Chem. Soc. Rev., 1991, **20**, 49–94

Electron Transfer across Vesicle Bilayers

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1 Introduction

For many years there has been considerable interest in better understanding the underlying physico-chemical mechanisms by which photosynthetic organisms capture and store the energy available in sunlight. Recently, significant advances have been made in this area.[1,2]

The phospholipid-based thylakoid membrane plays a vital role in photosynthesis. The spatially structured environment it provides allows the 'molecular cogs' of the 'green machine' to operate with incredible efficiencies.

The complexity of biological systems often necessitates the construction of simpler models to help develop and evaluate theories about the form and function of the photosynthetic apparatus. As a nearly spherical bilayer of amphipathic molecules, the vesicle assembly provides an excellent supramolecular model of the thylakoid membrane. Studies with these less complex microheterogeneous structures can assist in the elucidation of the physical principles underlying light and electron conduction *in vivo*. They are also important in our attempts to produce 'artificial' photo-transducers capable of converting light energy into chemical potential. Photo-assisted electron transfer across vesicle bilayer is of great interest to this area, not least because it offers the possibility of separating the resultant redox products. Various applications can be envisaged, including separation of the sites of hydrogen and oxygen production in the photochemical decomposition of water.[3,4]

In this paper we review research carried out on electron transfer reactions in vesicle systems, covering first ground state electron transfer reactions across phospholipid-based vesicle bilayers. We will then overview excited state electron transfer reactions in predominately (a) 'natural product' (phospholipid) and (b) 'synthetic' (surfactant) vesicle assemblies, in each case considering charge separation phenomena localized at one or both bilayer surfaces before examining transmembrane redox reactions.

To begin with, however, it is worthwhile outlining some of the more important features of Nature's light harvesting centres, as well as those of their much simpler model counterparts.

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[1] J. Diesenhofer and H. Michel, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 829.
[2] R. Huber, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 845.
[3] J. G. Calvert, *Ohio J. Sci.*, 1953, **53**, 293.
[4] M. Calvin, *Photochem. Photobiol.*, 1983, **37**, 349.

2 Biological Transmembrane Redox Systems

Life, as we know it, depends on solar energy, in particular that in the visible region of the solar spectrum, both as a source of free energy and information. However, without a means to capture, transform, and utilize visible light, no ecosystem can be supported by it. Over three thousand two hundred million years ago, Nature, through its long evolution, had perfected a process known as photosynthesis[5–7] by which visible light could be converted to electrical and chemical energy.

All photosynthetic organisms, whether they are higher plants, algae, or lower bacteria, contain so-called reaction centres, which are the site for solar energy-driven photosynthetic processes.[8,9] Significant progress on the nature of these biophysical processes has been made possible over the last thirty years only by an increased understanding of the gross structure and chemical composition in and around these centres.[10]

Crucial to the design of all Nature's photo-transducers is the bilayer lipid membrane; specifically, it is the molecular organization of the proteins, lipids, and pigments that constitute photosynthetic membranes that make photosynthesis possible.

In green plants the photosynthetic membrane, the thylakoid membrane,[8–10] is highly convoluted and forms sacs, called thylakoids, enveloping a region of fluid, the lumen. Thylakoids are arranged in stacks, termed grana, in the fluid medium (stroma) of cell organelles known as chloroplasts (Figure 1).

On the basis of such observations and many physiological experiments, it is now generally accepted that photosynthesis consists of two photosystems in series, both centred on the thylakoid membrane, and a dark reaction that takes place in the stroma. The basis of our present understanding of photosynthesis is represented by the so-called 'Z' scheme[4,11,12] (Figure 2). In simple terms, photosynthesis can be described as the light-assisted oxidation of water to oxygen and reduction of carbon dioxide to carbohydrates, and such a process cannot be driven by the energy available from a single photon of visible light.[5,7]

Whilst photosystem II is involved with the oxidation of water near the inner surface of the thylakoid membrane,[13] it also instigates the vectorial transfer of electrons to photosystem I (the first to evolve on Earth), located nearer the outer surface of the membrane. This 'downhill' transfer process, and that subsequent to

[5] 'Light, Chemical Change and Life a source book in photochemistry', ed J D Coyle, R R Hill, and D R Roberts, Open University Press, Milton Keynes, 1982, p 355
[6] 'Solar Power and Fuels', ed J R Bolton, Academic Press, London, 1977, p 53
[7] H T Tien, *Prog Surf Sci*, 1989, **30**, 1
[8] 'Progress in Photosynthesis Research', ed J Biggins, Martinas Nijhoff Publishers, Dordrecht, Netherlands, 1987
[9] J M Anderson, *Biochim Biophys Acta*, 1975, **416**, 191
[10] 'Fifth International Congress on Photosynthesis', ed G Akoyunoglou, Balaban International Press, Rehovot, Israel, 1981, **1**, 254
[11] 'Bioenergetics of Photosynthesis', ed Govindjee, Academic Press, New York, 1975
[12] J H Fendler, *J Phys Chem*, 1985, **89**, 2730
[13] 'Photochemical Conversion and Storage of Solar Energy', ed J S Connolly, Academic Press, 1981, p 1

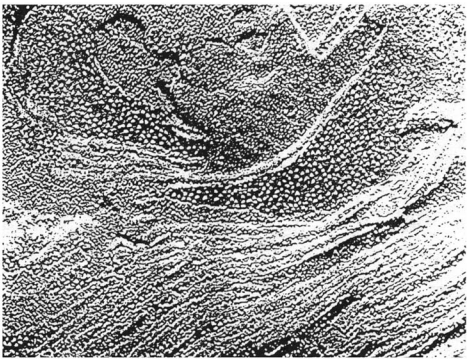


Figure 1 *Electron micrograph of chloroplast lamallae*
(Reproduced with permission from ref 4)

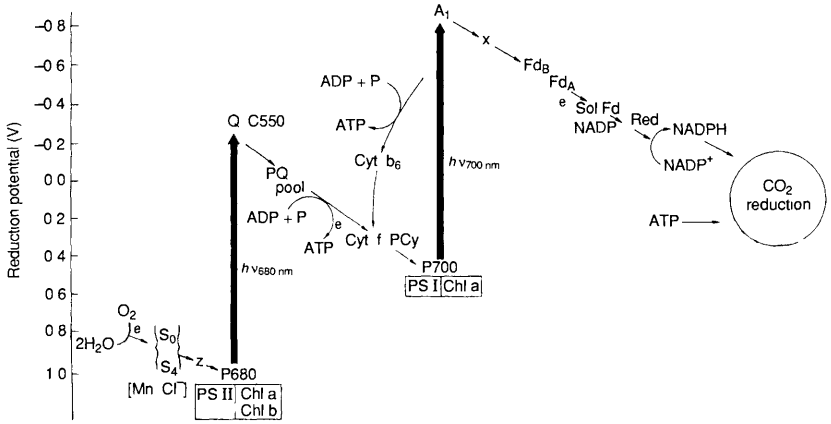


Figure 2 *Photosynthetic Z-scheme of electron transport*
(Reproduced with permission from ref 4)

the further light activation that follows, together provide the reduced adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) required for CO_2 reduction [14 15] The chemiosmotic hypothesis, developed concurrently with the 'Z' scheme, proposed that it is the electrochemical gradient of protons generated across membranes by light-assisted electron transport that acts as the driving force for phosphorylation (energy transduction),[16] as well as the active transport of ions [17]

[14] Biochemistry of Photosynthesis ed R D F Gregory Wiley New York 1978
[15] Photosynthesis in Relation to Model Systems Topics in Photosynthesis ed J Barber 3 vol Elsevier/North Holland Amsterdam and New York 1979
[16] P Mitchell *Biol Rev* 1966 **41** 445
[17] Biological Transport H N Christensen Benjamin Press New York 1975

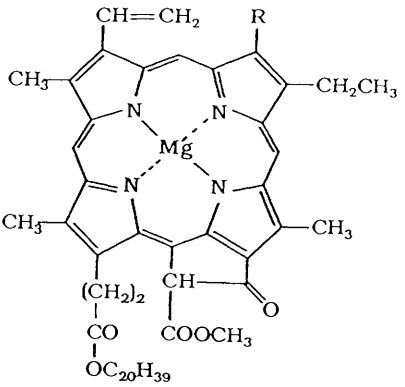


Figure 3 *Structure of chlorophyll* a (R = CH_3) *and chlorophyll* b (R = CHO)

The highly organized, precisely defined spatial relationships of the membrane components create a supramolecular device of incredible efficiency and low photo-degradation, which trap and utilize the four photons of visible light required to produce one molecule of oxygen in only one-millionth-millionth of a second.[18]

In gross chemical composition, thylakoid membranes consist of 60% proteins, 20% lipids and pigments, 4% nucleic acids and others, but their precise distribution is uncertain.[9]

Monogalactolipids, sulpholipids, phosphatidylglycerol, lutein, plastoquinone, and some pigments are present on the outer surface of the thylakoid membrane, whilst digalactolipids and pigments are located at the inner surface. At physiological pH, both the phospholipids and sulpholipids are negatively charged.[14,15]

Extrinsic proteins, such as water-soluble ferredoxin, ferredoxin-NADP oxidoreductase (a flavoprotein), and ATP synthetase (CF_1) lie near the stroma. Towards the thylakoid interface with the lumen, water-splitting manganese proteins of photosystem II have been identified, together with an extrinsic, loosely-bound copper protein, plastocyanin.[8,10] Although little is known of these proteins in their native state, it is thought that the specific orientation of proteins with the membrane, including those of the numerous intrinsic pigment-protein complexes involved in light absorption, facilitate both primary charge separation and vectorial electron flow.[19]

Most of the 300 or so chlorophyll molecules (Figure 3) and other membrane pigments contained within each photosynthetic unit are not involved in any photochemistry, but act as light-harvesting antennae which transfer absorbed electronic excitation energy (by 'resonant transfer') to the reaction centre at which electron transfer occurs.[1]

[18] L. Milgrom, *New Scientist*, 2nd February 1984, p. 26.
[19] G. R. Fleming, J. L. Martin, and J. Breton, *Nature*, 1988, **333**, 190.

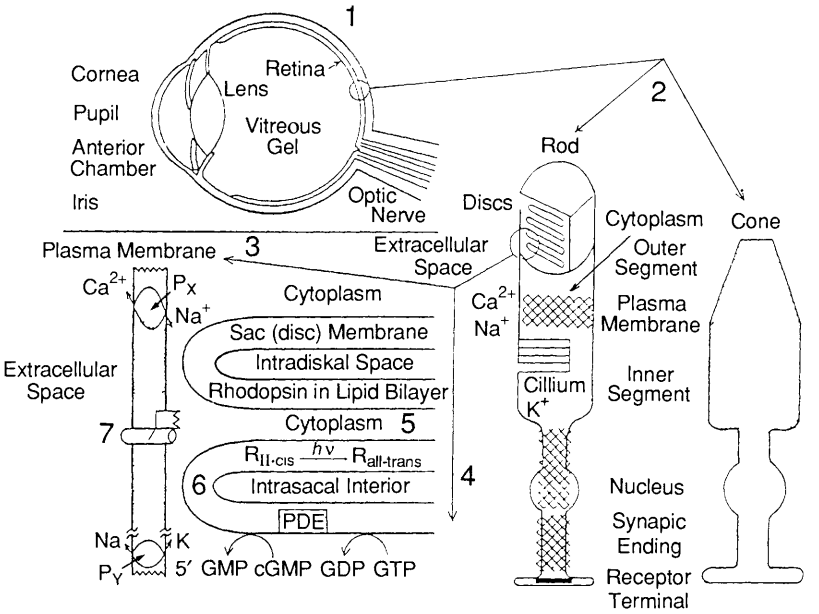


Figure 4 *Schematic drawings of the eye and its photoreceptor membranes.* (1) *Vertebrate eye.* (2) *The retina, a sheet of light sensitive tissue, consists of two kinds of photoreceptors, rods, and cones.* (3,4) *The structural details of a rod and its photobiophysics and photobiochemistry in terms of the plasma and sac membranes are shown in the lower left.* (5) *In the dark,* Na^+/Ca^{2+} *pumps in the outer segment and* Na^+/K^+ *pumps in the inner segment maintain a high* K^+ *and low* Ca^{2+} *concentration inside of the plasma membrane which has a high permeability to* Na^+. (6,7) *Under illumination, a single photon absorbed by a rhodopsin* (R) *isomerizes it to* R*, *which initiates the* cGMP *cascade that blocks the inward flow of* Na^+ *and* Ca^{2+} *in the outer segment*
(Reproduced with permission from ref. 7)

It is thought that chlorophyll molecules form a monolayer array on the surface of membrane-spanning intrinsic proteins; the orientation and separation of the hydrophobic chlorin rings, buried within the folds of the protein, are maintained for maximum energy migration,[20] whilst inhibiting concentration quenching.[21] Strongly exciton-coupled 'special pairs' of chlorophyll molecules act to trap excitons from other chlorophyll antennae, and in this state are able to mediate the vectorial transfer of an electron, ultimately derived from water.[21]

Research into photosynthesis can be operationally divided into the biochemical and photophysico-chemical. The 'dark' enzymatic reactions of the former, which comprise the Calvin cycle, are well understood.[14,15] However, in spite of extensive efforts, a detailed explanation of almost all of the major physiochemical membrane events remains some time away. Those aspects most actively

[20] M. D. Archer, in 'Photochemistry', Specialist Periodical Report, ed. D. B. Smith, Royal Society of Chemistry, London, 1976, vol. 7, chapter 5, p. 561.
[21] M. D. Archer, in 'Photochemistry', Specialist Periodical Report, ed. D. B. Smith, Royal Society of Chemistry, London, 1977, vol. 8, chapter 5, p. 571.

researched at present are the primary steps of quantum conversion,[22,23] photophosphorylation and electron trnasport, and oxygen evolution.[24]

Other notable examples of pigmented organelle membranes are the outer segment sac membrane of retinal rods in vertebrate eyes, where light is transduced and utilized as a signal to trigger a sensory response[7,25] (Figure 4), and the 'purple membrane' of *Halobacterium halobium*, capable of both photoconversion and photodetection.[7] The electron microscope reveals all light-transducing membranes as surprisingly similar in gross construction; a lipid bilayer in which photoactive pigments are embedded.

3 Models of Transmembrane Electron Transfer[26b]

Clearly, until our knowledge of biomembrane transductors has vastly improved, alternative and conflicting explanations can be offered for almost any experimental findings in the technically demanding area of *in vivo* biomembrane research. As a result, workers have attempted to gain insights into the nature of the complex biotransducers by studying simpler, more easily interpretable, models comprising artificially constructed lipid bilayers. Two types of bilayer system have been constructed; the bilayer lipid membrane (BLM)[6,7,26,27] and the vesicle.[27] The first consists of a planar BLM of similar thickness to biological membranes (~4—6 nm), separating two aqueous solutions. The real power of the BLM system is that one can precisely characterize membranes and membrane phenomena by measurement of the subtle changes in membrane capacitance, impedance, and conductivity.[28,29]

Careful alteration in reactant concentrations allows investigation of a whole spectrum of experimental parameters on a single membrane, and application of a single and varying electrical potential across the membrane permits identification of effects associated with the charge character of ionic concentration gradients.[30] A surface charge may be conferred on a BLM by incorporation of anionic or cationic compounds. Importantly, asymmetric BLM's can be constructed,[26] and any BLM membrane can be analysed, in whole or in part, by spectroscopic methods.

The major problems with the BLM as a model for a biological membrane stem from its method of preparation.[26,28] Very significant amounts of organic solvents remain in the membranes, and their exact composition is unclear.[31] Their small

[22] J Deisenhofer and H Michel, *Angew Chem*, 1989, **28**, 829
[23] R Huber, *Angew Chem, Int Ed Engl*, 1989, **28**, 848
[24] D T Sawyer and M E Bodini, *J Am Chem Soc*, 1975, **97**, 6588
[25] 'The Eye', A Knowles and M J A Dartnell, ed H Dawson, Academic Press, New York, 1977, p 425
[26] (*a*) H T Tien, 'Bilayer Lipid Membranes (BLM) Theory and Practice', Dekker Inc, New York, 1974, (*b*) J H Fendler, 'Membrane Mimetic Chemistry', Wiley, New York, 1982
[27] A D Bangham, N W Hill, and N G A Miller in 'Methods in Membrane Biology', ed E D Korn, vol 1, Plenum Press, New York, 1974, chapter 1
[28] D S Berns, *Photochem Photobiol*, 1976, **24**, 117, and ref therein
[29] H T Tien, *Photochem Photobiol*, 1972, **16**, 271
[30] J S Huebner, A E Popp, and K R Williams, *J Chem Educ*, 1988, **65**, 102
[31] R E Pagano, J M Ruysschaert, and I R Miller, *J Membrane Biol*, 1972, **10**, 11

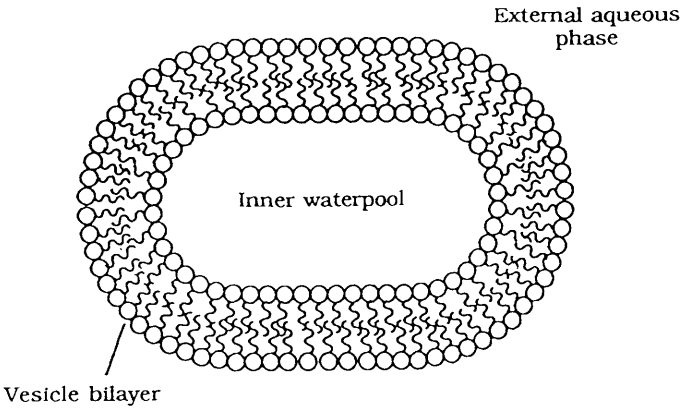


Figure 5 *Idealized representation of a lipid vesicle assembly in cross section.* ○ *hydrophilic headgroup.* ∿∿ *hydrophobic hydrocarbon tail*

surface area makes them unsuitable for the measurement of diffusion rates and chemical reactions in general. Furthermore, BLM's over *ca.* 3mm lack reproducible stability over long periods,[26] and binding studies with BLM's have generally proved to be impractical.

The alternative tool in model systems that obviates most of these problems is the lipid vesicle, which is an approximately spherical bilayer lipid membrane that encloses a small volume of aqueous solution[27] (Figure 5). Formed in great numbers, their combined surface area can be 10^{10} times greater than the largest BLM. This, together with their great stability, makes them suitable for precise diffusion and spectrophotometric measurements, binding studies, and gas evolution experiments. They are amenable to study by a variety of biochemical and biophysical methods, including gel filtration, electrophoresis, ultracentrafugation, calorimetry, fluorescence, nuclear magnetic resonance, and electron spin resonance.[28] By way of its form, the vesicle bilayer mimics many biological membranes in possessing an inherent structural dissymmetry, permitting steric, electrostatic, and other orientation factors to be considered and evaluated.

However, the major advantage of vesicle bilayers is that they can be generated with a precisely defined composition, without the incorporation of extraneous materials.

Accurate analytical measurements and area/volume-related estimates of vesicle systems are only possible, however, with unilamellar assemblies of uniform size distribution, requirements that cannot be fulfilled in many instances, particularly with naturally-derived lipids.[28] Another disadvantage with vesicle systems is the difficulty in experimental manipulation; electrical measurements cannot be made across these bilayers, and, once formed, the vesicle interior solution cannot be readily altered.

Naturally, while no single model can be expected to represent faithfully all

aspects of a biomembrane, the complementary approaches of the BLM and vesicle model systems, together with related systems such as organized multilayers,[32–35] have already contributed significantly to our understanding of many physiochemical events in complex biological ensembles.[22,23] The insights gained will be invaluable in the construction of artificial devices, based on the 'membrane principle' of supramolecular organization, for practical applications such as solar energy utilization.

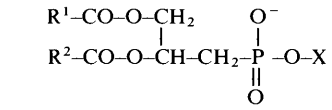
It is our intention in the remaining part of this paper to review the most notable advances in the area now known as 'biomimetic chemistry', as they related to electron transfer processes in vesicular systems. It seems sensible, therefore, first to discuss in more detail the preparation, form, and behaviour of these microheterogeneous assemblies.

A. Vesicle Assemblies.—Vesicles are quasi-spherical, multimolecular aggregates of surface active (surfactant) molecules in which a lipid bilayer separates an inner aqueous compartment from the bulk aqueous phase[35] (Figure 5). Multilamellar vesicles, or liposomes, were first prepared by shaking naturally derived cell phospholipids with water,[36] but since then many other natural lipids have been found to form vesicles or liposomes.[37]

The thermodynamics of self-organization are complex,[38] but the phenomenon is essentially a result of the 'hydrophobic effect'[39] in balance with hydrophilic and geometric factors. In general, surfactant molecules carrying two long alkyl chains form vesicles, while those with a single chain assemble as micelles. The kinetic stability of vesicles is far greater than that of micelles, and, once formed, they cannot be destroyed by dilution.[40]

As a reflection of their natural abundance, the majority of physical studies of biological lipid assemblies have been on phospholipids (Figure 6). With increasing water content, these molecules assume, sequentially, homogeneous and crystalline phases, followed by heterogeneous dispersions of vesicles or (usually) liposomes of broad size distribution (*ca.* 100—1800nm in diameter).[28,41] Ultrasonic dispersal[42] of these liposomes leads to smaller, unilamellar vesicles (bilayer thickness ~4—6nm)[37] of narrow size distribution (*ca.* 30—100nm diameter), although this technique may accelerate autodioxidation of phospholipids containing unsaturated bonds.[28] Other means of preparation

[32] I Yamazaki, N Tamai, and Y Fujita, *J Am Chem Soc*, 1988, **92**, 5035, and ref therein
[33] R L Eissler and H J Dutton, *Photochem Photobiol*, 1981, **33**, 385
[34] T Miyasaka, T Watanabe, A Fujishima, and K Honda, *Surf Sci*, 1980, **101**, 541
[35] K Kalyanasundaram, 'Photochemistry in Microheterogeneous Systems', Academic Press, London, 1987
[36] A D Bangham, *Prog Biophys Mol Biol*, 1968, **18**, 29
[37] 'Liposomes in Biological Systems', ed G Gregoriadis and A C Allison, Wiley, New York, 1978
[38] R Nagarajan, *Chem Eng Commun*, 1987, **55**, 251
[39] C Tanford, 'The Hydrophobic Effect', Wiley, New York, 1973, J H Fendler and E J Fendler, 'Catalysis in Micellar and Macromolecular Systems', Academic Press, New York, 1975
[40] J H Fendler, *Chem Rev*, 1987, **87**, 877, and ref therein
[41] H Ringsdorf, B Schlarb, and J Venzmer, *Angew Chem, Int Ed Engl*, 1988, **27**, 113 and ref therein
[42] C D Tran, P L Klahn, A Romero, and J H Fendler, *J Am Chem Soc*, 1978, **100**, 1622



R^1, R^2 hydrocarbon tails
$R^1 = R^2$ = myristoyl (C_{14}) DMPX
= palmitoyl (C_{16}) DPPX
= stearoyl (C_{18}) DSPX
= oleolyl (C_{18}(1,9-*cis*)) DOPX
= elaidoyl (C_{18}(1,9-*trans*)) DEPX

phosphotidic acid (PA),
X = –H

phosphotidylcholine (PC),
X = $-CH_2CH_2\overset{+}{N}(CH_3)_3$

phosphotidylserine (PS),
X = $-CH_2CH_2\overset{+}{N}H_3COO^-$

phosphotidylethanolamine (PE),
X = $-CH_2CH_2\overset{+}{N}H_3$

phosphotidylinositol (PI),
X = $-C_6H_5(OH)_5$

Figure 6 *Structure of the more abundant phospholipids*

include slow injection of an organic lipid solution into an aqueous solution,[43] cholate dialysis,[44] gel filtration, and ultracentrifugation.[28]

Considered the most sophisticated model of the biological membrane, the vesicle lipid bilayer is currently viewed in terms of the fluid mosaic (liquid crystalline) model proposed for cell membranes.[45]

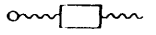
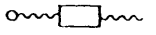
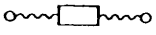
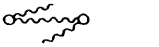
In the liquid crystalline state, fast lateral diffusion of lipids within the plane of the vesicle bilayer ($D \sim 10^{-8}\,cm^2\,s^{-1}$) and rapid *cis*/*trans* segmental motion of the hydrophobic chains contrast their extremely slow transversal, so-called 'flip-flop' motion.[40] On lowering the temperature, the bilayer undergoes a characteristic thermotropic phase transition (T_c) to a gel-like state where the chains are not fully extended and tilt to the normal plane of the bilayer; all modes of mobility, particularly rotational, decrease in magnitude.[35,46,47]

The gel-crystalline phase transition temperature reflects the fluidity, and consequently the permeability, of the bilayer, and is controlled by the chain length, the degree of unsaturation and polymerization,[48] the headgroup structure (including electrical charges), and the presence of solute(s).[49] In agreement with the chemiosmotic hypothesis,[16] significant pH gradients can be maintained across phospholipid bilayers, with permeability coefficient for H^+ and OH^- around only $10^{-4}\,cm\,s^{-1}$, but six orders of magnitude greater than that measured for Na^+.[50]

Although extensively investigated as the most closely-related models of biological membranes, the complexities and chemical instabilities of natural product vesicle assemblies necessitated the development of simpler, yet functional, synthetic membrane mimetic agents.

[43] L. A. M. Rupert, D. Hoekstra, and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1985, **107**, 2628.
[44] L. T. Mimms, G. Zampighi, Y. Nozaki, C. Tanford, and J. A. Reynolds, *Biochem.*, 1981, **20**, 833.
[45] S. L. Singer and G. L. Nicolson, *Science*, 1975, **175**, 720.
[46] I. Tabushi, I. Hamachi, and Y. Kobuke, *Tetrahedron Lett.*, 1987, **28**, 5899.
[47] E-S. Wu, K. Jacobson, and D. Papahadjopoulos, *Biochemistry*, 1977, **16**, 3936.
[48] J. M. Gebicki and M. Hicks, *Nature*, 1973, **243**, 232.
[49] J. H. Fendler, *Acc. Chem. Res.*, 1980, **13**, 7, and ref. therein.
[50] M. Rossignol, P. Thomas, and C. Grignon, *Biochim. Biophys. Acta*, 1982, **684**, 195.

Table 1 *Morphologies of various amphiphilic molecules which form vesicles in aqueous solution*

Surfactant type	*Molecular structure*	*Type of membrane formed*	*Tail*
Single chain		Bilayer	Hydrocarbon
Single chain		Bilayer	Fluorocarbon
Single chain		Monolayer	Hydrocarbon
Double chain		Bilayer	Hydrocarbon
Double chain		Bilayer	Fluorocarbon
Triple chain		Bilayer	Hydrocarbon
Triple chain		Bilayer	Fluorocarbon
Mixed chain		Monolayer	Hydrocarbon
Mixed chain		Monolayer	Hydrocarbon

The formation of bilayer structures in simple surfactant dispersions had been inferred from their phase diagrams[48] before the first were recognized in 1976 on shaking thin films of oleic and linoleic acids in aqueous buffers.[51] Known as ufasomes, their formation was inhibited by electrolytes, they were unstable outside the pH 6—8 range, did not concentrate on centrifugation, were open to oxidative decomposition, and retained substrates poorly.[52]

Since then, the aggregational behaviour of many hundreds of surfactant molecules has been examined,[41,53,54] many of which can be be prepared in the pH 1—13 range, where they remain stable for months whilst retaining substrates in substantial amounts.[42] Based on the numerous surfactant studies performed, three essential structural elements of a surfactant have been identified for its assembly into a bilayer structure.[54,55] Firstly, a flexible tail consisting of a linear methylene chain (C_7 or longer) or a related structure; secondly, a rigid segment, and, lastly, a hydrophilic headgroup, consisting of groups such as quaternary ammonium, phosphate, or sulphonate. It has also been found that the presence of additional spacer groups (such as methylene groups, C_{10} or more) inserted between the rigid segment and the headgroup, and interacting groups (such as esters) promotes vesicle formation. Table 1 illustrates the morphologies of some surfactant structures studied.

[51] J M Gebicki and M Hicks, *Chem Phys Lipids*, 1976, **16**, 142
[52] M Hicks and J M Gebicki, *Chem Phys Lipids*, 1977, **20**, 243
[53] T Kunitake, N Kimizuka, N Higashi, and N Nakashima, *J Am Chem Soc*, 1984, **106**, 1978, and ref therein
[54] T Kunitake, Y Okahata, Y Shiomomura, S Yasumuni, and K Takarabe, *J Am Chem Soc*, 1981, **103**, 5401, and ref therein
[55] A Kumano, T Kajiyama, M Takayanagi, T Kunitake, and Y Okahata, *Ber Bunsenges Phys Chem*, 1984, **88**, 1216

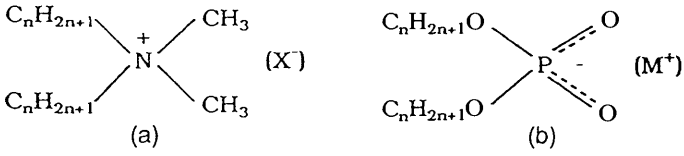


Figure 7 *Structures of vesicle forming molecules: (a) dialkyldimethylammonium halide (b) dialkylphosphate*

Notably, vesicles comprising a *single* surfactant monolayer can be formed;[38,56] such molecules stabilize other bilayers, imitating Nature's membrane-spanning lipids.[41] The need for increased bilayer stability together with controllable permeability and morphology has led to the development (by a variety of approaches) of the most sophisticated system in the armoury of the membrane mimetic chemist, the polymerized surfactant vesicle.[41,57,58] Chemical and photochemical treatment of suitable preformed vesicles can further differentiate opposite sides of the vesicle membrane.[41]

Surfactant vesicles exhibit most of the characteristic properties of natural lipid vesicles, such as thermotropic phase transitions[59,60] and osmotic activity.[49] However, generally, they are more easily destabilized by high salt concentrations ($>0.1\,mol\,dm^{-3}$) or by the presence of oxyanions or polyions.[35] Both ionic strength and pH can strongly affect their size and permeabilities,[60] and so their preparation, handling and usage warrants careful scrutiny. Particular care is necessary when using charged surfactant vesicles in the presence of multicharged counterions, since these can bring about coagulation of the vesicles at low concentrations.

Photochemical studies with surfactant vesicles have been principally with those composed of dialkylammonium halides or dialkylphosphates[41,49] (Figure 7). Vesicle morphology lends itself ideally to the compartmentalization and organization of the components of such systems, in the inner and outer aqueous phases, in the hydrophobic bilayer itself, and in the interfaces. The geometrical and compositional differences between each side of the bilayer are reflected by many surface-differentiating chemical reactions[61] and spectral phenomena.[62]

By a judicious combination of preparatory techniques, the properties of a vesicle system can be tailored to requirements and then examined under a variety of conditions. The understanding gained from the simplest of vesicle systems can be applied and developed for evermore complex assemblies, where the fine tuning of form and function necessary in practical supramolecular devices (*e.g.* for targeted drug delivery, solar energy conversion, *etc.*) should be possible.

[56] J-H. Fuhrhop, V. Liman, and V. Koesling, *J. Am. Chem. Soc.*, 1988, **110**, 6840.
[57] J. Stefely, M. A. Markowitz, and S. L. Regen, *J. Am. Chem. Soc.*, 1988, **110**, 7463, and ref. therein.
[58] N. Higashi, T. Adachi, and M. Niwa, *J. Chem. Soc., Chem. Commun.*, 1988, **1573**, and ref. therein.
[59] Y. Okahata, R. Ando, and T. Kunitake, *Ber. Bunsenges Phys. Chem.*, 1981, **85**, 789.
[60] A. Kumano, T. Kajiyama, M. Takayanagi, T. Kunitake, and Y. Okahata, *Ber. Bunsenges Phys. Chem.*, 1984, **88**, 1216.
[61] R. A. Moss, S. Bhattacharya, and S. Chatterjee, *J. Am. Chem. Soc.*, 1989, **111**, 3680.
[62] Y-M. Tricot, D. N. Furlong, A. W-H. Mau, and W. H. F. Sasse, *Aust. J. Chem.*, 1985, **38**, 527.

4 Ground State Electron Transfer Across Vesicle Bilayers

Transmembrane electron transport plays an important role in the respiratory and photosynthetic systems of mitochondria and thylakoid membranes, respectively. The study of these processes *in vivo* is, however, hindered by our limited knowledge of the relative locations and orientations of the electrochemical prosthetic groups involved;[63,64] many of the electron transporting units are tightly bound in complex, multi-enzyme systems.[65,66] In an effort to better understand the molecular mechanism of electron transport in bioenergetic membranes, model systems based on vesicle assemblies have been extensively studied.

In biological systems, the electrochemical potential gradient across the cell membrane is maintained by the inside–outside unequal distribution of lipids and proteins bound to the cell membrane. A central feature of the chemiosmotic hypothesis of oxidative phosphorylation in natural systems is that this potential difference induces the generation of a proton (or other ion) concentration gradient across the membrane *via* coupling to a 'downhill' chemical reaction.[67,68] The pH gradient drives many important biological functions such as active transport,[17] stimulus response,[69] and ATP synthesis.[70] In the absence of a permeant ion this theory predicts that a membrane potential should develop to retard electron transfer.

By constructing a model cell system comprising phosphatidylcholine vesicles containing ferricyanide as an electron acceptor (midpoint potential 0.36 V *versus* SHE, similar to ferrocene) in the inner waterpools, and ascorbate (-0.186 V *versus* SHE) as an electron donor in external aqueous solution, Hinkle was able to demonstrate that membrane-soluble ferrocene acted as a moderately effective *mobile* electron carrier between donor and acceptor.[71] Importantly, the rate of photoassisted reduction of ferricyanide underwent a further five-fold increase in the presence of catalytic amounts of carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), and other compounds which had been shown to increase the proton permeability of several natural and artificial membranes[72,73] (Figure 8). The same effect was observed in the presence of gramicidin, a carrier of both protons and sodium ions.[73,74] Benzoquinone, on the other hand, catalysed electron transport across the vesicle bilayer at the higher rate unaided, with FCCP having little effect.

[63] R A Capaldi, F Malatesta, and V Darley-Usmar, *Biochim Biophys Acta*, 1983, **726**, 135
[64] G Hauska, E Hurt, N Gabellini and W Lockan, *Biochim Biophys Acta*, 1983, **726**, 97
[65] B L Trampower, *J Bioenerg Biomembr*, 1981, **13**, 1
[66] T G Traylor, *Acc Chem Res*, 1981, **14**, 102
[67] P Mitchell, *Nature*, 1961, **191**, 144
[68] P Mitchell, *Biol Rev*, 1966, **41**, 445
[69] 'Biochemistry The Chemical Reactions of Living Cells', Academic Press, New York, 1977, p 265
[70] J M Lehn and J P Behr, *J Am Chem Soc*, 1973, **95**, 6108
[71] P Hinkle, *Biochem Biophys Res Commun*, 1970, **41**, 1375
[72] J Bielawski, T E Thompson, and A L Lehninger, *Biochem Biophys Res Commun*, 1966, **24**, 948
[73] P J F Henderson, J D McGivan, and J B Chappell, *J Biochem*, 1969, **111**, 521
[74] P Mueller and D O Rudin, *Biochem Biophys Res Commun*, 1967, **26**, 398

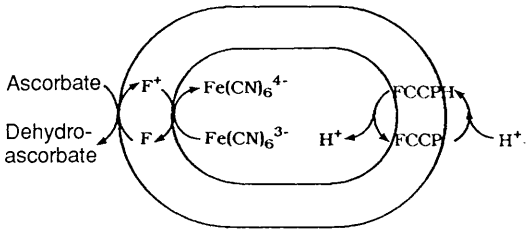


Figure 8 *Mechanism of FCCP-stimulated, ferrocene-mediated transbilayer ground state electron transport*[72,75]

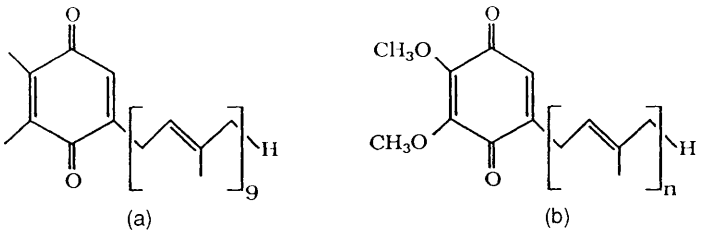


Figure 9 *Structures of (a) plastoquinone* (PQ) *(b) ubiquinone* (UQ_n)

Hinkle's results were explained in line with the chemiosmotic hypothesis:[68] the ferrocene/ferricinium couple is electrogenic; that is, capable of electron transport only, and requires the presence of a so-called 'uncoupling agent' to affect a higher level charge-compensating ion influx. Conversely, benzoquinone is a hydrogen carrier, and crosses the membrane in an electrically neutral cycle, transferring hydrogen atoms into the vesicle interior.[75]

The action of benzoquinone is interesting in view of the known role of plastoquinone and ubiquinone (Figure 9) in electron transport in plant photosynthesis[11] and respiration,[76] and bacterial photosynthesis,[77] respectively. In fact, as soon as the concept of oriented loops of electron transport had been developed for biomembranes,[67,68] the excess, mobile 'pools' of quinones present in these systems were implicated in proton translocation.[78,79] Some argued,[80] however, that the isoprenoid side chains of plastoquinone and ubiquinone, both of which are long enough (in an all-*trans* form) to span the lipid bilayer, would restrict their mobility perpendicular to the plane of the membrane; others suggested that they, in fact, acted as Nature's 'molecular wire'.

[75] P. C. Hinkle. *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1973, **32**, 1988.
[76] S. Papa, *Biochim. Biophys. Acta.* 1976, **456**, 39.
[77] F. M. Harold in 'Current Topics in Bioenergetics', ed. D. R. Sanadi, Academic Press. 1976. vol. 6. p. 83.
[78] F. L. Crane, *Annu. Rev. Biochem.*, 1977, **46**, 439.
[79] S. S. Anderson, I. G. Lyle, and R. Paterson, *Nature*, 1976, **259**, 147.
[80] R. N. Robertson and N. K. Boardman, *FEBS Lett.*, 1975, **60**, 1.

In a series of papers,[81–83] Hauska and others studied the effect of increasing the isoprenoid side chain of quinones on their ability to transport electrons/protons across the bilayer of a model system similar to Hinkle's (except that the 'external' donor was dithionite, $E_{\frac{1}{2}}$ −1.13 V *versus* SHE).[84] They found that they did act as hydrogen atom carriers; the efficiency of ubiquinones, all of which were membrane bound, actually increased with isoprenoid chain length, with a dramatic rise above two isoprene units. This was in spite of the higher activation energy of reaction with the longer chain molecules, suggesting that the mechanism of hydrogen atom transport altered with increasing side chain length.

Ubiquinones possessing no, or a very short, isoprenoid side chain, for which the kinetics of electron transfer were all pseudo first-order, could be regarded as acting like benzoquinone. The apparently higher order for the kinetics of the long chain ubiquinones (and plastoquinone) was explained as arising from the heterogeneous occupancy of the lipid membrane by 'quinone domains'; the isoprenoid side chain exerting a tendency to form clusters of higher molecular structure in the bilayer—remarkably similar to the 'quinone pool' proposed for natural systems.[78,85]

Semiquinone transients were only detectable for the lower quinones, an observation attributed to the faster disproportionation of the higher intermediates in the ordered domains.[83] The concept of the 'molecular wire' was not supported by these studies,[82] which found that saturation of the quinone side chain had no effect on the efficiency of catalysis.

Although most of the electron-transport components of biological systems are known, the actual mechanism of electron transfer is not understood in detail for the vast majority of the many redox reactions that occur. Cytochromes are known to play a major role; the most knowledge is available for the interaction of cytochrome *c* with cytochrome oxidase,[86] but the latter is a large, poorly-defined multi-redox centred complex.

Cytochromes incorporate metal centres, most notably heme proteins, and it was for this reason that Runquist *et al.* investigated the efficiency of hemin dimethyl ester in the transport of electrons across the bilayer of phosphatidylcholine vesicles (from external indigotetrasulphonic acid to internal ferricyanide).[87] Their studies showed that the iron porphyrin catalysed electron transport 10 times faster than Hinkle's ferrocene model system—equivalent to 240 electrons/molecule of hemin dimethyl ester per minute. The rate of electron transfer exhibited a first order dependence on the iron porphyrin concentration, eliminating aggregation effects. Electron transport was found not to be electrogenic, but rather involved a coupled, neutral system, in which charge-compensation was affected by the catalytic ferric hemin dimethyl ester, but where

[81] G Hauska, *FEBS Lett*, 1977, **79**, 345
[82] A Futami, E Hurt, and G Hauska, *Biochim Biophys Acta*, 1979, **547**, 583
[83] A Futami and G Hauska, *Biochim Biophys Acta*, 1979, **547**, 597
[84] I Hamachi, Y Kobuke, and I Tabushi, *Bull Chem Soc Jpn*, 1988, **61**, 3613
[85] J C Salerno, H J Harmon, H Blum, J S Leigh, and T Ohnishi, *FEBS Lett*, 1977, **82**, 179
[86] S Ferguson-Miller, D L Brautigen, and E Margoliash, *J Biol Chem*, 1978, **253**, 149
[87] J A Runquist and P A Loach, *Biochim Biophys Acta*, 1981, **637**, 231

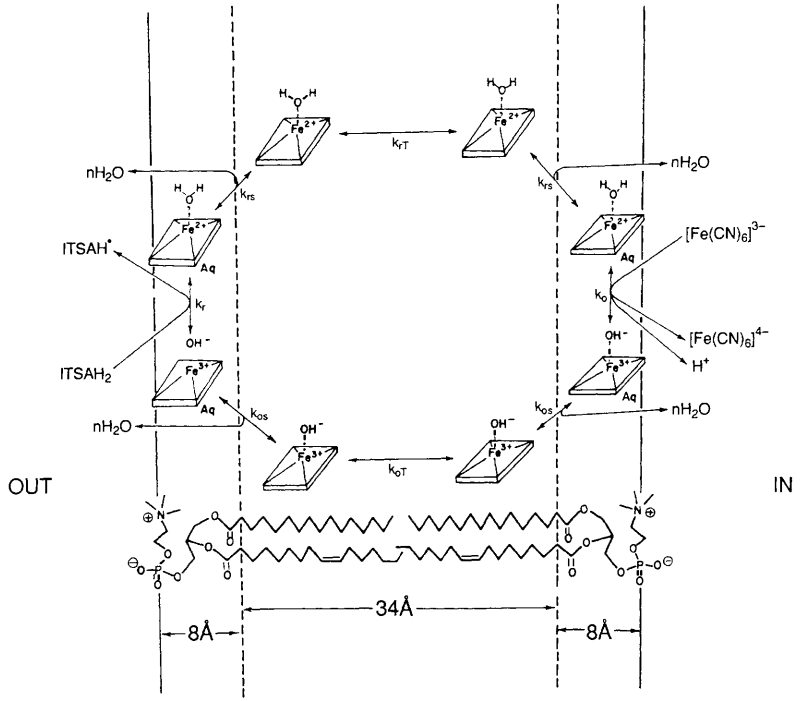


Figure 10 *Schematic representation of hemin dimethyl ester-catalysed electron transport across PC vesicle bilayer. The rhombi represent the expected size of the conjugated π-system of the porphyrin*
(Reproduced with permission from ref. 87)

net proton flux was affected through the coordination of water to the ferrous porphyrin (Figure 10). Interestingly, when the phosphatidylcholine vesicles were, like many natural membranes, made negatively charged (by the incorporation of 20% cardiolipin), the rate of hemin dimethyl ester-catalysed electron transport increased almost threefold.

In Nature, it is imagined that the cytochrome components are immobilized in a highly ordered matrix, with electrons hopping between redox centres. Since, *in vivo*, cytochromes often play a discriminating role in only allowing electrons (and not protons) to be transported, providing an effective 'electron wire' across the membrane,[88] natural cytochromes must incorporate some kind of 'gate', perhaps involving a conformational change in a heme protein, that can prevent coupled electron/proton flux.

Cytochrome c_3 (cyt-c_3), a bacterial electron carrier, has a unique structure of four heme units in a single protein ($M_w \sim 14\,000$ for *Desulphoribrio vulgaris*),[89]

[88] P. Mitchell, *Science*, 1979, **206**, 1148.
[89] T. Yagi and K. Maruyama, *Biochim. Biophys. Acta*, 1971, **243**, 214.

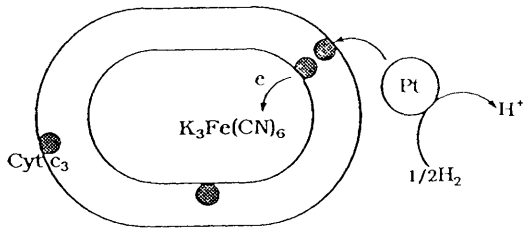


Figure 11 *Cytochrome* c_3*-mediated electron transport across a phospholipid vesicle bilayer from 'external'* H_2, *via colloidal platinum, to 'internal' ferricyanide*[95]

with strong intra- and inter-protein heme–heme interactions, as shown by the ability of cyt-c_3 to conduct electricity in a solid-state film.[90] By incorporating cyt-c_3 in the membrane of phosphatidylcholine vesicles (*ca.* 25 nm diameter, containing inner waterpool ferricyanide, with external dithionite), Tabushi and co-workers showed that cyt-c_3 catalysed transbilayer electron transport in a second order manner,[91] with a rate twice that of the hemin dimethyl ester or plastoquinone systems.

Analysis of the kinetic data suggested that, when in close proximity, molecules of cyt-c_3 (each $\sim$3 nm in diameter) on opposite sides of the vesicle bilayer (4—5 nm wide) associate for a brief period (at least), forming an aggregate that acts as a highly effective 'electron channel' across the membrane; a process strikingly like that proposed for biological membranes. In more detailed studies[92] the electron influx was shown to be coupled to net proton (*i.e.* H^+ and/or OH^-) transport 10—100 times larger than passive H^+/OH^- permeability,† creating a pH gradient of 4 across the vesicle bilayer, large enough to affect ATP synthesis.[93]

In sulphate-reducing bacteria, hydrogenase, and cyt-c_3 catalyse the metabolism of H_2 or other in/organic reducing agents for the purpose of ATP synthesis. Upon bubbling H_2 through a suspension of phosphatidylcholine/cyt-c_3 vesicles containing entrapped ferricyanide with external colloidal platinum[94] known to act like hydrogenase in lowering the potential barrier for the conversion of H_2 to H^+ by weakening the H–H bond, Tabushi and Nishiya were able to mimic the bacterial process by demonstrating cyt-c_3 catalysed transmembrane electron transport using H_2 as an electron source[95] (Figure 11). Again, electron transport was afforded by the self-aggregation of cyt-c_3; the concommitant ion flux (mostly OH^-) generated a pH gradient large enough to effect ATP synthesis. If the latter is possible, a complete H_2/ATP-metabolizing artificial cell may be available.

† $P_{H^+/OH}$ was estimated as 1.1×10^{-8} cm/s under these conditions

[90] Y Nakahara, K Kimura, H Inokuchi, and T Yagi, *Chem Lett*, 1979, 877
[91] I Tabushi and T Nishiya, *J Am Chem Soc*, 1981, **103**, 6983
[92] I Tabushi and T Nishiya, M Shimomura, T Kunitake, H Inokuchi, and T Yagi, *J Am Chem Soc*, 1984, **106**, 219
[93] A T Jagendorf and E Uribe, *Proc Natl Acad Sci USA*, 1966, **55**, 170
[94] I Tabushi and A Yazaki, *J Am Chem Soc*, 1981, **103**, 7371
[95] I Tabushi and T Nishiya, *Tetrahedron Lett*, 1981, **22**, 4989

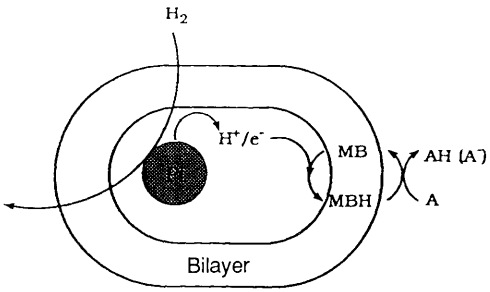


Figure 12 *The use of polymerized vesicle-entrapped colloidal platinum in transmembrane ground state electron transfer*[97]

In a related study,[96] mixed vesicles of dipalmitoylphosphatidylcholine (DPCC) and a polymerizable man-made surfactant (either $[(H_2C{=}CHC_6H_4NHCO\text{-}(CH_2)_{10})(C_{16}H_{33})N(CH_3)_2]Br$ or $[CH_2{=}CH(CH_2)COO]_2NPO(OH)_2$ containing K_2PtCl_4 in the inner waterpool were produced. Upon irradiation with uv-light, colloidal platinum was formed, and the vesicle bilayer underwent polymerization. After incorporation of methylene blue (MB) or 10-methyl-5-deazaisoalloxazine-3-propanesulphonic acid (MAPS) into the bilayer, bubbling hydrogen gas through the system brought about the platinum-catalysed reduction of MB (or MAPS)[97] by hydrogen atoms (*i.e.* protons and electrons) originating from H_2 (Figure 12). Added ferric chloride was subsequently reduced, regenerating MB (or MAPS). These reduction and oxidation cycles could be repeated many times.

This system illustrates the usefulness of organized assemblies in providing the compartmentalization of precursors required for the structural, spatial, and chemical control of cluster generation and stabilization.[40] For example, the intravesicle platinum particles previously described were far more separated than those formed in homogeneous solution in the absence of a stabilizer. The latter particles precipitated in only a few days; conversely, vesicle-entrapped colloidal platinum remained stable for over a month.

Another biologically important group of membrane-bound electron transporters are the flavoproteins,[98] electron-transducing enzymes that are involved in the initial stage of many metabolic systems, such as amino acid oxidase, NADH dehydrogenase, and NADH cytochrome reductase.[99] In these, and other, systems, the flavin unit accepts electrons from various reducing substrates and transfers them to acceptors, such as quinones, heme proteins, and iron–sulphur clusters. The flavin unit functions not only as an efficient one- and two-electron transfer catalyst, but sometimes as a hydrogen transfer catalyst;

[96] K. Kurihara and J. H. Fendler, *J. Am. Chem. Soc.*, 1983, **105**, 6152.
[97] A. J. G. Visser and J. H. Fendler, *J. Phys. Chem.*, 1982, **86**, 2406.
[98] 'Flavins and Flavoproteins', ed. T. P. Singer, Elsevier, Amsterdam, 1976.
[99] P. Hemmerich, G. Nogelschneider, and C. Veeger, *FEBS Lett.*, 1970. **8**, 69.

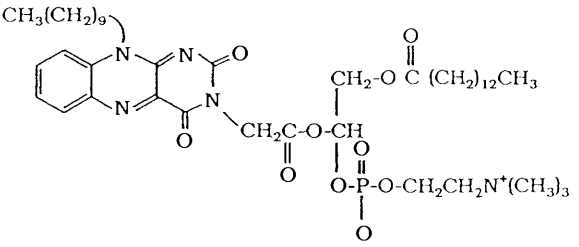


Figure 13 *An artificial flavolipid*

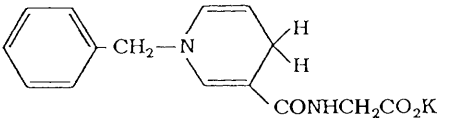


Figure 14 *Structure of* N-(*carboxymethyl*)-1-*benzyl*-1,4-*dihydronicotinamide*

the half and fully reduced flavins having pK_a values close to the physiological pH value.[100]

In order better to understand the sophisticated function of the membrane-bound flavin, Tabushi and his co-workers covalently attached a synthetic flavin unit near the polar head group of a natural lipid (phosphatidylcholine) to give a 'flavolipid'[101] (Figure 13), which was then incorporated into phosphatidylcholine vesicles (containing waterpool-entrapped ferricyanide with 'externally' added dithionite).[100] Flavolipid-catalysed, transbilayer electron transport was found to occur with twice the efficiency of the cyt-c_3 systems.

As with the cyt-c_3 systems, electron transfer was shown to occur through the formation of transient 'electron channels' between *opposing* flavolipid molecules, rapidly diffusing in the plane of the bilayer (10^{-8}—10^{-5} $cm^2 s^{-1}$), and demonstrating the effectiveness of the 'half channel' mechanism.[100] In the corresponding dipalmitoylphosphatidylcholine (DPPC) vesicle-containing system, the rate of catalysis by the flavolipid increased almost 100-fold on increasing the temperature of the system above its phase transition temperature (38—39 °C);[46] a dramatic rate enhancement related to a similar increase in the lateral diffusion rate.[47]

The flavolipid/phosphatidylcholine vesicle system was modified further by replacing dithionite with a hydrophilic NADH model compound, potassium *N*-(carboxymethyl)-1-benzyl-1,4-dihydronicotinamide (BzNAHCOOK,[102,103] Figure 14). BaNAHCOOK ($E_{\frac{1}{2}}$ −0.36 V *versus* SHE) closely mimics the redox properties of dihydronicotinamide (NADH, $E_{\frac{1}{2}}$ −0.32 V), an important donor in the primary events of the respiratory chain.[104] High overpotentials, such as that

[100] I Tabushi, I Hamachi, and Y Kobuke, *J Chem Soc*, *Perkin Trans I*, 1989, 383
[101] I Tabushi and I Hamachi, *Tetrahedron Lett*, 1986, **27**, 5401
[102] I Tabushi and I Hamachi, *Tetrahedron Lett*, 1987, **28**, 3363
[103] I Hamachi, Y Kokube, and I Tabushi, *Bull Chem Soc Jpn*, 1988, **61**, 3613
[104] P Mitchell, *Eur J Biochem*, 1979, **95**, 1

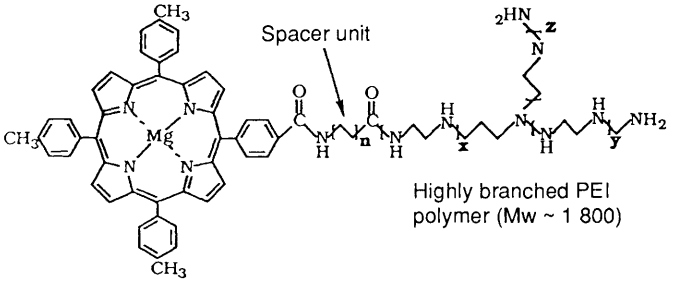


Figure 15 *Schematic representation of a poly(ethylenimine)-C$_n$(aliphatic spacer)-linked manganese porphyrin*

of dithionite ($E_{\frac{1}{2}}$ −1.13 V) over the flavin ($E_{\frac{1}{2}}$ −0.12 V and −0.37 V), are avoided in native systems. As efficient transmembrane electron transfer was observed, the flavolipid-BzNAHCOOK coupling in this system provided a simplified, isotopochemical, model of the NADH dehydrogenase-flavin interaction found in the mitochondrial inner membrane.

In vivo redox processes involving large differences in electrochemical potential are rare.[105] This is unsurprising, since they require a large physical separation of donor and acceptor to minimize side reactions, and besides the additional longer range organization required for such a system, the lower number of redox reactions involved would severely limit the opportunity for coupling to energy-utilization reactions. It seems likely, therefore, that for effective coupling of energy-releasing, electron-transfer reactions, to energy-utilization reactions in bioenergetic membranes, electron flow must be efficiently directed from one redox centre to another.[106] Should these vectorial redox reactions be 'short circuited' by competing, long-range electron transfer reactions, there would be a major loss in efficiency or coupling might not occur at all.

The distance/dependence on electron transport between isoenergetic redox centres in a bilayer membrane of phosphatidylcholine vesicles was evaluated by Dannhauser *et al.*[106] They linked a hydrophobic manganese porphyrin to an extremely hydrophilic, highly branched, poly(ethylenimine) polymer through a linear hydrocarbon spacer (Figure 15). They demonstrated that with the highly charged polymer remaining in the aqueous phase, the penetration of the porphyrin into the bilayer was controlled by the length of the spacer group, permitting systematic variation of the minimum distance separating two such derivatives incorporated from opposite sides of the bilayer. They reported that significant electron (and proton) transfer between 'external' indigotetrasulphonic acid and 'internal' ferricyanide was observed by a 'half-channel' type mechanism only when the edge-to-edge distance separating two opposed porphyrins was approximately 0.4 nm or less; supporting the presently held view that vectorial electron flow across most biomembranes involved sequential electron transfer between *many* redox centres.

[105] L. Y-C. Lee and J. K. Hurst, *J. Am. Chem. Soc.*, 1984, **106**, 7411.
[106] T. J. Dannhauser, M. Nango, N. Oku, K. Anzai, and P. A. Loach, *J. Am. Chem. Soc.*, 1986, **108**, 5865.

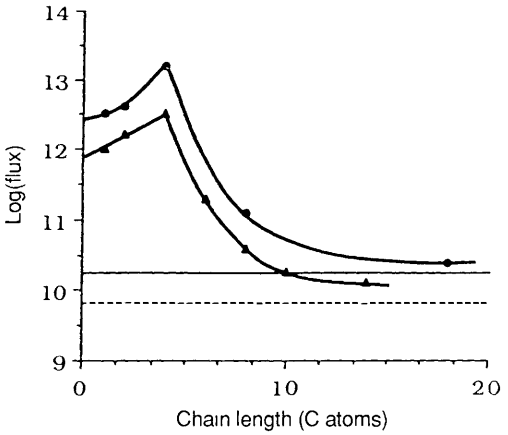


Figure 16 *Variation of electron flux across phosphatidylcholine vesicle bilayers (electrons* s^{-1} cm^{-2}*) from external dithionite to internal ferricyanide* (●) *(with control —) or flavin* (▲) mononucleotide *(with control)*[108]

In another phospholipid vesicle system, a number of manganese porphyrin-linked quinones were evaluated for their ability to catalyse transmembrane ground state electron transfer, which was also found to be inversely related to the distance between the porphyrin and quinone moieties [107]

In an interesting report, Lee and Hurst proposed an 'electron hopping' conduction model to explain the long range (*ca* 4nm) electron transfer observed between $[(NH_3)_5$ Ru-4-(1,1 -dodecenyl) pyridine$]^{3+}$ ions located at opposite sides of phosphatidylcholine vesicle bilayers [105] The suggested mechanism of electron transfer, which had a rate constant two orders of magnitude lower than that of the manganese porphyrin-linked polymer system, was reported to involve the tunnelling of electrons to intermediary sites located within the hydrocarbon phase at the bilayer alkyl chain interface However, conceptual problems with this mechanism have led others to question its validity [106]

Alkyl viologens have proven to be some of the most important non-biologically based ground state electron carriers [92 103 108] A study of the effect of increasing alkyl chain length of alkyl viologens on their effectiveness to catalyse electron transfer across phosphatidylcholine vesicle bilayers (from 'external' dithionite to internal ferricyanide) showed an increase in the overall rate of electron transport for C_1 to C_4, and thereafter a decrease to C_{18} [108] (Figure 16) Further research revealed that the overall electron transport rate was primarily controlled by the phase transfer of alkyl viologen cation radicals from the 'exterior' aqueous phase to the bilayer for C_1 to C_4, and by the phase transfer of (more hydrophobic) cation radicals from the bilayer to the 'internal aqueous phase for C_4 to C_{18}

[107] M Nango H Kryu and P A Loach *J Chem Soc Chem Commun* 1988 697
[108] I Tabushi and S I Kugimiya *Tetrahedron Lett* 1984 **25** 3723

These processes were best balanced, providing the maximum electron flow, at C_4.

It is apparent from the work reviewed in this section that chemical models based on vesicle assemblies do have relevance to biological membranes, and can help increase our limited knowledge of the organization and function of natural systems.

5 Photoredox Processes in Natural Product-containing Vesicles

A. Charge Separation.—(i) *Pure Natural Product Vesicles.* The photochemical properties of pigmented phospholipid bilayer membranes have been extensively studied in recent years. The goals of this work have been to understand better the form and function of the thylakoid membrane, eventually allowing construction of artificial systems for the photochemical conversion of solar energy.

Amongst other decay processes, molecular excited states, either singlets or triplets, can ionize to give radical ions, equation 1, or undergo electron transfer reactions with other species, either by oxidative or reductive quenching,[35] equations 2 or 3:

$$S^* \rightleftharpoons S^+ + e^-_{solv} \quad (1)$$

$$S^* + Q \rightleftharpoons Q\,S^+ + Q^- \quad (2)$$

$$S^* + Q \rightleftharpoons S^- + Q^+ \quad (3)$$

S = Sensitizer, Q = Quencher

The photoionization process, which can occur by mono- or bi-photonic pathways, is often observed in polar solvents, due to the enthalpy gain available from the solvation of the photoproducts. Photoredox reactions are readily adaptable to photosensitized redox processes:

$$D + A \overset{h\nu/\text{sensitizer}}{\rightleftharpoons} D^{+\bullet} + A^{-\bullet}$$

D = Donor, A = Acceptor

Light energy utilization can only be achieved if net charge separation is effected, and this is largely dependent upon the rate of back electron transfer. In photosynthesis, electron transfer across the membrane phase boundary is *the* key to effective energy conversion; here, the 'excited' electron can transverse the phase boundary (where it is captured), but the ground state electron cannot.[109]

Many studies in this area were concerned with the photoelectric effects of planar pigmented bilayer membranes.[7] However, the advantages of microheterogeneous, particularly vesicle, systems were recognized in the study of heterogeneous photoredox processes.

Nichols *et al.* were the first to demonstrate the photoredox activity of chlorophyll (a and b) incorporated into phospholipid vesicles by showing that

[109] M. Calvin, *Acc. Chem. Res.*, 1978, **11**, 369.

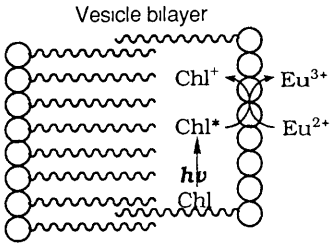


Figure 17 *An example of phospholipid* ⌒ *bilayer-facilitated charge separation of chlorophyll*[112]

photo-excited chlorophyll caused the oxidation of added aqueous-phase ferrocytochrome *c*.[110] Later, Tomkiewicz and Corker detected light-driven chlorophyll radical cation monomer formation in chlorophyll-containing phosphatidylcholine vesicles in the presence of aqueous phase electron acceptors such as $Fe(CN)_6^{4-}$, Sm^{3+}, or Eu^{3+} (at 77 K) by ESR spectroscopy, concluding that the lipid/water *interface* was unique in imparting some stability to photochemical charge separation (Figure 17).[111] More detailed room temperature ESR studies on chlorophyll-containing vesicles were reported by Oettmeier *et al.*[112] Of the wide variety of quinoid and non-quinoid molecules they employed as acceptors, they found that those having access to the membrane, such as Fe^{3+}, pyrophosphate, and methylviologen (MV^{2+}), gave rise to chlorophyll radical cation formation under illumination.

Mangel was the first to present evidence suggesting the presence of chlorophyll aggregates in vesicles,[113] which is significant in view of the importance of chlorophyll association in photosynthesis. Moreover, he has shown that vesicles containing chlorophyll and β-carotene (known to quench the chlorophyll triplet state *in vivo*[114]) are capable of light-induced charge transport on introduction of a redox potential gradient across the vesicle membrane.[35] Similarly, Sudo and Toda observed photoreduction of 'fast red' dyestuff by ascorbate (in and/or on the vesicle?) using stearylanthraquinone-2-sulphonate as a sensitizer.[115]

Ford and Tollin used laser flash photolysis to measure the triplet quenching efficiencies, radical yields, and radical recombination kinetics of chlorophyll-incorporated phosphatidylcholine vesicle suspensions in the presence of charged electron acceptors, located either in the internal aqueous volume of the vesicles or in the external, continuous aqueous phase.[116] The inner and outer interfaces of the vesicles displayed a striking asymmetry with regard to electron transfer reactions involving chlorophyll* and the electron acceptors. With methyl

[110] P Nichols, J West, and A D Bangham, *Biochim Biophys Acta*, 1974, **363**, 190
[111] M Tomkiewicz and G A Corker, *Photochem Photobiol*, 1976, **22**, 249
[112] W Oettmeier, J R Norris, and J J Katz, *Z Naturforsch, Teil C*, 1976, **31**, 163
[113] M Mangel, *Biochim Biophys Acta*, 1979, **430**, 459
[114] P Mathis and C C Schneck in 'Carotenoid Chemistry and Biochemistry', ed G Britton and T W Goodwin, Pergamon Press, Oxford, 1982
[115] Y Sudo and F Toda, *Chem Lett*, 1978, 1011, *ibid*, *Nature*, 1979, **279**, 807
[116] W E Ford and G Tollin, *Photochem Photobiol*, 1982, **36**, 647

viologen (MV^{2+}) as the *symmetrically* distributed acceptor, for example, 52% of the total chlorophyll triplet population could be quenched from the inside, but only 16% from the outside (~32% being inaccessible from either side), in spite of the quenching rate constant for the outside reaction being *twice* that of the inside. Radical yields and recombination kinetics also displayed asymmetric behaviour: on the inside only 4% of quenched triplets gave rise to separated radicals, as opposed to 32% on the outside. Furthermore, the half life of $chl^{+\bullet}$ and $MV^{+\bullet}$ was approximately 100 times longer at the outer surface than at the inner.

These results were interpreted as evidence for an asymmetric distribution of chlorophyll across the bilayer, with most being located towards the outer surface: there is greater mobility in the outer monolayer of the membrane. This was seen as a reflection of the inherent structural and compositional differences between the inner and outer monolayers, coupled with an electrostatic bilayer asymmetry. Interestingly, this model is in total agreement with that proposed by Smalley *et al.* for the analogous asymmetric distribution of magnesium octaethylporphyrin in an equivalent phosphatidylcholine vesicle system.[117]

In a later study, Ford and Tollin investigated the effect of incorporating cholesterol into the earlier system.[118] Cholesterol, a neutral lipid which is an important constituent of many natural membranes, and controls the fluidity of the bilayer,[119] had two main effects. Firstly, it shifted the distribution of chlorophyll within the vesicle wall from one favouring the outer monolayer to one favouring the inner, and, secondly, it made all chlorophyll molecules (both ground and excited state) more accessible to water and to water-soluble quencher molecules. These effects, which occurred with levels of cholesterol above 15%, were largely attributed to the creation of spaces between the phospholipid headgroups.

The net charge separation efficiency was strongly affected by structural factors such as the location of donor and acceptor relative to the bilayer/water interface, bilayer surface charge distribution, and the degree of interaction between water and the bilayer surface. These structural factors have been shown to be controllable by changing the counterions, headgroups, the length of the hydrocarbon tail of the amphiphiles, or by adding salts or slightly water-soluble alcohols.[120]

In the second of a series of related publications, Kevan and his co-workers reported on the effect of varying levels of cholesterol in chlorophyll *a*/DPPC vesicles on the *photoionization* efficiency of chlorophyll *a* (at 77 K) in both the presence and absence of lipophilic and lipophobic electron scavengers. With lipophobic scavengers (including water), the efficiency of photoionization decreased with increasing cholesterol, while no effect was observed with the lipophilic scavengers.[120,121] In agreement with the work of Ford and Tollin,

[117] J. F. Smalley and S. W. Feldberg, *J. Phys. Chem.*, 1989, **93**, 2570
[118] W. E. Ford and G. Tollin, *Photochem. Photobiol.*, 1984, **40**, 249.
[119] D. Chapman in 'Membrane Fluidity in Biology', ed. R. C. Aloia, Academic Press, New York, vol 2. 1983, p. 15.
[120] I. Hiromitsu and L. Kevan, *J. Am. Chem. Soc.*, 1987, **109**, 4501, and refs. therein.
[121] N. Ohta and L. Kevan, *J. Phys. Chem.*, 1985, **89**, 3070.

increasing bilayer fluidity, causing chlorophyll *a* (and the lipophilic scavenger) to move away from the bilayer interface, was seen as the primary effect. A later study on the same system showed that a decrease in the amphiphile chain length (C_{18} to C_{14}) led to an increase in the photoionization efficiency of chlorophyll *a* to water-soluble scavengers,[122] and this was interpreted as indicating a decrease in the average locus of chlorophyll *a* position relative to the membrane surface.

Using their earlier system, Kevan *et al.* have correlated an increase in the rate of intersystem crossing between the singlet and triplet state of chlorophyll *a* with an increase in the photoionization yield upon addition of metal chloride salts.[123] This was given as evidence that the photoionization of chlorophyll *a* involves the triplet state of chlorophyll *a* as a precursor, at least in the presence of the salts.

In their most recent study, Kevan *et al.* have investigated the effect of medium chain length alcohols, metal chloride salts, the presence of an unsaturated surfactant tail, and the addition of dimethyl sulphoxide or glycerol (cryoprotective agents), on the photoionization efficiency of chlorophyll *a* in a number of different phospholipid vesicles in the presence and absence of electron scavengers.[124] Variations in the photoionization yield *versus* these structural parameters were discussed in terms of the solubilization site of chlorophyll *a*, loss of integrity of the vesicle structure, and differences in the degree of headgroup hydration.

Photo-assisted charge separation between a number of pyrene derivatives, which acted as sensitizer, and *N,N*-diethylaniline (DEA) *within* the bilayer of DPPC vesicles occurred in yields much lower than in homogeneous solution.[125] This was attributed to the non-polar, microviscous environment of the membrane, which hindered the formation and separation of photoproducts. Other studies of trans-boundary electron transfer have been performed with inorganic ion derivatives as sensitizers.[126]

Photo-assisted charge separation across the bilayer-water interface of phospholipid vesicle assemblies has been employed towards water-splitting. Manganese (IV) dioxide incorporated into DPPC vesicles has been found to form a polynuclear complex capable of catalysing dioxygen evolution from water in the presence of the oxidant $[Ru(bpy)_3]^{3+}$.[127] Manganese is believed to be at the active site of O_2 evolution in the inner thylakoid membrane of chloroplasts,[128] it seems that a sealed membrane is required for both oxygen evolution and photophosphorylation.[15,129,130]

[122] T Hiff and L Kevan, *J Phys Chem*, 1988, **92**, 3982
[123] I Hiromitsu and L Kevan, *J Phys Chem*, 1989, **93**, 3218
[124] T Hiff and L Kevan, *J Phys Chem*, 1989, **93**, 3227
[125] S Neumann, R Korenstein, Y Barenholtz, and M Ottolenghi, *Isr J Chem*, 1982, **22**, 125
[126] M Calvin, I Willner, C Laane, and J Otvos, *J Photochem*, 1981, **17**, 195, J H Fendler, *J Photochem*, 1981, **17**, 303
[127] N P Luneva, E I Knerelman, V Ya Shafirovich, and A E Shilov, *J Chem Soc, Chem Commun*, 1987, 1504
[128] 'Photosynthetic Oxygen Evolution', ed H Metzner, Academic Press, New York, 1978
[129] W Stillwell and H T Tien, *Photobiophys Photobiochem*, 1981, **2**, 159
[130] W Stillwell and H T Tien, *Biochim Biophys Res Commun*, 1978, **81** 212

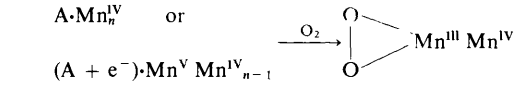


Figure 18 *Reaction scheme for the formation of a possible photosynthetic oxygen-evolving active site intermediate*[131]

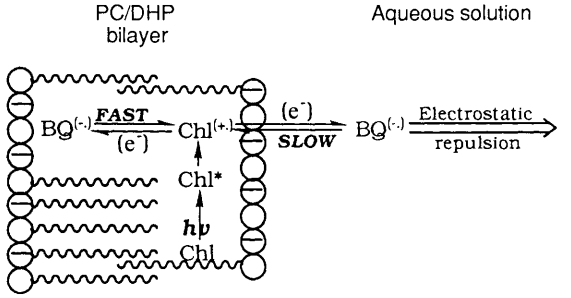


Figure 19 *Negatively charged* PC:DHP *bilayer/water interface-facilitated charge separation of chlorophyll/benzoquinone photoredox products* (chl$^{+\bullet}$, BQ)[132]

The actual membrane-associated, oxygen-evolving, enzyme involved has proved to be one of the most unstable and elusive entities in all of biology, being sensitive to mild biochemical treatments, ageing, heat, ultraviolet light, organic solvents, and high salt concentrations. Spectroscopic analogies were drawn between the state of manganese in the natural and model system, suggesting that the active site in both may involve an intermediate of the type shown in Figure 18.[131]

(ii) *Mixed Biological/Synthetic Lipid Vesicles.* Using a system of otherwise electrically neutral phosphatidylcholine/chlorophyll vesicles incorporating various amounts of negatively and/or positively charged 'synthetic' surfactants in aqueous solutions of varying ionic strength, Fang and Tollin investigated the effect of vesicle bilayer surface charge on the photo-assisted charge separation events between chlorophyll and the lipophilic electron acceptor, benzoquinone (BQ).[132] Laser flash photolysis studied showed that in the phospholipid vesicles recombination (back electron transfer) of the charge separation products, chl$^{+\bullet}$ and BQ$^{-\bullet}$, was biphasic, with fast recombination *within* the bilayer, and much slower recombination across the bilayer-water interface.

However, when the vesicles contained a sufficient quantity of the negatively charged surfactant, dihexadecylphosphate ($\sim 20\%$), and the ion concentration of the surrounding solution was low, the chl$^{+\bullet}$/BQ$^{-\bullet}$ fast component disappeared, and all of the radical decay proceeded *via* the slow interfacial process (Figure 19). This was attributed to the electrostatic repulsion of BQ$^{-\bullet}$ from the negatively

[131] M. Calvin, *J. Chem. Soc., Faraday Disc. II*, 1980, **70**, 383.
[132] Y. Fang and G. Tollin, *Photochem. Photobiol.*, 1983, **38**, 429.

charged bilayer, inhibiting the fast recombination pathway, and the concomitant electrostatic stabilization of the cationic chlorophyll species that remained. High counterion concentrations neutralized surface charge. These combined effects led to a 35% increase in the radical yield, without significantly affecting triplet quenching.

Incorporation of relatively small quantities of positively charged surfactants (didodecyldimethylammonium bromide or cetylpyridinium chloride, $\leqslant 20\%$) had the reverse effect, largely due to the restricted escape of the more mobile transient, $BQ^{-\bullet}$, after charge separation. When the anionic and cationic surfactants were present in equimolar amounts, the radical yields and decay kinetics were relatively unaffected, but a large effect was observed on the radical difference spectrum, indicating the clustering of oppositely charged molecules within the bilayer.

In a subsequent publication, Ford and Tollin continued work on these charged vesicle systems, examining the charge separation and radical recombination effects of salt ions distributed asymmetrically between the interior and exterior aqueous phases of the vesicles.[133] They showed that millimolar levels of asymmetrically distributed counterions had a much greater effect on radical yields and lifetimes than counterions symmetrically distributed at 100 times the concentration. Their results were interpreted mainly in terms of surface-specific counterion neutralization leading to tighter packing of the lipid monolayers; particularly the larger, less restricted external monolayer, making separation of light-induced ion-radical pairs more difficult.

In an important publication, Tollin *et al.* further utilized the surface charge of their mixed DPPC/charged surfactant/chlorophyll vesicle system to influence the reaction dynamics of photo-excited chlorophyll further by employing *electrically charged* electron acceptors, either positively charged methyl viologen(2+) or negatively charged sulphonated quinones(1−).[134] The charge of the acceptor both before *and* after charge transfer now became an important factor in determining the degree of charge separation. The authors reported a 100% conversion of chlorophyll triplet to radical cation for negatively charged vesicles with *negatively* charged acceptors; enhanced repulsion of the reduced acceptor (which showed an increased half life) and simultaneous stabilization of $chl^{+\bullet}$, led to efficient charge separation (Figure 20).

Recent work by Hiff and Kevan has shown that the *photoionization* yield of chlorophyll *a* in mixed surfactant/phospholipid vesicles *decreases* with increasing negative surface charge.[135] This was seen as arising from the unfavourable negative electric field which had to be overcome by the electron for it to be solvated. As expected, the inclusion of a positively charged synthetic surfactant, had the opposite effect.

Hiff and Kevan also correlated an increase in the average oligomer number of chlorophyll with a decrease in the volume of the lipid amphiphile headgroups—

[133] Y Fang and G Tollin, *Photochem Photobiol*, 1984, **39**, 685
[134] V Senthilathipan and G Tollin, *Photochem Photobiol*, 1985, **42**, 437
[135] T Hiff and L Kevan, *J Phys Chem*, 1989, **93**, 2069

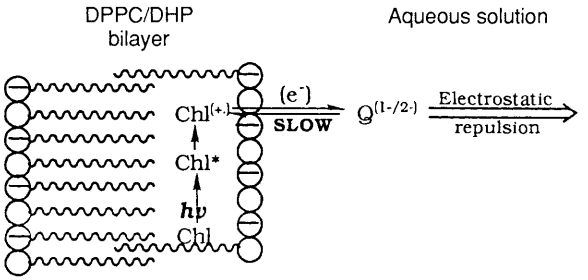


Figure 20 *Negatively charged* DPPC:DHP *bilayer/water interface-facilitated charge separation of chlorophyll/(negatively charged) sulphonated quinone photoredox products* ($chl^{+\cdot}$, Q^{2-})[134]

interesting in view of the fact that bacterial photosynthesis is known to be initiated by the photoionization of a chlorophyll dimer.[136] Presumably, the smaller headgroups increase the volume available for chlorophyll solubilization, giving a greater probability of oligomer formation.

Clearly, electrostatic control of the pathway of light-harvesting phenomena may well be useful in future practical applications of membranous photochemistry for solar energy conversion. It is perhaps significant in this context that chloroplast thylakoid membranes contain a proportion ($\sim 10\%$) of negatively charged (sulpho)lipids.

B. Photo-assisted Transmembrane Electron Transfer.—In 1976, Mangel demonstrated that photo-assisted electron transport could occur across the bilayer of phospholipid vesicles.[113] By incorporating chlorophyll as sensitizer and β-carotene as electron mediator into the bilayer of phosphatidylcholine vesicles, Mangel effected electron transfer from waterpool-entrapped ascorbate to 'external' Fe^{3+} with a quantum efficiency of 0.075 on illumination of the vesicles (Figure 21). His spectroscopic data indicated that some chlorophyll aggregates were present in the vesicle bilayer,[137] but that these aggregates did not form in the equivalent planar lipid bilayer.[138] Furthermore, he showed that disruption of the chlorophyll aggregates (by pyridine or increased temperature) led to a 75% drop in quantum yield, suggesting that the chlorophyll aggregates may play an important role in the conversion of photonic energy to electronic energy.

A year later, Toyoshima *et al.* reported the light-induced oxidation of water on the 'outer' surfaces of phosphatidylcholine vesicles incorporating chloroplast extracts (68% chlorophyll *a*, 22.8% chlorophyll *b*, pigments such as carotene and xanthophyll, 9.2%), the proton carrier carbonylcyanide *p*-trifluoromethoxylphenylhydrazone (FCCP) in the bilayer, with potassium

[136] A. J. Hoff in 'Light Reaction Path of Photosynthesis', ed. F. K. Fong, Springer-Verlag, New York, 1982.
[137] E. Rabinowitch in 'Primary Processes in Radiation Biology', ed. R. Mason and I. Augenstein, Academic Press, New York, 1964.
[138] A. Hani and D. S. Berns, *J. Membrane Biol.*, 1972, **8**, 333.

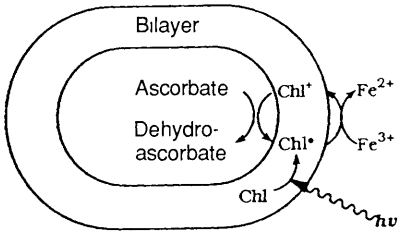


Figure 21 *Photo-assisted mediation of electrons across phospholipid vesicle bilayer between 'internal' ascorbate and 'external'* $FeCl_3$ [113]

ferricyanide entrapped in the inner waterpool.[139] The rate of oxygen production (4.2×10^{-4} per mole at 10^5 lux) was proportional to light intensity, and they reported that charge exchange occurred between chlorophyll molecules on opposite sides of the vesicle bilayer:

$$\text{chl} + h\nu \longrightarrow \text{chl}^*$$

$$\text{chl}_{in} + Fe^{3+} \rightleftharpoons \text{chl}_{in}^{+\bullet} + Fe^{2+}$$

$$\text{chl}_{in}^{+\bullet} + \text{chl}_{out} \text{ (or } \text{chl}_{out}^*) \longrightarrow \text{chl}_{in} + \text{chl}_{out}^{+\bullet}$$

$$\text{chl}_{out}^{+\bullet} + \tfrac{1}{2}H_2O \longrightarrow \text{chl}_{out} + \tfrac{1}{4}O_2 + H_{out}^+$$

These results, however, have not been successfully reproduced by other workers,[129,130] who have suggested that the observed 'oxygen' was, in reality, a heating effect on the oxygen electrode. Also, since this study was performed in the presence of a tris-(hydroxymethyl)aminomethane ('Tris') buffer, which is known to permeate bilayers,[140] it is unclear whether FCCP was necessary in preventing charge accumulation inside the vesicles.

In a more defined system, Kurihara *et al.* showed that chlorophyll itself could act catalytically as both sensitizer and electron mediator in the photoassisted transport of electrons across a phosphatidylcholine bilayer between 'internal' ascorbate (or water?) and 'external' Cu^{2+}, without requiring β-carotene or other proposed electron carriers.[141]

In a later study, the same authors reported the catalytic reduction of ferricyanide in the continuous aqueous phase outside illuminated phosphatidylcholine/chlorophyll vesicles.[142] This was enhanced by, but apparently did not necessarily require, the presence of an added reductant in the inner waterpools of the vesicles, suggesting the possibility of H_2O or OH^- oxidation. A tris buffer was employed in this study, but the rate of electron transfer was enhanced in the

[139] Y Toyoshima, M Morino, H Motoki, and M Sukigara, *Nature*, 1977, **265**, 187
[140] T Yamashita and W L Butler, *Plant Physiol*, 1969, **44**, 435
[141] K Kurihara, M Sukigara, and Y Toyoshima, *Biochim Biophys Acta*, 1979, **547**, 117
[142] K Kurihara, and Y Toyoshima, M Sukigara, *Biochim Biophys Res Commun*, 1979, **88**, 320

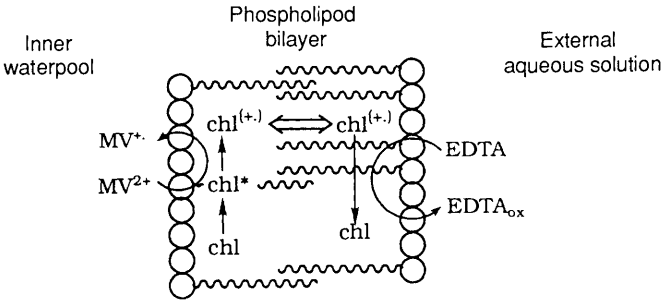


Figure 22 *Proposed mechanism for photo-assisted, chlorophyll-mediated electron transport across a PC vesicle bilayer from 'external' EDTA to 'internal' methyl viologen*[143]

presence of the uncouplers FCCP, 2,4-dinitrophenol (DNP) or the anion tetraphenylboron (TPB), but not with the lipid-soluble dimethyldibenzylammonium (DDA) *cation.* The presence of the uncoupling agents led to a faster decrease in the pH of the external solution on photolysis of the system, indicating an increased rate of charge-compensating proton transfer out of the vesicles.

Ford and Tollin employed laser flash and steady state photolysis to carry out detailed kinetic studies on the reaction processes involved in the photo-assisted, chlorophyll-mediated transport of electrons across phosphatidylcholine vesicle bilayers from 'external' ethylenediaminetetraacetate (EDTA) to 'internal' methyl viologen (MV^{2+}).[143] The magnitude of the rate constant of electron transfer through the membrane ($>10^4 s^{-1}$), and the rate/chlorophyll concentration dependence were both interpreted as indicating that electron transfer involved electronic, rather than molecular, carriers. That is, opposing chlorophyll molecules, which are predominantly orientated with their chlorin rings close to the membrane–water interfaces, and their phytyl chains embedded in the membrane, exchanged electrons across the bilayer (Figure 22). Electron exchange between chl and $chl^{+\cdot}$ in solution has been estimated at $(1.2 \pm 0.9) \times 10^8 dm^3 mol^{-1} s^{-1}$,[144] which contrasts with the slow transmembrane diffusion ($k \sim 10^{-2} s^{-1}$) of a nitroxide spin-labelled chlorophyll *b* derivative.[145]

In an extension of this work, Ford and Tollin reported a similar rate of electron transfer across the bilayer of phosphatidylcholine/chlorophyll *a* vesicles separating a water-soluble naphthoquinone acceptor [*S*-(2-methyl-1,4-naphthoquinonyl-3)-glutathione] in the inner waterpools from an 'external' thiol donor (glutathione).[146] The modified system had a quantum yield of 0.2, with a rate constant for electron exchange between chl and $chl^{+\cdot}$ in the inner lipid *monolayer* estimated at $3.2 \times 10^6 dm^3 mol^{-1} s^{-1}$. They also concluded that the

[143] W. E. Ford and G. Tollin, *Photochem. Photobiol.*, 1982, **35**, 809.
[144] G. L. Closs and E. V. Sitzmann, *J. Am. Chem. Soc.*, 1981, **103**, 3217.
[145] G. B. Birrell, S. A. Boyd, J. F. W. Keana, and H. O. Griffith, *Biochim. Biophys. Acta*, 1980, **603**, 213.
[146] W. E. Ford and G. Tollin, *Photochem. Photobiol.*, 1983, **38**, 441.

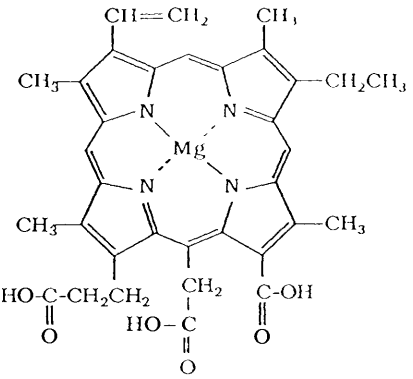


Figure 23 *Structure of chlorophyllin*

inherent asymmetry of chlorophyll distribution in the vesicle bilayer favoured net photo-assisted electron transfer *into* the vesicles.[116]

Interestingly, the reverse was found to be true when chlorophyllin *a*[147] (Figure 23), a water-soluble saponification product of chlorophyll *a* with a similar photochemical reactivity[148] was used as a membrane-bound sensitizer/mediator in phosphatidylcholine vesicle bilayers separating 'internal' ascorbate from 'external' methyl viologen; no transmembrane electron transfer was observed when donor and acceptor were distributed oppositely.[149] Chlorophyllin *diffusion* was proposed as the mechanism of electron transport across the bilayer, since, unlike chlorophyll, chlorophyllin possesses no long alkyl (phytyl) chain, and is mobile across the bilayer.[150]

Notably when buffered with tris, the rate of transmembrane electron transport in the chlorophyllin system was unaffected by FCCP, but an enhancement was observed when a 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid (HEPES) buffer was used. It appeared that tris, which is known to be permeable across membranes,[140] and possesses a primary amine group, acted as the uncoupling agent; HEPES is impermeable to the membrane.[151]

In the same work, replacement of the central magnesium atom of chlorophyllin with zinc doubled the rate of methyl viologen reduction; the equivalent copper-containing pigment was inactive as a sensitizer/electron mediator. As the spectra of these pigments are similar, their unequal photocatalytic activities were attributed to the different redox potentials and/or lifetimes of the excited pigments.

Totally synthetic chlorophyll analogues, Mg-P^{3+} and Mg-P (Figure 24) were

[147] G Oster, S B Broyde, and J S Bellin, *J Am Chem Soc*, 1964, **86**, 1309
[148] D Brune and A S Peitro, *Arch Biochim Biophys*, 1970, **141**, 371
[149] S Hidaka, E Matsuomota, and F Toda, *Bull Chem Soc Jpn*, 1985, **58**, 207
[150] S Hidaka and F Toda, *Chem Lett*, 1983, 1333
[151] N E Good, *Arch Biochim Biophys*, 1962, **96**, 653

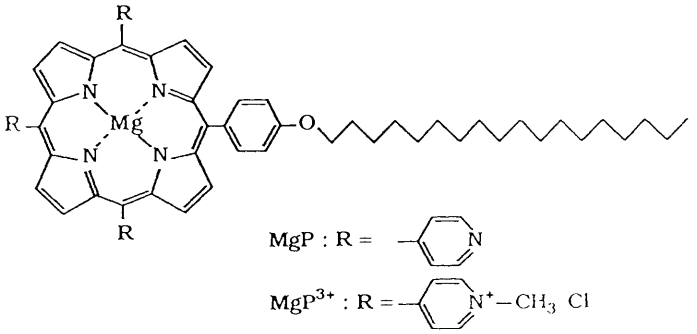


Figure 24 *Synthetic magnesium porphyrins:*
MgP; 5,10,15-*tris*(4-*pyridyl*)-20-[(*octadecyloxy*)*phenyl*]*porphinatomagnesium*, $MgP^{3+\bullet}$, 5,10,15-*tris*(1-*methylpyridinium*-4-*yl*)-20-[4-(*octadecyloxy*)*phenyl*]*porphinatomagnesium*

found to be effective photocatalysts for the transport of electrons across the bilayer of DPPC vesicles (from entrapped EDTA to 'external' MV^{2+}).[152] The higher activity of Mg-P^{3+} over Mg-P (30:1) was explained in terms of the position of the porphyrin headgroup relative to the bilayer–water interface;[153] the charged Mg-P^{3+} headgroup residing close to the more polar (surface) region of the bilayer, facilitating electron transfer between donor and acceptor. During photolysis of these systems, the radical *cation* (and not the radical anion) of the magnesium porphyrins was detected, and this was interpreted as indicating a concerted *two* step mechanism for transbilayer electron transfer (as Figure 25).

Magnesium octaethylporphyrin (MgOEP) acts as both sensitizer and transmembrane redox mediator in phospholipid vesicles.[154] MgOEP, having no long alkyl tail is free to diffuse across the bilayer, and it was found that in this case the neutral, protonated MgOEP 'anion' was the likely charge carrier, and not the MgOEP cation or its protonated form.

Matsuo *et al.* proposed a concerted two-step activation of the amphipathic zinc porphinato complex $ZnC_{12}TPyP$ (Figure 25), analogous to the Z scheme in photosynthesis, to explain electron transduction across illuminated vesicles of DPPC from 'internal' EDTA to an 'external' acceptor, disodium-9,10-anthraquinone-2,6-disulphonate (2,6-AQDS).[155]

The mechanism of transmembrane electron transport was established, in part, by the detection of $ZnC_{12}TPyP^{+\bullet}$ (and not $ZnC_{12}TPyP^{-\bullet}$) and by comparison of the rate of electron transport with that of the *diffusive* mechanism of the corresponding acriflavin-containing model,[156] which was less than one percent of the $ZnC_{12}TPyP$-containing systems. However, the authors did not report the expected quadratic dependence of 2,6-AQDS reduction on incident light intensity.

[152] T. Katagi, T. Yamamura, T. Saito, and Y. Sasaki, *Chem. Lett.*, 1982, 417.
[153] T. Katagi, T. Yamamura, T. Saito, and Y. Sasaki, *Chem. Lett.*, 1981, 1451.
[154] A. Ilani, M. Woodle, and D. Mauzerall, *Photochem. Photobiol.*, 1989, **49**, 673.
[155] T. Matsuo, K. Itoh, K. Takuma, K. Hashimoto, and T. Nagamura, *Chem. Lett.*, 1980, 1009.
[156] J. J. Grimaldi, S. Boileau, and J. M. Lehn, *Nature*, 1979, **279**, 807.

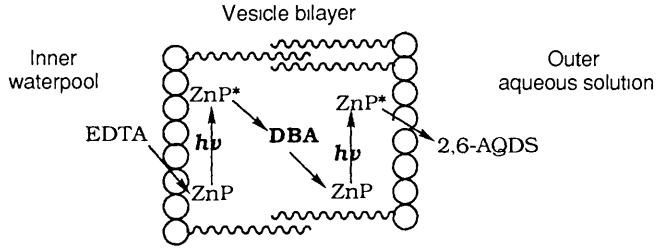


Figure 25 *Proposed two-quantum mechanism of transbilayer electron transport by* ZnP {5,10,15-*tris*(4-*pyridyl*)-20-[4-(*dodecylpyridinium*)]*porphinato zinc*} *in the presence of the electron mediator* DBA[155]

Significant enhancements in the rate of reduction of 2,6-AQDS were observed in the presence of neutral mediators, such as 1,3-dibutylalloxazine (DBA) and 1,3-didodecylalloxazine (DDA). Vitamin K_1 had little effect. In view of the similar mobilities of the mediators, their effectiveness was related to their redox potentials (*versus* SHE): DBA (−0.55 V) < DDA (−0.49 V) < VK_1 (−0.39 V). Another amphiphilic zinc porphyrinato complex, 5,10,15-tris(1-methylpyridinium-4-yl)-20-(4-stearoxyphenyl)porphyrinatozinc(II) trichloride (ZnP^{3+}) was found to behave like ZnTPyP (5,10,15,20-tetrakis(4-pyridyl)porphinatozinc) as a sensitizer/mediator when incorporated *symmetrically* across DPPC vesicle bilayers between EDTA and methylviologen.[157] Again, vitamin K_1, which has often been used as an electron mediator,[156,158] caused no enhancement of the electron transfer rate, although vitamin K_3 did (10%), probably because of its more suitable redox potential.[153]

It is likely that ZnP^{3+}, with three hydrophilic pyridinium groups and one hydrophobic stearoxyphenyl group, would be orientated 'tail in' with its porphyrin headgroup close to the bilayer–water interface, reminiscent of the chlorophyll locus.

In a later study, an *asymmetric* ZnP^{3+}/DPPC vesicle system, with ZnP^{3+} present only in the outer monolayer of the vesicles, was used to test the effectiveness of potential mediators.[153] Of the quinones tested, ubiquinone Q_{10} (UQ_{10}) and benzoquinone (BQ) had suitable redox properties, but only UQ_{10} was active; benzoquinone was seen as reluctant to access the more hydrophilic regions of the membrane. The hydrophobic tetraphenyl porphyrins, ZnTPP and H_2TPP, were shown to be more effective as mediators for ZnP^{3+} than as combined sensitizer/mediators by themselves.

ZnTPP has been employed by Parmon and his co-workers to transport electrons across phospholipid vesicle bilayers from internal EDTZ (or NADH) to external methyl viologen with a quantum yield of *ca.* 0.1%.[159,160] They observed a quadratic dependence of the rate of $MV^{+\bullet}$ accumulation on light intensity,

[157] K Katagi, T Yamamura, T Saito, and Y Sasaki, *Chem Lett*, 1981, 503
[158] W E Ford, J W Otvos, and M Calvin, *Nature*, 1978. **274**, 507
[159] V N Parmon, S V Lymar, I M Tsvetkov, and K I Zamaraev, *J Mol Cat*, 1983. **21**. 353
[160] K I Zamaraev, S V Lymar, M I Khramov, and V N Parmon, *Pure Appl Chem*, 1988. **60**. 1039

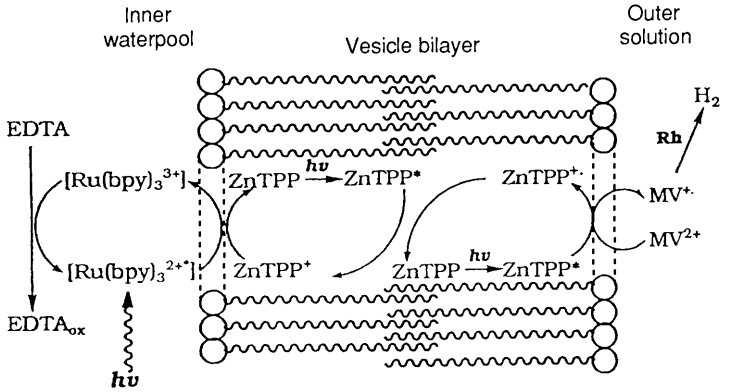


Figure 26 *Proposed mechanism for photo-assisted,* ZnTPP-*mediated electron transport across a phospholipid vesicle bilayer from 'internal'* EDTA, *via* $[Ru(bpy)_3^{2+*}]$, *to 'external' water,* via *methyl viologen*$^{+\cdot}$[159]

favouring a two-quantum mechanism for the photo-assisted transport of electrons. In the presence of hydrogenase or polymer-supported rhodium particles, the $MV^{+\cdot}$ produced evolved dihydrogen with a maximum quantum yield (for $\lambda > 500$ nm) of *ca.* 10^{-2} %. If $[Ru(bpy)_3]^{2+}$ was included in the inner waterpools, the quantum yield of $MV^{+\cdot}$ increased to 0.57%, which corresponded to a three-fold increase in the quantum yield, even when the entire light absorption was taken into consideration. This effect was ascribed first to the spectral sensitization due to increased band absorption, and, second, to the apparent energy (or electron) transfer from $[Ru(bpy)_3]^{2+*}$ to ZnTPP. The latter phenomenon is remarkably similar to the action of the chlorophyll 'antennae' in the thylakoid membrane (Figure 26).

When EDTZ was replaced by $CoCl_2$, a known homogeneous catalyst for dioxygen evolution, small amounts of $MV^{+\cdot}$ could still be generated photochemically.[161] It has been suggested that this system provides the basis for cyclic water cleavage; but oxygen has not been detected as a product.

Parmon *et al.* used the previously noted properties of $[Ru(bpy)_3]^{2+*}$, and the increased hydrophobicity of viologens upon reduction, to construct a light-transducing phospholipid vesicle system with the photosensitizer, $[Ru(bpy)_3]^{2+*}$, present with EDTA in the inner waterpool.[160] Cetylviologen (CV^{2+}) was embedded in the vesicle bilayer, and ferricyanide was dissolved in the continuous aqueous phase. On irradiation, electrons were transferred from EDTA to ferricyanide with a quantum efficiency of *ca.* 15%. This was attributed to efficient charge separation at the inner surface (since the more hydrophobic $CV^{+\cdot}$ species will move deeper into the membrane), and the rapid reduction of $[Ru(bpy)_3]^{3+}$ by EDTA. Transbilayer electron transfer was reported to be affected mainly by

[161] L. B. McGowan and J. O'M. Bockris, 'How to Obtain Clean Energy', Plenum Press, New York, 1980.

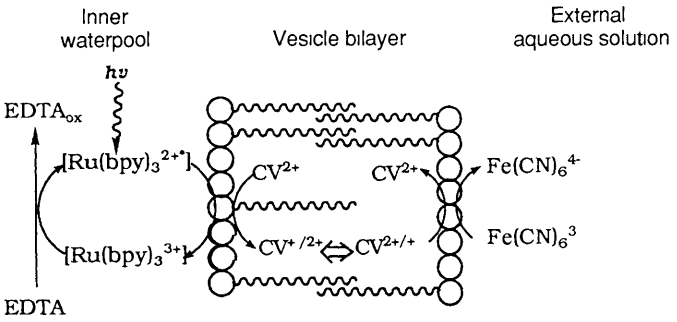


Figure 27 *Proposed mechanism for the viologen-mediated transfer of electrons across a phospholipid vesicle bilayer from internal* $[Ru(bpy)_3]^{2+*}$ *to exernal ferricyanide* [160]

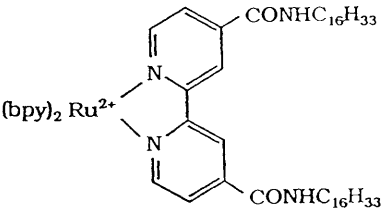


Figure 28
(N,N -di(1-*hexadecyl*)-2,2 -*bipyridine*)-4,4 -*dicarboxamide*)-*bis*(2,2 -*bipyridine*)*ruthenium*(II)

electron exchange between opposing inner $CV^{+\cdot}$ and outer CV^{2+}, involving electron tunnelling through the central hydrophobic region of the membrane (Figure 27)

A similar system involving inner waterpool $[Ru(bpy)_3]^{2+}$ (or $ZnTMPyP^{4+}$), membrane-incorporated octyldecylviologen, and an external oxidant (methylene blue, ferricyanide, $[\{Ru(bpy)_2(H_2O)\}_2O]^{4+}$ or $[SiMo_{12}O_{42}]^{8-}$) were shown to behave in a similar manner [162]

Tabushi and Kugimiya studied the effect of the alkyl chain length of moderately hydrophobic viologens on their ability to transfer electrons from EDTA (donor) and $ZnTSO_3NaP$ (as photocatalyst) to flavin mononucleotide (FMN), separated by phosphatidylcholine vesicle bilayers [163] For these viologens, electron transport was controlled by flux conjugation of the radical cation across the two bilayer/water interfaces The overall quantum yield of FMNH showed a characteristic biphasic dependence on hydrophobicity, in which optimum flux conjugation took place at C_4, the same as for ground state electron transport [108]

Ford *et al* constructed phosphatidylcholine vesicles containing a surfactant analogue of $[Ru(bpy)_3]^{2+}$, $RuC_{16}(bpy)_3^{2+}$ (Figure 28), vitamin K_1 (as a hydrogen carrier), decachloro-*m*-carborane (as proton carrier), and cetylviologen [158]

[162] E E Yablonskaya and V Y Shafirovich *Nouv J Chim* 1984 **8** 117
[163] I Tabushi and S Kugimiya *J Am Chem Soc* 1985 **107** 1859

The ruthenium complex was shown to photocatalyse electron transfer from 'internal' EDTA to 'external' methyl viologen, up a free energy gradient.[164]

In later studies, the same authors reported that the same ruthenium complex could photocatalyse electron transport across the bilayer of phosphatidylcholine vesicles from 'internal' EDTA to 'external' heptylviologen *without* any additional components.[165] The quantum yield dependence on the phosphatidylcholine:ruthenium complex mole ratio was shown to be consistent with an electron exchange mechanism between ruthenium complexes located in opposing lipid monolayers (again, electron tunnelling may have been involved), estimated to have a rate constant in the order of $10^4 - 10^6 s^{-1}$. This is many orders of magnitude faster than the transmembrane diffusion of lipids. The same conclusion was drawn from a comparison of the estimated activation energies of the two processes.[164] However, no clear cut explanation was given for the mechanism by which electrons were transferred from the bilayer interior to the external acceptor, *i.e.* whether the transbilayer electron transfer process was mono- or bi-photonic.

The rate of transmembrane electron transfer to ferricyanide in this system was increased 6.5-fold with the addition of the potassium ionophore valinomycin in the presence of K^+, while a 3-fold stimulation by the proton carriers gramicidin or FCCP was also observed.[166] These results indicated that the rate of photoinduced electron transfer across the vesicle bilayer was limited by the co-transport of cations in the absence of ion carriers. Further rate enhancements could be achieved by generating suitable transmembrane potentials with K^+ gradients in the presence of valinomycin, giving an 11-fold increase in quantum yield (4.4×10^{-3}).

Using a related system incorporating the C_{12} analogue of Ford's C_{14} surfactant ruthenium complex into the bilayer of DPPC vesicles separating 'internal' EDTA from 'external' methyl viologen, Matsuo *et al.* demonstrated photo-assisted transmembrane electron transfer, but only if the *photo-excited*, reduced form of the ruthenium complex was produced at the outer liposomal interface.[167] On the basis of these results, they proposed a two-photon mechanism for the transport of electrons across the bilayer for both the C_{12} *and* C_{16} ruthenium complexes.

Coutts and Patterson constructed an asymmetric vesicle system based on natural products.[168] When dissolved in the inner waterpool of unsaturated phosphatidylcholine (or dioleolylphosphatidylcholine) vesicles, flavin mononucleotide (FMN), a prosthetic group of the flavoprotein enzymes, acted as an electron source (from EDTA) for 'external' cytochrome c^{III} in the presence of light when either coenzyme Q_{10} or vitamin K_1 were present in the vesicle bilayer. In the absence of these quinones or when the bilayer was below its T_c, electron

[164] H. D. Mettee, W. E. Ford, T. Sakai, and M. Calvin, *Photochem. Photobiol.*, 1984, **39**, 679.
[165] W. E. Ford, J. W. Otvos, and M. Calvin, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3590.
[166] C. Laane, W. E. Ford, J. W. Otvos, and M. Calvin, *Proc. Natl. Acad. Sci USA*, 1981, **78**, 2017.
[167] T. Matsuo, K. Takuma, Y. Tsutsui, and T. Nishijima, *J. Coord. Chem.*, 1980, **10**, 187.
[168] D. Coutts and R. Patterson, *J. Membrane Sci.*, 1986, **27**, 275.

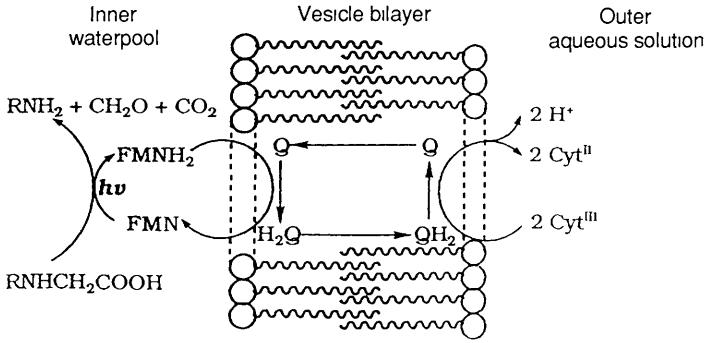


Figure 29 *Schematic representation of photochemically activated transbilayer electron/proton transport, mediated by* Q_{10} *from 'external' cytochrome* c^{III} *to 'internal' flavin mononucleotide* (FMN)[168]

(and proton) transport was not observed; a diffusive mechanism was proposed (Figure 29). Interestingly, particularly rapid cyt c^{III} reduction was achieved using a vesicle membrane with approximately the composition of the inner mitochondrial membrane.

Sudo and Toda have demonstrated transbilayer photo-assisted electron transfer reactions across phosphatidylcholine vesicle bilayers mediated by a variety of simple dyestuffs. Methylene blue, for example, has been shown as an effective electron (and proton) mediator catalyst between ascorbate (E'_0 = −0.17 V) and ferricyanide (E'_0 = 0.33 V), a thermodynamically 'up hill' reaction that does not occur in homogeneous or micellar solution.[115,169] Being both water-soluble and lipophilic, photosensitizers of this type readily permeate the bilayer, and consequently their ability to mediate electrons is strongly dependent upon their redox properties.[170]

6 Photoredox Processes in Surfactant Vesicles

A. Charge Separation.—The naturally-occurring phospholipids known to form vesicle assemblies in aqueous solution are typically zwitterionic, containing predominantly long-chain phosphatidylcholines. Synthetic surfactant vesicles can, like their phospholipid counterparts, accommodate and organize a substantial number of molecules, lower ionization potentials, and facilitate electron transfer across their interfaces. In the latter respect, the high surface charge density of many synthetic surfactant vesicles can allow kinetic control of photosensitized charge separations.

The architecture of vesicles with charged interfaces can be exploited to advantage in photoionization and photoredox processes. The photoionization of pyrene was enhanced greatly when localized within the hydrophobic bilayers of anionic dihexadecylphosphate (DHP) vesicles.[171] In this environment, the

[169] Y Sudo and F Toda, *J Chem Soc , Chem Commun* , 1979, 1044
[170] Y Sudo, T Kawashima, and F Toda, *Chem Lett* , 1980, 355
[171] J R Escabi-Perez, A Romero, S Lukac, and J H Fendler, *J Am Chem Soc* , 1979, **101**, 2231

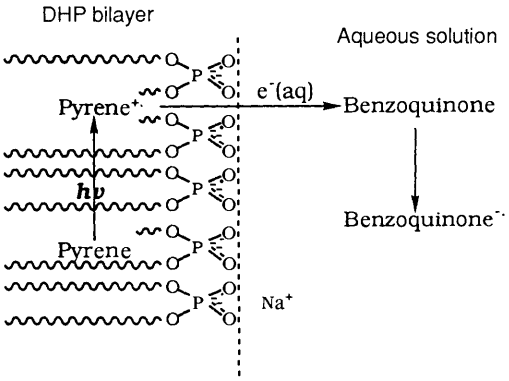


Figure 30 *Laser-induced photoionization of pyrene, and subsequent electron transfer*[171]

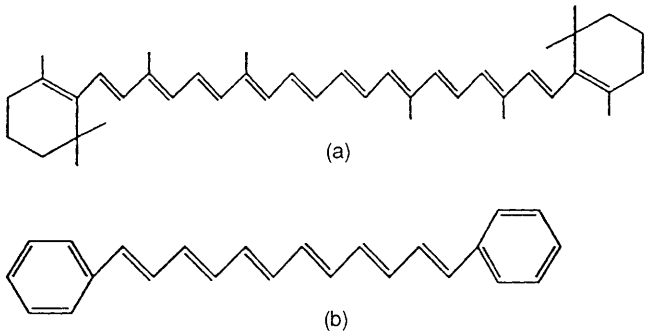


Figure 31 *Structures of all* trans *(a)* β*-carotene and (b) diphenylhexatriene*

ionization of pyrene increased substantially. Importantly, the anionic interface served both to stabilize the pyrene cations generated, and promote the ejection of electrons into the aqueous phase. Here, they could be accepted by benzophenone, which was subsequently repelled from the DHP surface (Figure 30). The polarity gradient provides the driving force for the exit of the electron, and the net charge on the DHP vesicles prevents charge recombination.

The photoionization yield of chlorophyll *a* (at 77K) in cationic dioctadecyldimethylammonium chloride (DODAC) vesicles was found to be twice that with zwitterionic phospholipid vesicles, which was itself greater than with anionic DHP vesicles.[135] The results were discussed in terms of electrostatic barriers to electron transfer into the aqueous phase, and were consistent with the findings of Lanot and Kevan for the photoionization of ZnTPP in anionic and cationic vesicles.[171a]

Electron transfer from the radical anion of carotene and a shorter polyene, diphenylhexatriene (Figure 31), both formed on reaction with radiolytically

[171a] M. P. Lanot and L. Kevau, *J. Phys. Chem.*, 1989, **93**, 998.

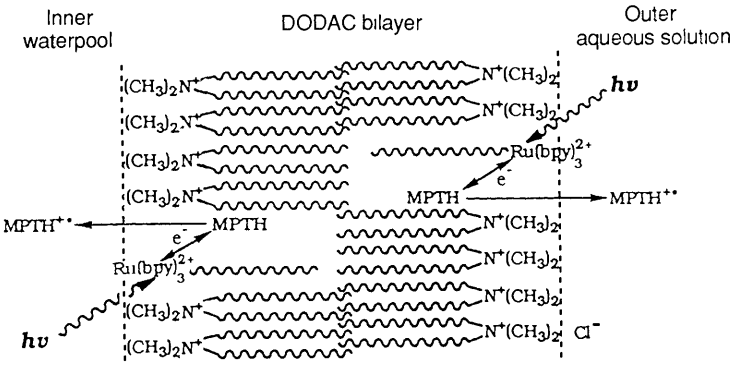


Figure 32 *Possible reaction pathways between* DODAC *intercalated* $[RuC_{18}(bpy)]^{+\cdot}$ *and* $MPTH^{+\cdot}$[174]

produced e_{aq}^-, in cationic didodecyldimethylammonium bromide vesicles was studied.[172] Carotenoid polyenes are believed to play a role as protective agents and accessory pigments in natural photosynthetic membranes, and may also be involved in electrical conduction through their extended π-electron systems. Electron transfer was found to occur from the membrane-stabilized polyene radical anions to externally-bound (Fe)EDTA with a second order rate constant of $1 \times 10^{10}\,dm^3\,mol^{-1}\,s^{-1}$. By contrast, no evidence of electron transfer to Eu^{3+} was obtained.

The organizational ability of cationic DODAC vesicles facilitated Förster-type energy transfer from bilayer-incorporated lysopyrene to externally bound pyranine with an efficiency of up to 43%.[173]

Photosensitized electron transfer and charge separation in DODAC vesicles has been studied in detail using a surfactant derivative of tris(2,2′-bipyridine)ruthenium perchlorate, $[RuC_{18}(bpy)_3]^{2+}$, as the photoactive electron acceptor, and *N*-methylphenothiazine (MPTH) as donor.[174]

Subsequent to the reductive quenching of the excited ruthenium complex (anchored onto the inner and outer vesicle surfaces) by MPTH, distributed throughout the hydrophobic layer;

$$[RuC_{18}(bpy)_3]^{2+*} + MPTH \longrightarrow [RuC_{18}(bpy)_3]^{+} + MPTH^{+\cdot}$$

three pathways were recognized for the reaction of $[RuC_{18}(bpy)_3]^{+\cdot}$ with $MPTH^{+\cdot}$. Firstly, geminate recombination of the cation radicals could occur at the site of generation. Secondly, repulsion of $MPTH^{+\cdot}$ into the inner waterpools, and, due to spatial confinement, rapid recombination with $[RuC_{18}(bpy)_3]^{+\cdot}$ at the inner surface of the vesicle. Finally, escape of $MPTH^{+\cdot}$ into the bulk aqueous phase, where it could survive for extended periods (several milliseconds) through mutual electrostatic repulsion with the vesicle surface (Figure 32).

[172] M Almgren and J K Thomas, *Photochem Photobiol*, 1980, **31**, 329
[173] T Nomura, J R Escabi-Perez, J Sunamoto, and J H Fendler, *J Am Chem Soc*, 1980, **102**, 1484
[174] P P Infelta, M Gratzel, and J H Fendler, *J Am Chem Soc*, 1980, **102**, 1479

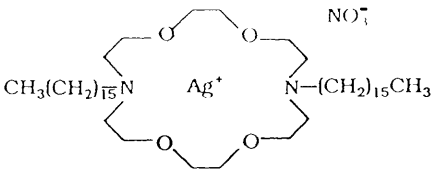


Figure 33 *A vesicle-forming dialkyldiaza crown ether*(II)

The effect of MPTH and NaCl concentrations on these pathways was examined. At low MPTH concentrations, $MPTH^{+\bullet}$ was preferentially partitioned into the waterpools, but higher $MPTH^{+\bullet}$ levels created a potential gradient that caused further $MPTH^{+\bullet}$ to be ejected into the bulk aqueous phase. Increasing the MPTH concentration, therefore, resulted in more efficient charge separation, and a decrease in the outer surface charge density of the DODAC vesicles.

Addition of chloride ions to the vesicle sytem also decreased the funtional positive charges on the outer DODAC vesicle surface. This had three important consequences:

(*i*) The number of sites where the local electrostatic field prevented the exit of $MPTH^{+\bullet}$ was reduced.

(*ii*) A dissymmetry was created between the inner and outer surface potentials, favouring the exit of $MPTH^{+\bullet}$ into bulk aqueous solution.

(*iii*) The reduced net charge on the aggregates increased the rate of the back-reaction.

By judicious addition of electrolyte, the amount of $MPTH^{+\bullet}$ produced and expelled into the bulk aqueous phase could be maximized. Under this condition, sufficient electrostatic repulsion existed between $MPTH^{+\bullet}$ and the vesicle surface significantly to hinder undesirable charge recombination reactions. Similar counterion effects have been noted on photosensitized electron transfer between surfactant vesicle solubilized chlorophyll and benzoquinone.[175]

Electron transfer from the photoactive ZnTPP to duroquinone (or $C_{11}DQ$), both solubilized in DODAC vesicles have been studied.[176] Again, salt addition favoured ejection of the (porphyrin) radical cation from the bilayer by decreasing the outer surface potential, but radical yields were decreased.

Functionalized surfactant vesicles have proved an important advance in achieving efficient photo-assisted charge separation. When complexed with silver(I) ions, the surfactant dialkyldiaza crown(II) ether, shown in Figure 33, aggregates spontaneously into vesicles.[177] Photoinduced electron transfer from two sensitizers, either a cyanine dye or the surfactant $[RuC_{16}(bpy)_3]^{2+}$ complex, occurred extremely rapidly, forming zerovalent silver, which was stabilized by the microenvironment of the vesicles.

The oxidative quenching of $[Ru(bpy)_3]^{2+}$ by viologens showed striking differences to that observed in homogeneous solution when redox-active

[175] Y. Fang and G. Tollin, *Photochem. Photobiol.*, 1984, **39**, 685.
[176] M-P. Pileni, *Chem. Phys. Lett.*, 1980, **71**, 317.
[177] K. Monserrat, M. Grätzel, and P. Tundo, *J. Am. Chem. Soc.*, 1980, **102**, 5527.

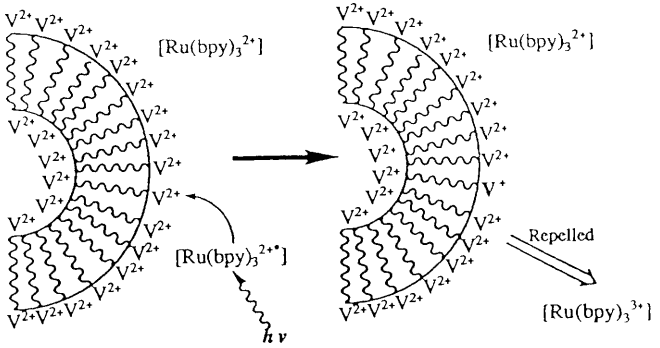


Figure 34 *Schematic representation of the role of the surface charge of surfactant-viologen vesicles in facilitating charge separation between a reduced surfactant-viologen and* [Ru(bpy)$_3$]$^{3+}$][178]

surfactant alkylviologen ((R,Me)V^{2+}) vesicles were employed.[178] The forward reaction was facilitated by high local concentrations of donor and acceptor, while the back-reaction was retarded by the electrostatic repulsion of [Ru(bpy)$_3$]$^{2+}$ from the cationic vesicle surface (Figure 34). The close proximity of the viologen headgroups may result in electron transfer between neighbouring viologens.

DODAC vesicles have been used to promote electron transfer from aqueous-phase, excited-state [Ru(bpy)$_3$]$^{2+}$ or zinc tetramethylpyridyl porphyrin (ZnTMPyP^{4+}), (regenerated by EDTA) to an alkyl-substituted methylviologen, $C_{14}MV^{2+}$.[179] The reduced form of acceptor, $C_{14}MV^{+\bullet}$, is extremely hydrophobic, and was rapidly incorporated into the vesicle bilayers, which, through electrostatic repulsion of the sensitizer cation, provided an effective electrostatic barrier for the back-reaction (50 times slower than in vesicle-free solution). Unlike in cationic micelles, $C_{14}MV^{+\bullet}$ existed in DODAC as a multimer, which could be generated even in aerated solution. Furthermore, in the presence of a colloidal platinum catalyst, this multimer was found to generate hydrogen from water:

$$(C_{14}MV^{+\bullet})_n + nH_2O \longrightarrow 0.5nH_2 + nOH^- + nC_{14}MV^{2+}$$

In an interesting report, Hamachi and Kobuke have investigated the reduction of an artificial, membrane-bound flavolipid (Figure 13) by dihydronicotinamide adenine dinucleotide (NADH).[180] NADH is one of the main electron donors to flavoproteins in many biological redox processes, but has not been widely studied with flavins because of the slowness of the reaction. In the presence of DODAC, NADH oxidation by the flavolipid was accelerated 4.6×10^4 times compared with that in zwitterionic phosphatidylcholine vesicles. Fluorescence studies indicated that electrostatic binding of NADH to DODAC altered the conforma-

[178] K Kurihara, D Tundo, and J H Fendler, *J Phys Chem*, 1983, **87** 3777
[179] K Monserrat and M Gratzel, *J Chem Soc, Chem Commun*, 1981, 183
[180] I Hamachi and Y Kobuke, *J Chem Soc, Chem Commun*, 1989, 130

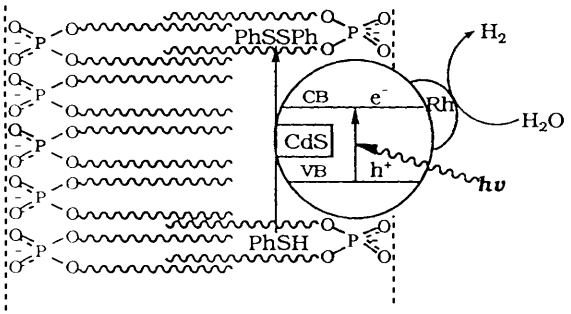


Figure 35 *Idealized model for the* CdS-*sensitized photoreduction of water by* PhSH *in aqueous* DHP *vesicles*[181]

tion of NADH to an open one, with its dihydropyridine ring extended into the membrane phase, where it could interact with the flavin unit.

In a series of papers, Fendler and his co-workers have reported extensive and detailed studies on the *in situ* formation of colloidal semiconductor particles in charged surfactant vesicle bilayers, which provided size, geometrical control, and stabilization of the clusters through compartmentalization of the precursors.[40] In an early report, rhodium-coated cadmium sulphide particles of narrow size distribution (~4 nm) were produced in DHP vesicles by reduction of Cd^{2+} by H_2S, and subsequent irradiation of Rh^{3+} by uv light.[181] In the presence of thiophenol as a sacrificial electron source, photolysis of the system caused band-gap excitation of electrons in the CdS particles (2.4 eV), leading to the reduction of water through surface-deposited rhodium (Figure 35).

Similar particles were generated in DODAC, DODAB,[182] and polymerizable vesicles[183] (from a Cd/EDTA complex), and hydrogen production was optimized. Later, a thiol-functionalized surfactant was employed as a recyclable electron donor in the DODAC system.[184] Further enhancement in hydrogen production was achieved using benzyl alcohol as donor.[185,186]

Semiconductor particles, comprising either homogeneous mixed crystals of $Zn_xCd_{1-x}S$ or crystals of CdS coated with ZnS, were generated in, and stabilized by, DHP vesicles, allowing control of the semiconductor band gap.[186] ZnS-coated CdS particles (Cd:Zn ratio 1:1) generated hydrogen at five times the rate ($\varphi = 0.0186$) of pure CdS particles, possibly by removing ineffective, low-lying surface states on CdS.

[181] Y-M. Tricot and J. H. Fendler, *J. Am. Chem. Soc.*, 1984, **106**, 7359.
[182] R. Rafaeloff, Y-M. Tricot, F. Nome, and J. H. Fendler, *J. Phys. Chem.*, 1985, **89**, 533.
[183] Y-M. Tricot, A. Emeren, and J. H. Fendler, *J. Phys. Chem.*, 1985, **89**, 4721.
[184] R. Rafaeloff, Y-M. Tricot, F. Nome, P. Tundo, and J. H. Fendler, *J. Phys. Chem.*, 1985, **89**, 1236.
[185] H-C. Youn, Y-M. Tricot, and J. H. Fendler, *J. Phys. Chem.*, 1987, **91**, 581.
[186] H-C. Youn, S. Barai, and J. H. Fendler, *J. Phys. Chem.*, 1988, **92**, 6320.

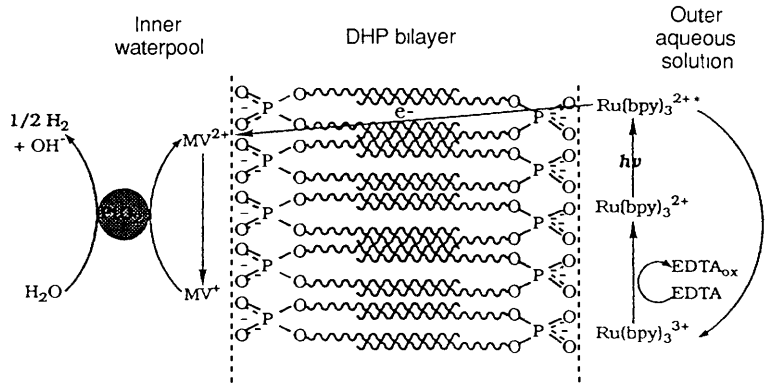


Figure 36 *Originally proposed model for transmembrane electron transfer from* EDTA *to* MV^{2+} *via* $[Ru(bpy)_3]^{2+}$ [187]

B. Transmembrane Electron Transfer.—Charged surfactant vesicle bilayers offer particular advantages over their zwitterionic phospholipid counterparts towards transmembrane electron transfer processes; not least, greater stability, and more efficient charge separation in light-assisted reactions.

Tunuli and Fendler reported electron transfer between photoexcited $[Ru(bpy)_3]^{2+}$, located on the outer surface of anionic DHP vesicles, and methylviologen (MV^{2+}), located at the inner, *in the absence of charge carriers.*[187] With 'external' EDTA present as sacrificial electron donor, reduction of MV^{2+} proceeded with a quantum efficiency of 2.4×10^{-2}. Additionally, if PtO_2 was entrapped in the DHP inner waterpools, hydrogen evolution and concomitant reoxidation of $MV^{+\bullet}$ was observed (Figure 36).

It was subsequently shown, however, that in the presence of 'Tris' buffer used in the study, methylviologen underwent extensive photoinduced diffusion across the DHP bilayers.[188,189] Consequently, the photoredox reaction between $[Ru(bpy)_3]^{2+*}$ and MV^{2+} in fact occurred at the *outer* surface of the vesicles. In a recent study, it has been reported that both $MV^{+\bullet}$ and $[Ru(bpy)_3]^{2+}$ show significant leakage rates across the DHP bilayers.[190] All these findings may have relevance to studies of transmembrane electron transfer in phospholipid vesicle systems.

Photoinduced electron transfer from CdS particles embedded in the outer monolayer of DHP vesicles to methylviologen in the inner waterpools in the absence of buffer has been demonstrated with a quantum yield of 0.05, although only at high CdS concentration, and under strong illumination.[190] The reduced methylviologen that resulted subsequently diffused through the vesicle bilayer (Figure 37).

[187] M S Tunuli and J H Fendler, *J Am Chem Soc*, 1981, **103**, 2507
[188] L Y-C Lee, J K Hurst, M Politi, K Kurihara, and J H Fendler, *J Am Chem Soc*, 1983, **105**, 370
[189] B C Patterson, D H Thompson, and J K Hurst, *J Am Chem Soc*, 1988, **110**, 3656
[190] Y-M Tricot and J Manassen, *J Phys Chem*, 1988, **92**, 5239

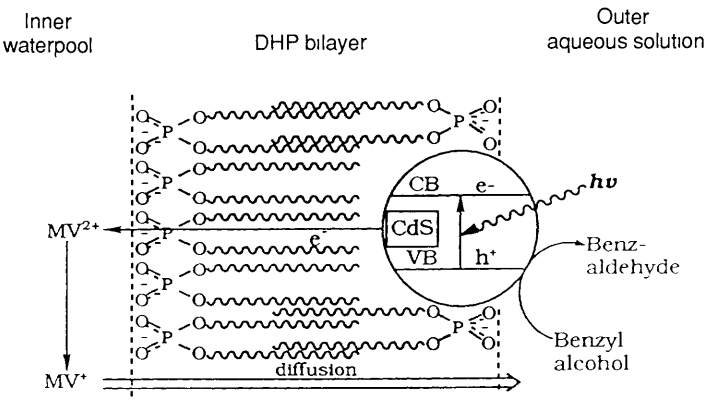


Figure 37 *Proposed mechanism for electron transfer across* DHP *vesicle bilayer from embedded* CdS to 'internal' MV^{2+} [190]

Indirect evidence of transmembrane electron transfer across DHP bilayers from the 'external' water-soluble photosensitizer [5,10,15,20-tetrakis(4′-sulphonatophenyl)-porphinato]zinc(II) (together with external amine donor) to 'internal' ferricyanide *mediated* by a hydrophilic methylviologen ($C_{16}MV^{2+}$) was reported by Hurst *et al.*[191] When ferricyanide was present, $C_{16}MV^{+\bullet}$ formation occurred more slowly, and its appearance was preceded by a pronounced induction period, suggesting ferricyanide oxidation of the reduced viologen radical. Ferricyanide itself was shown not to diffuse across the vesicle bilayer during the course of the studies. The 'transmembrane' reaction required $C_{16}MV^{2+}$ on *both* membrane surfaces, indicating an electron exchange mechanism between opposing mono- and dications. However, this simple transmembrane reaction mechanism was not supported by a more recent kinetic analysis of this system; an alternative explanation of the complex rate equations derived was not given.[192]

These studies clearly illustrate some of the permeability problems associated with synthetic surfactant vesicles which mean that unambiguous demonstration of genuine vectorial transmembrane photoredox processes are difficult to demonstrate because they are masked by homogeneous reactions associated with diffusion of the electron donor and/or acceptor across the vesicle bilayer.

Recently, in a series of papers[193–198] concerning the redox properties of

[191] J K Hurst, L Y-C Lee, and M Gratzel, *J Am Chem Soc*, 1983, **105**, 7048
[192] D H Thompson, W C Barrette, and J K Hurst, *J Am Chem Soc*, 1987, **109**, 2003
[193] C Dainty, D W Bruce, D J Cole-Hamilton, and P Camilleri, *J Chem Soc, Chem Commun*. 1984, 1324
[194] P Camilleri, A Dearing, D J Cole-Hamilton, and P O'Neill, *J Chem Soc, Perkin Trans 2*. 1986. 569
[195] J N Robinson, D J Cole-Hamilton, and P Camilleri, *J Chem Soc, Chem Commun*, 1988, 1410
[196] J N Robinson, D J Cole-Hamilton, P Camilleri, C Dainty, and V Maxwell, *J Chem Soc, Faraday Trans 1*, 1989, **85**, 3385
[197] J N Robinson, D J Cole-Hamilton, M K Wittlesey, and P Camilleri, *J Chem Soc. Faraday Trans*. 1990, **86**, 2897
[198] J N Robinson, D J Cole-Hamilton, M K Whittlesey, and P Camilleri, to be published

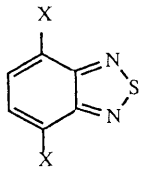


Figure 38 *Structure of benzothiadiazoles used for combined chromophores and electron transfer catalysts* Y = CN, BTDN, Y = COOEt, BTDE, Y = COOBu, BTDB

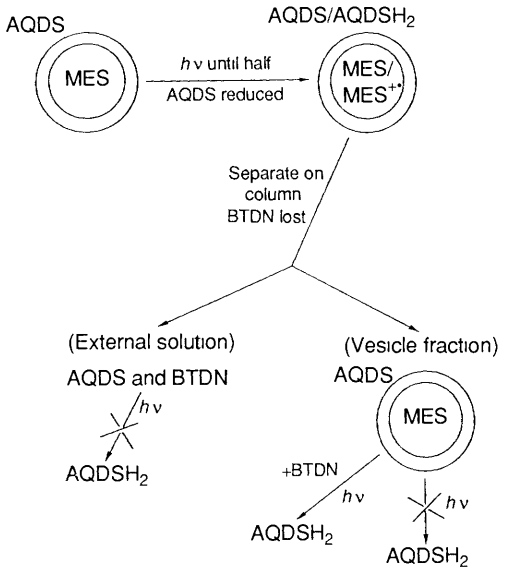


Figure 39 *Schematic representation of the procedure by which asymmetric* DODAB *vesicles were tested for leakage of* MES *or* AQDS [197]

2,1,3-benzothiadiazole-4,7-dicarbonitrile (BTDN) (Figure 38, X = CN), Robinson, Cole-Hamilton, and co-workers have presented convincing evidence that this combined chromophore and electron transfer agent can transfer electrons in a photochemical reaction from 4-morpholineethanesulphonic acid (MES) entrapped in the inner water pools of DODAB vesicles to anthraquinonedisulphonate salts (AQDS) in the bulk water, and that this reaction occurs *via* a genuine vectorial electron transport [195 197] In a key experiment, they photolysed the unsymmetrical vesicle system until *ca* 50% of the AQDS had been reduced They then separated the vesicles from the bulk water by chromatography on a Sephadex column The vesicle fraction contained MES and AQDS (BTDN was lost on the column) but reduction of AQDS did not occur on photolysis, confirming that AQDS had not diffused through the bilayer but was bound to the outer surface of the vesicle On addition of BTDN

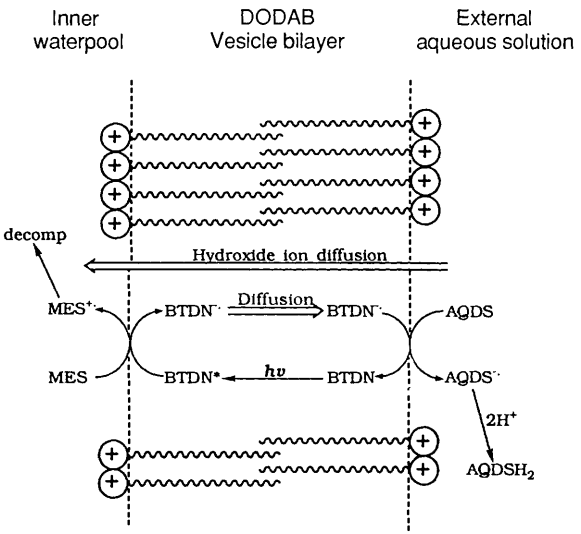


Figure 40 *Schematic representation of the proposed mechanism by which* BTDN *mediates electron transport across the bilayer of* DODAB *vesicles. Charge compensation occurs by diffusion of* OH^- *or* H^+*; only that involving* OH^- *is shown*[197]

to these vesicles, reduction of AQDS again occurred on photolysis, confirming that the MES had remained inside the vesicles and that the vesicles had retained their integrity. Photolysis of the chromatographed fraction which contained the bulk water again did not lead to reduction of AQDS, confirming that MES had not leaked out of the inner water pools[193,197] (Figure 39).

Detailed mechanistic studies on this system suggested that electrons were transported across the vesicle bilayer by diffusion of $BTDN^{\bullet -}$, which is only stable in ordered assemblies such as vesicle bilayers or micelles, not in water, but that in continuous photolysis experiments, the overall rate of this process was determined by concomitant charge compensating diffusion of OH^- or H^+ across the bilayer (Figure 40).[193,197]

Using the diethyl or dibutyl esters of 2,1,3-benzothiazole-4,7-dicarboxylic acid (Figure 38, X = COOEt or COOBu), electron transfer was again observed in similar unsymmetrical DODAB systems. In both cases, the rate of electron transfer was very similar to that observed for BTDN, despite the esters having different properties in terms of solubility in the bilayer, ability to absorb visible light and redox potential. The similarity in rates of electron transport for the three systems was interpreted as further evidence that diffusion of OH^- or H^+ across the vesicle bilayer is the overall rate determining step.[198,199]

[199] J. N. Robinson, Ph.D. Thesis, University of St. Andrews, 1989.

7 Concluding Remarks

Most of the work reviewed in this paper has been published only in the last ten years, covering just one aspect of the scientific literature involving vesicle assemblies However, it is clear even from this discussion that the design of the multi-molecular bodies, studied either as biomimetic or purely 'synthetic' structures, has grown very rapidly in sophistication in only a few years The same can be said of the experimental techniques employed for analysis of these increasingly complex microheterogeneous systems

Our understanding of the relationships between the structure and behaviour of bilayer membranes and the (physio-)chemical processes that occur in and around them have been considerably enhanced by utilizing 'tailor made' model vesicle systems

The knowledge gained from such studies has already proven extremely valuable in other areas of membrane science Given the current rate of progress, it may soon be possible to develop devices based on 'artificial' membranes for applications in a variety of areas, such as medicine (drug delivery, photodynamic therapy), solar energy conversion (electron/proton pumps), and extraction mineralogy (ion transport)