the data symbols. Slope = $(2.46 \pm 0.12) \times 10^{-4}$ M; intercept = 1.542 ± 0.035 .

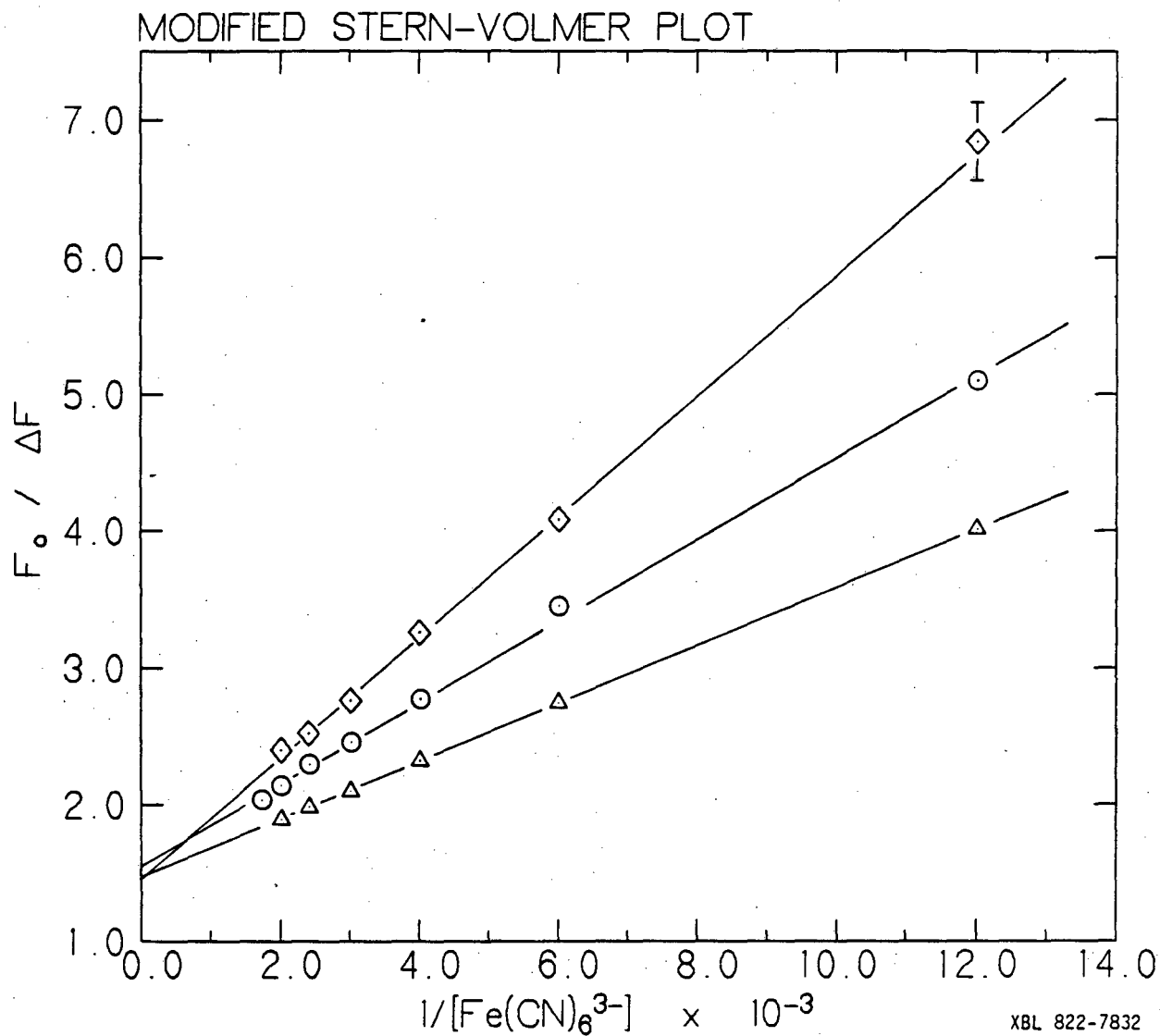


FIGURE 6. Modified Stern-Volmer plots: Luminescence quenching of *Ru-surf in vesicle walls by $\text{Fe}(\text{CN})_6^{3-}$ in the continuous aqueous phase. Three vesicle solutions differing in membrane composition are represented here: PC/Ru-surf = 11 (\triangle); 21 (\odot); 32 (\diamond). Error bar lengths are smaller than the data point symbols except for the one shown. See Table 1 for data extracted.

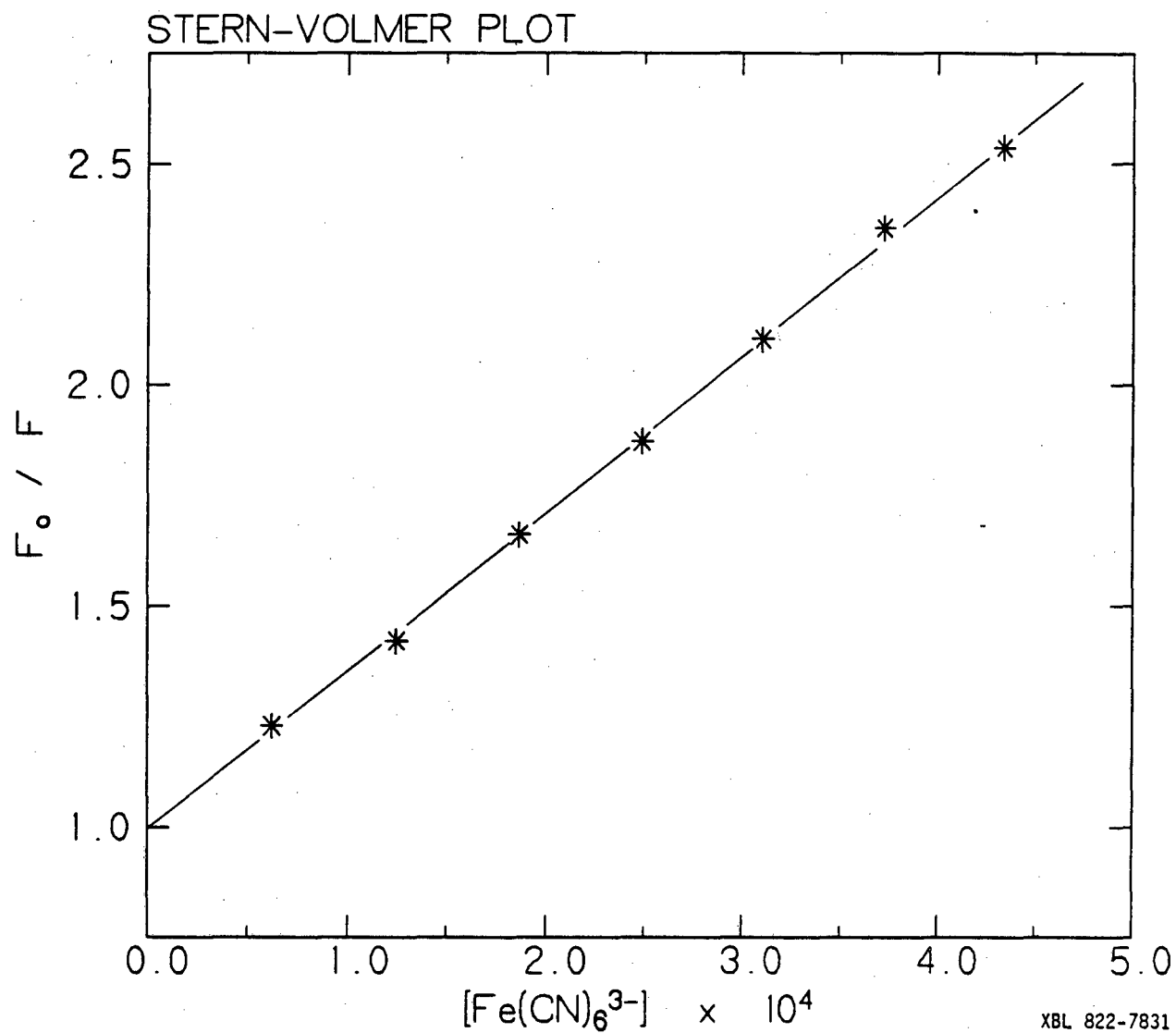


FIGURE 7. Stern-Volmer plot: Luminescence quenching of *Ru-surf by $\text{Fe}(\text{CN})_6^{3-}$ inside and outside vesicles (PC/Ru-surf = 31). Excitation wavelength = 481 nm; emission wavelength = 658 nm. Least square line data: Slope = 3.56×10^{-3} ; intercept = 0.993.

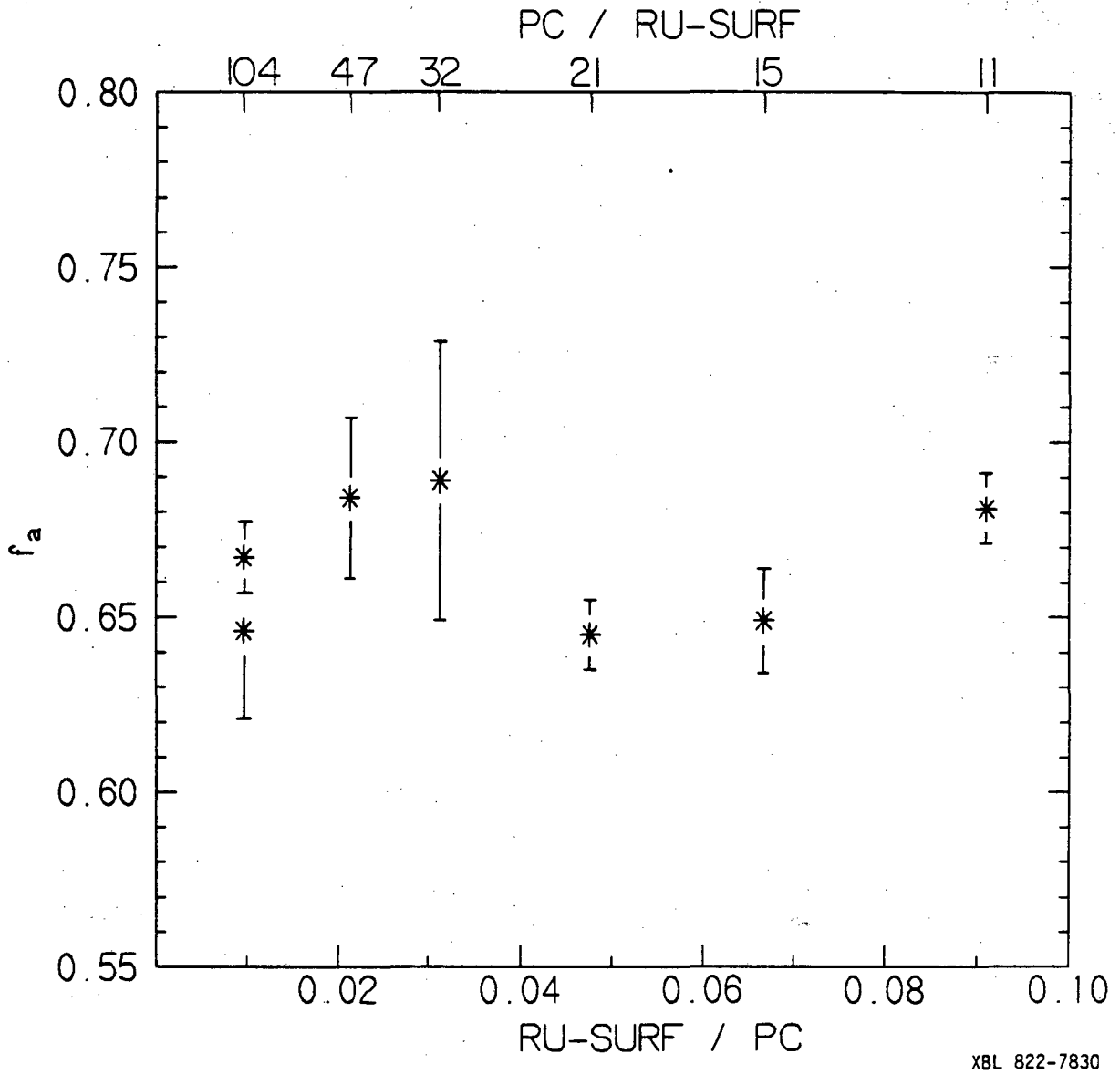
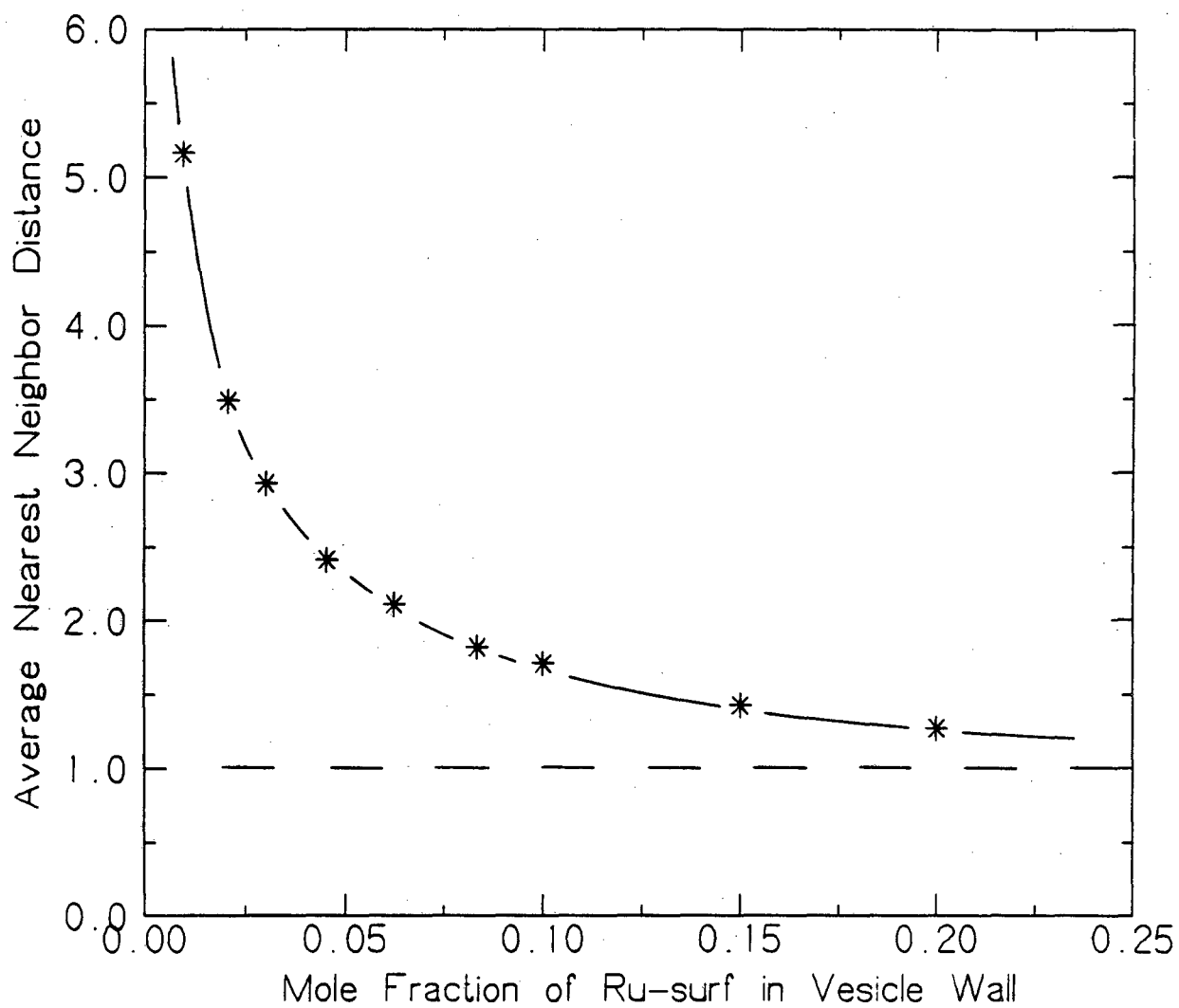
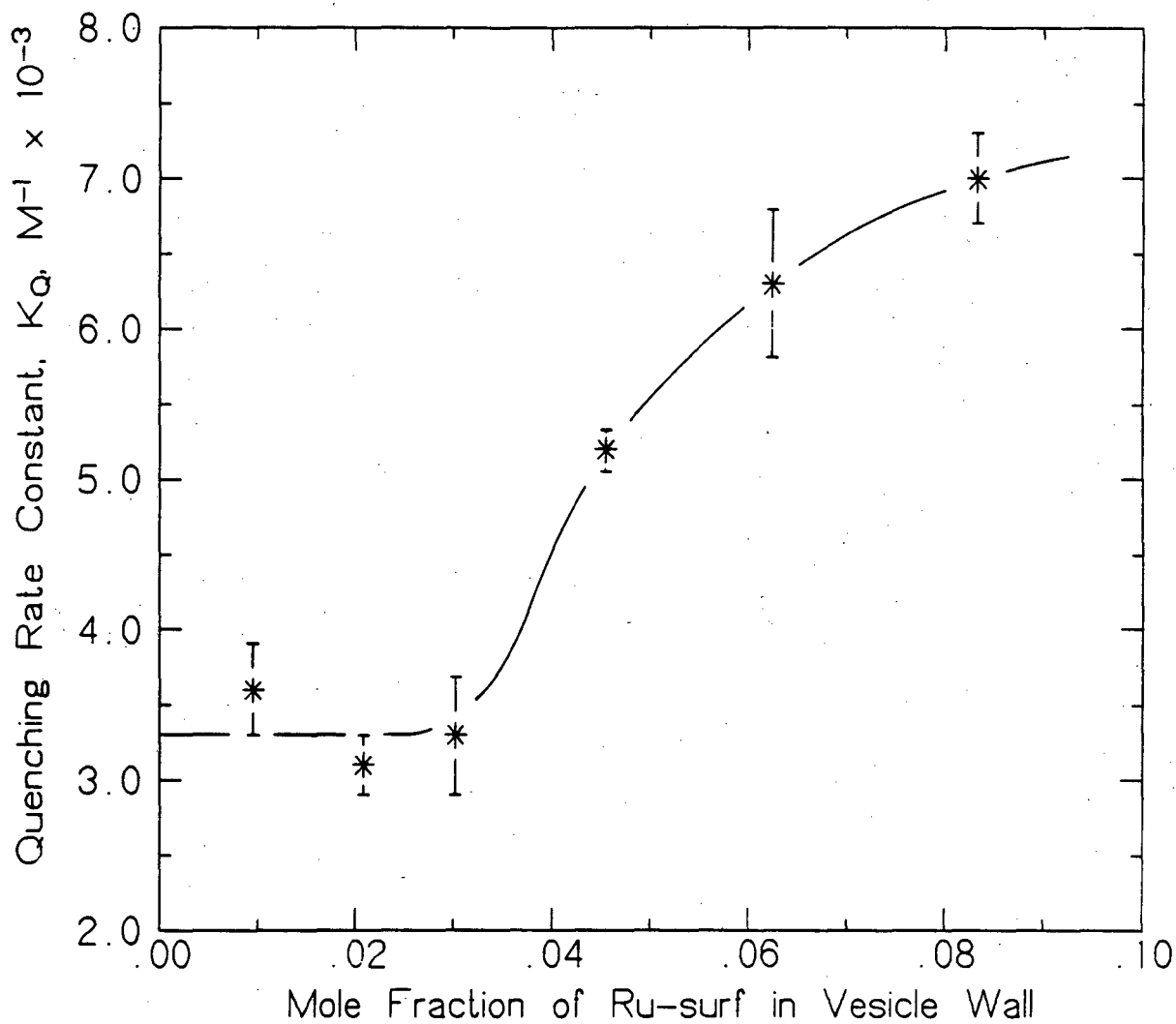


FIGURE 8. Fraction of quenchable fluorescence vs. Vesicle composition. No dependence demonstrated. Average $f_a = 0.666$ (std. dev. = 0.019). Vesicle composition is expressed in terms of molar ratios of PC and Ru-surf.



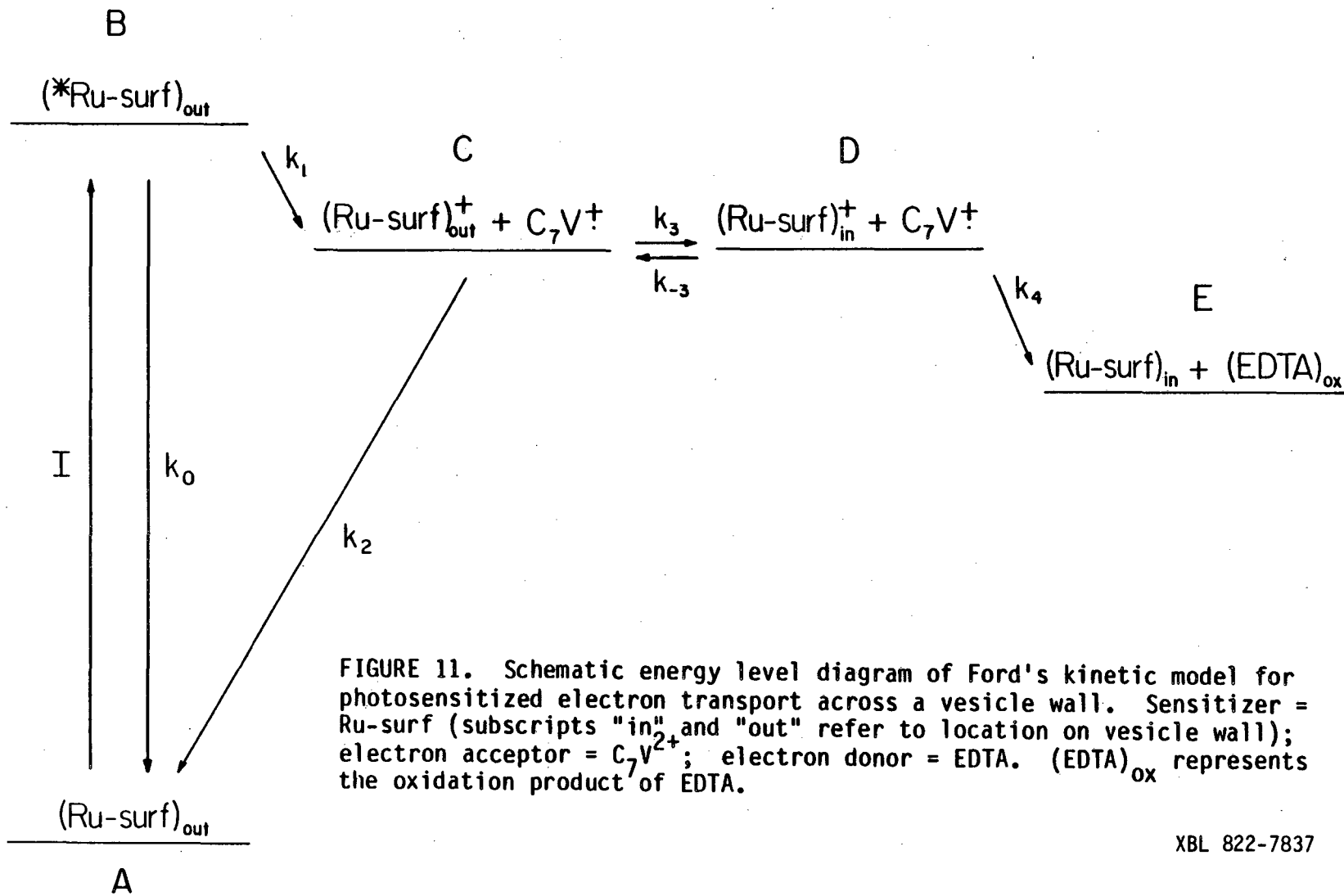
XBL 825-10282

FIGURE 9. Computer-simulated, average nearest neighbor distances between Ru-surfs in a vesicle wall as a function of Ru-surf concentration in the wall (mole fraction of Ru-surf = moles Ru-surf/moles PC + Ru-surf). Unit distance is one PC molecular span (=8.5 Å).



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FIGURE 10. The quenching rate constant as a function of Ru-surf concentration in the vesicle wall, derived from luminescence quenching of *Ru-surf in vesicles by $Fe(CN)_6^{3-}$ in the continuous aqueous phase.



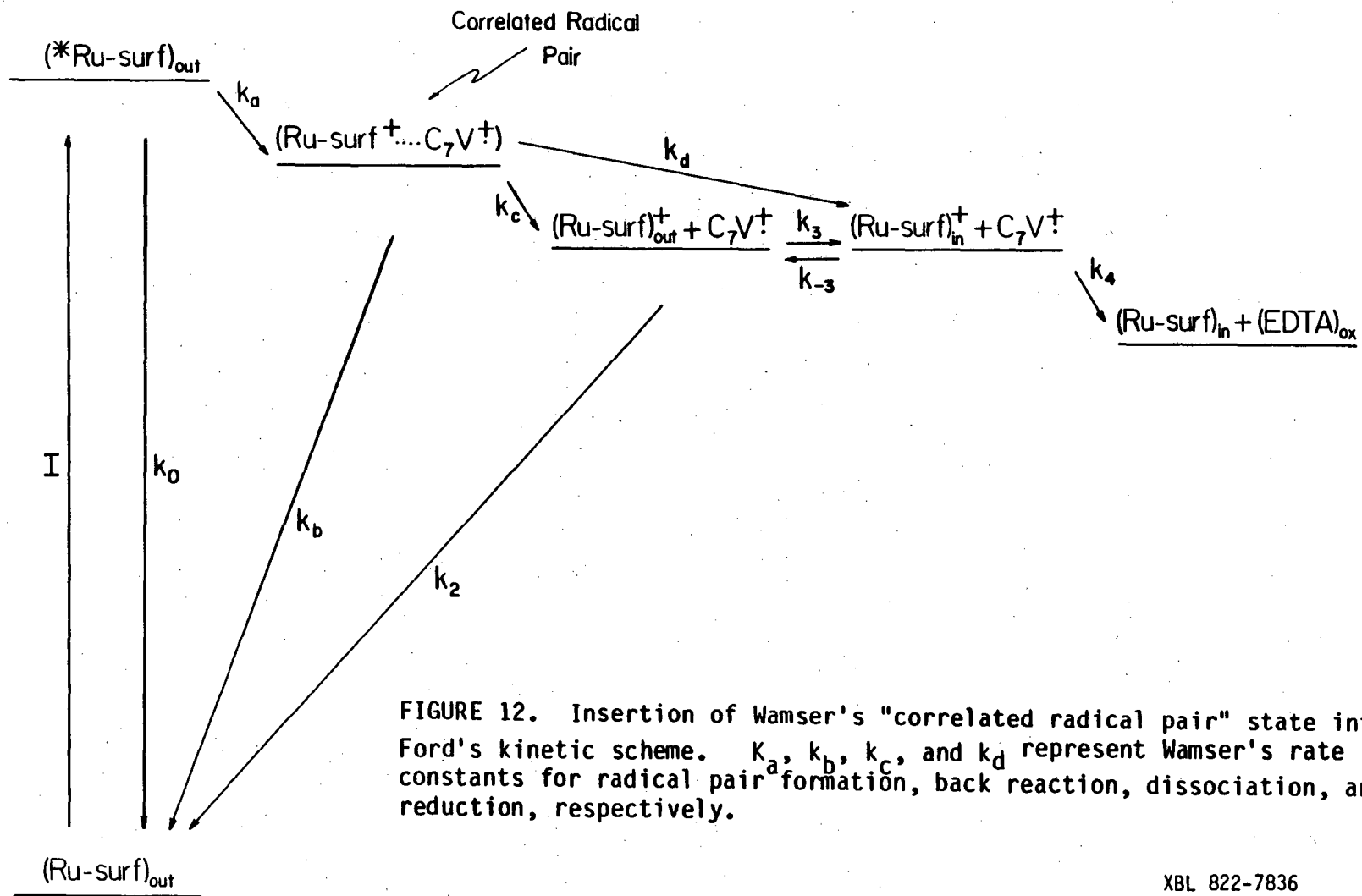


FIGURE 12. Insertion of Wamser's "correlated radical pair" state into Ford's kinetic scheme. k_a , k_b , k_c , and k_d represent Wamser's rate constants for radical pair formation, back reaction, dissociation, and reduction, respectively.

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 $K_Q = 2 \times 10^3 \text{ M}^{-1}$ for heptyl viologen quenching of *Ru-surf in PC vesicles (PC/Ru-surf = 31). Under the same conditions,
 $K_Q = 3 \times 10^3 \text{ M}^{-1}$ for $\text{Fe}(\text{CN})_6^{3-}$ (Table 1).
22. Other differences exist between Wamser's kinetic scheme (ref. 7) and Ford's (ref. 5). However, so as not to obscure the point being made in this paper, only the differences that are relevant to the argument are mentioned. Also, to aid comparison, Wamser's rate constant subscripts have been translated into Ford's corresponding subscripts.
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