

# The design and synthesis of artificial photosynthetic antennas, reaction centres and membranes

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Artificial antenna systems and reaction centres synthesized in our laboratory are used to illustrate that structural and thermodynamic factors controlling energy and electron transfer in these constructs can be modified to optimize performance. Artificial reaction centres have been incorporated into liposomal membranes where they convert light energy to vectorial redox potential. This redox potential drives a Mitchellian, quinone-based, proton-transporting redox loop that generates a  $\Delta\mu$ H<sup>+</sup> of *ca*. 4.4 kcal mol<sup>-1</sup> comprising  $\Delta$ pH *ca*. 2.1 and  $\Delta\psi$  *ca*. 70 mV. In liposomes containing CF<sub>0</sub>F<sub>1</sub>–ATP synthese, this system drives ATP synthesis against an ATP chemical potential similar to that observed in natural systems.

Keywords: artificial photosynthesis; artificial antenna; artificial reaction centres

#### 1. INTRODUCTION

Natural photosynthesis has inspired chemists to mimic aspects of this successful energy-transducing process in the laboratory. One of many approaches to the assembly of an artificial reaction centre is to use synthetic pigments, electron donors and electron acceptors that are related to the natural pigments (chlorophylls, carotenoids and quinones), but to employ covalent bonds in place of the proteins that function as scaffolds, organizing the pigments in the natural systems. Like the natural protein matrix, the covalent bonds of the artificial antennae and reaction centres control the separations, angular relationships and electronic coupling among the various active moieties, and thus control the rates and yields of energy and electron-transfer processes (Wasielewski 1992; Gust et al. 1993a, b, 1999, 2000, 2001a, b; Gust & Moore 2000). In § 3, we will discuss biomimicry of the various functions of carotenoids in photosynthesis. Later, in § 5, the preparation and function of artificial reaction centres will be described. Finally, in §6 we will review progress in the construction of artificial photosynthetic membranes.

#### 2. CAROTENOIDS IN NATURAL PHOTOSYNTHESIS

## (a) Carotenoid pigments as photosynthetic antennae

As accessory light-harvesting pigments, carotenoids are found in the chlorophyll-binding antenna proteins of photosynthetic organisms where they absorb light in the blue–green spectral region and transfer energy to nearby chlorophylls.

$$\operatorname{Car} \xrightarrow{n_c} {}^{1}\operatorname{Car},$$
 (2.1)

$$\operatorname{Car} + \operatorname{Chl} \to \operatorname{Car} + {}^{1}\operatorname{Chl}.$$
 (2.2)

Light energy collected in this way is funnelled to special structures in the membranes, known as reaction centres, where energy conversion to electrochemical potential takes place. The unique photophysics of carotenoids (*vide infra*) places severe constraints on aspects of the structure of carotenoid–chlorophyll-binding proteins that control the interactions of the pigments. As described below, many of these structural constraints have been identified in model systems by making systematic changes in the linkages connecting carotenoid pigments to tetrapyrroles and measuring the effect on energy-transfer rates and yields. We begin with a brief review of carotenoid photophysics to set the stage for interpreting energy-transfer experiments on model antenna systems.

#### (b) Carotenoid photophysics

This discussion applies to the genre of carotenoid pigments having nine or more conjugated double bonds, including oxy- and hydroxy-substituted compounds, although there are certainly small differences between pigments. Carotenoid absorption of light in the 400-550 nm spectral region gives rise to their intense yellow-orange colour, a consequence of high extinction coefficients (ca.  $10^5 \,\mathrm{M^{-1}\,cm^{-1}})$  in this region. This strongly electric dipole allowed transition is from the ground state  $(S_0)$  to the second excited singlet state  $(S_2)$  of the carotenoid. As expected for an upper excited singlet state, the  $S_2$  species has a very short lifetime of 200 fs or less (Truscott 1990; Shreve et al. 1991, 1992; Kandori et al. 1994; Macpherson & Gillbro 1998). Because of the short lifetime, the quantum yield of fluorescence emission from this state is very low (less than  $10^{-4}$ ) in spite of the strongly allowed nature of the transition and its correspondingly large radiative rate constant (ca. 109 s<sup>-1</sup>). Surprisingly, this state is important as an energy donor in photosynthesis (Trautman et al. 1990; Ricci et al. 1996).

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The transition from  $S_0$  to the lowest excited singlet state of carotenoids,  $S_1$ , is electric-dipole forbidden (Kohler 1991) and is not observed in the usual single-photon absorption experiment. It is readily populated by relaxation from  $S_2$ . The lifetime of the  $S_1$  state, as measured by transient absorption techniques, is *ca*. 10–40 ps for most carotenoids (Wasielewski & Kispert 1986; Trautman *et al.* 1990). The state decays essentially exclusively via internal conversion. Fluorescence from  $S_1$  is virtually undetectable (quantum yield less than  $10^{-4}$ ) and intersystem crossing to the triplet state is not observed. The energy of the  $S_1$ state is uncertain for most of the carotenoids involved in photosynthesis because the forbidden nature of the  $S_0 \rightarrow S_1$  transition makes it difficult to detect.

Although carotenoid triplet states are not formed in appreciable amounts by the usual intersystem crossing pathway, they can be produced via triplet-triplet energy transfer from other species or by radical-pair recombination processes in artificial reaction centres (Gust et al. 1992; Liddell et al. 1997; Carbonera et al. 1998). Thus, their transient absorption characteristics are well known (Mathis & Kleo 1973; Bensasson et al. 1976). Typically, they have strong absorption maxima at about 540 nm. In most organic solvents in the absence of oxygen, carotenoid triplet states have lifetimes of 5-10 µs. Phosphorescence from these triplets has not been reported, and therefore the energies of the carotenoid triplet states have not been measured by direct optical means. Energy-transfer studies have demonstrated that the energy of the lowest-lying carotenoid triplet is below that of singlet oxygen at ca. 1.0 eV. Results from our laboratory were obtained using a hybrid spectroscopic-calorimetric technique (photoacoustic spectroscopy) that has located the lowest-lying triplet state of a representative carotenoid (similar to the carotenoid moiety of structure 10 (see Appendix A) except containing one more conjugated double bond) at  $0.65 \pm 0.04 \text{ eV}$ (Lewis et al. 1994). Thus, the expected origin of the phosphorescence would be ca. 1900 nm.

#### (c) Photoprotection

In the photosynthetic process, carotenoids have functions that are ancillary to the basic energy-conversion reactions and are crucial to the protection of photosynthetic membranes from photochemical damage (Cohen-Bazire & Stanier 1958; Sieferman-Harms 1987; Frank & Cogdell 1996). Under certain conditions, most photosynthetic organisms unavoidably generate chlorophyll triplet excited states when exposed to light. Often, these are produced in the reaction centre via recombination of charge-separated states. Chlorophyll triplet states are excellent sensitizers for formation of singlet oxygen from the oxygen groundstate triplet as in the following equation:

$${}^{3}\mathrm{Chl} + {}^{3}\mathrm{O}_{2} \rightarrow \mathrm{Chl} + {}^{1}\mathrm{O}_{2}. \tag{2.3}$$

Singlet oxygen, with an energy of ca. 1 eV above the ground state, is a highly reactive species that can react with and destroy lipid bilayer membranes and other vital components of cells. Clearly, singlet oxygen production in photosynthetic organisms is potentially injurious (Krinsky 1979; Cogdell & Frank 1993). Such organisms have developed dual defence mechanisms based upon the low energy of the carotenoid triplet state mentioned above. Carotenes can quench chlorophyll triplet states at a rate

that precludes singlet oxygen generation via a triplettriplet energy-transfer process (equation (2.4)). Additionally, carotenoids can quench singlet oxygen itself through energy transfer (equation (2.5)) or chemical reaction (Foote *et al.* 1970).

$$^{3}$$
Chl + Car  $\rightarrow$  Chl +  $^{3}$ Car, (2.4)

$${}^{1}O_{2} + Car \rightarrow {}^{3}O_{2} + {}^{3}Car.$$
 (2.5)

Because the carotenoid triplet state is much lower in energy than singlet oxygen, it returns harmlessly to the ground state with the liberation of heat.

From this overview, it is readily appreciated that carotenoids were selected by the evolving photosynthetic apparatus because of their unique photophysical properties. Their strong absorption is closely matched to the maximum of the solar flux, although their triplet levels are well below the energy of singlet oxygen.

#### (d) Regulation of photosynthesis by carotenoids

It has been found that carotenoid polyenes are involved in a mechanism whereby some photosynthetic organisms dissipate excess chlorophyll singlet excitation energy (Yamamoto 1979; Demmig-Adams 1992; Yamamoto & Bassi 1995; Horton *et al.* 1996). This process is sometimes referred to as non-photochemical quenching because it is measured by *in vivo* fluorescence spectroscopy of plant leaves. It comes into play at high light levels and evidently helps to protect the organism from light-induced damage. Non-photochemical quenching is signalled by the conversion of violaxanthin to zeaxanthin, but the details of the quenching process are currently not understood.

#### 3. CAROTENOIDS IN BIOMIMETIC SYSTEMS

(a) Minicry of the antenna function of carotenoids As indicated in equation (2.2), in order to act as antennas for gathering light, carotenoid pigments must transfer singlet excitation energy to nearby chlorophyll molecules. Singlet energy transfer from carotenoid to chlorophyll can occur by at least two different pathways. At one limit, following absorption of light and internal conversion from  $S_2$ , the  $S_1$  level of the carotenoid is populated and then transfers energy to the  $Q_{\nu}$  (S<sub>1</sub>) of the chlorophyll. At the other limit, energy transfer from the  $S_2$  level of the carotenoid to the  $Q_x$  ( $S_2$ ) of the chlorophyll competes with internal conversion in the carotenoid. The short lifetime of the  $S_2$  state of the carotenoid does not a priori exclude the second possibility. Indeed, detailed spectroscopic studies on the subpicosecond time-scale of the pigment protein complexes that make up the lightharvesting antennas of photosynthetic organisms indicate that, in some cases, energy transfer from the carotenoid to the chlorophyll or bacteriochlorophyll may occur from the carotenoid  $S_2$  level rather that from the lower-energy S<sub>1</sub> excited state, as predicted by Kasha's rule (Trautman et al. 1990; Shreve et al. 1991; Koyama et al. 1996; Ricci et al. 1996; Krueger et al. 1998). The two pathways are shown schematically in figure 1.

However, the complexity of the natural systems containing multiple chromophores, coupled with the difficulty of the measurements on such a short time-scale, make the



Figure 1. Energy-level diagram showing both possible paths for energy transfer from the excited singlet states of a carotenoid to the  $S_1$  or  $S_2$  levels of a tetrapyrrole pigment.

pathway assignments uncertain. Structures 1 and 4 (see Appendix A) are examples of simple, well-defined molecular dyads that were designed to help in the understanding of the carotenoid to chlorophyll singlet–singlet energy-transfer process in natural systems.

Transient absorption and fluorescence experiments on structures 1 and 4 and the model pigments, structures 2, 3, 5 and 6 (see Appendix A), yielded convincing results about the energy-transfer pathway (Macpherson et al. 2002). In structure 1, the lifetime of  $S_2$  was measured by fluorescence upconversion to be 45 fs, whereas the  $S_2$  lifetime of model carotenoid 3 is 160 fs. The  $S_1$  lifetime was 8 ps in both structures 1 and 3. Moreover, the  $S_1$  rise time for tetrapyrrole (moiety of dyad 1) was found to be 62 fs by fluorescence upconversion. Therefore, only the carotenoid  $S_2$  state was quenched by the attached tetrapyrrole. The observation that the time constant associated with the decay of the energy donor (carotenoid  $S_2$ ) is consistent with that associated with the energy arrival at the acceptor (tetrapyrrole  $S_1$ ) solidly demonstrates the  $S_2$  energytransfer pathway. (The difference between carotenoid S<sub>2</sub> decay at 45 fs and tetrapyrrole  $S_1$  rise at 62 fs is due to the delay associated with relaxation to the fluorescent state in the tetrapyrrole.) Quantitatively, quenching of the carotenoid  $S_2$  state from 160 fs to 45 fs by energy transfer results in an efficiency of 70%, which matches that measured by steady-state fluorescence excitation spectroscopy.

Studies of dyad 4 illustrate that both pathways can be active. In initial experiments on dyad 4, the attached tetrapyrrole was found to quench the carotenoid  $S_2$  state from 95 fs in model 6 to 28 fs, and the carotenoid  $S_1$  level from 12 to 9 ps. The rise of the  $S_1$  level of the tetrapyrrole of structure 4, as measured by fluorescence upconversion, required a major exponential component (74%) of 41 fs

and a minor component (26%) of 4 ps. While the match between the 9 ps decay of the carotenoid  $S_1$  and the 4 ps rise component of the tetrapyrole  $S_1$  is only qualitative, these preliminary experiments do provide evidence that both states can be energy donors. Taken together, the carotenoid  $S_2$  and  $S_1$  kinetic data give an overall quantum yield of energy transfer of *ca.* 80%, in qualitative agreement with that observed by steady-state fluorescenceexcitation spectroscopy.

Why is the yield of the  $S_1$  pathway different in structures 1 and 4? There are undoubtedly subtle differences in the electronic coupling between the chromophores (*vide supra*) and the energy-transfer energetics are different. The  $S_1$  level of the tetrapyrrole in stucture 4 is about 270 cm<sup>-1</sup> higher than that of structure 1. Considering this small energy difference, the fact that the  $S_1$  carotenoid band is expected to be rather broad (Frank *et al.* 1996) and that the  $S_1$  levels of these carotenoids have not been measured but should be similar, the role of thermodynamics in controlling these energy-transfer processes remains speculative.

### (b) Carotenoid antenna for non-photosynthetic pigments

One of the clearest examples of the increased absorption cross-section for a photochemical process provided by carotenoid pigments is that observed in carotenoidbuckminsterfullerene dyad, structure 7 (see Appendix A) (Imahori et al. 1995). The carotenoid absorption spectrum is distinct and much stronger than that of the underlying  $C_{60}$  bands. Upon excitation of the carotenoid moiety of stucture 7 with a 150 fs pulse of 600 nm laser light, the carotenoid radical cation forms in ca. 800 fs via photoinduced electron transfer to the fullerene to yield  $C^{+}-C_{60}^{-}$ . The charge-separated species has a lifetime of ca. 500 ps and decays partially to the carotenoid triplet. Excitation of the carotenoid pigment provides two routes to the charge-separated species: energy transfer from the carotenoid to the C60 followed by photoinduced electron transfer to the C<sub>60</sub> S<sub>1</sub> and direct photoinduced electron transfer from the carotenoid  $S_1$  level. Although the contributions of the two pathways leading to  $C^{+}-C_{60}^{-}$  could not be separated, the overall quantum yield for the formation of  $C^{+}-C_{60}^{-}$  is unity. These results illustrate one way that primary photochemistry with a high yield can occur upon excitation of a carotenoid pigment, and keep the door open to the suggestion that carotenoids can serve as blue light photoreceptors (Quiñones et al. 1996).

#### (c) Mimicry of the regulation of photosynthesis: non-photochemical quenching

Regarding the role of carotenoid pigments in nonphotochemical quenching of photosynthesis mentioned in § 1, it is interesting to compare structures 1 and 4. Both demonstrate a high level of antenna function as measured by energy-transfer efficiencies of 70 to 80%, which are similar to those observed in many natural systems. Regarding the tetrapyrrole singlet energies, the S<sub>1</sub> level of structure 1 is slightly lower than that of structure 4. Although the carotenoid moieties in structures 1 and 4 are different and their S<sub>1</sub> levels are not known, there is no evidence from the observed absorption into S<sub>2</sub> that there are significant differences in their singlet energy levels. Interestingly, in structure 4 the tetrapyrrole fluorescence is quenched (by the attached carotenoid) ca. 10-fold over that of structure 1. This is consistent with a simple description of the tetrapyrrole energetics in that energytransfer quenching from the tetrapyrrole  $S_1$  to the carotenoid would be more likely in structure 4 because the tetrapyrrole  $S_1$  level is above that of the tetrapyrrole of structure 1, and is therefore more likely to be above that of the carotenoid  $S_1$ . However, this interpretation is inconsistent with the carotenoid  $S_1$  state acting as an energy donor in structure 4 and not in structure 1. If tetrapyrrole to carotenoid energy transfer in structure 4 is thermodynamically downhill, then carotenoid to tetrapyrrole energy transfer must be uphill and is more uphill in structure 4 than in structure 1. Although we do not have an explanation for the large quenching effect and enigmatic energy-transfer behaviour, structures 1 and 4 do illustrate that subtle changes in parameters such as the coupling between the pigments, their redox levels and their spectroscopic energies can modulate and direct the energy flow between carotenoids and tetrapyrroles.

What is the role of energy or electron transfer in the quenching of tetrapyrrole fluorescence by carotenoids? In other model studies, the redox levels of a porphyrin-carotenoid dyad have been shown to influence the quenching mechanism to the extent that electron transfer from the carotenoid to the excited porphyrin was shown to occur (Hermant *et al.* 1993). However, in a series of carotenoid–porphyrin dyads in which the number of conjugated carbon–carbon double bonds in the carotenoid moiety was systematically increased from 7 to 11, quenching could not be assigned uniquely to either electron- or energy-transfer processes (Cardoso *et al.* 1996). Unfortunately, these model studies have not produced a definitive answer to the electron–lenergy-transfer question.

#### (d) Mechanistic considerations of energy transfer

Regarding the role of carotenoids as antenna pigments, energy transfer from them to chlorophylls can be brought about by a variety of interactions. If the distance between the chromophores is larger than their transition dipole lengths, if the transition dipoles have sufficient strength and the correct orientation, and if the donor state has sufficient lifetime (usually expressed as fluorescence quantum yield), energy transfer may be described by the Förster mechanism (Förster 1948). As the chromophores are brought closer together, higher-order electrostatic terms must be included in the electronic coupling matrix elements. The inclusion of these terms can relax the need for strong, dipole-allowed transitions and relatively longlived states in the donor and can account for the carotenoid  $S_1$  and  $S_2$  levels acting as donor states (Krueger *et* al. 1998). If the chromophores are brought into van der Waals contact so that there is orbital overlap between the donor and the acceptor, the Dexter electron-exchange mechanism becomes important.

The Dexter mechanism does not depend on dipole or mutipole strengths and is the accepted mechanism for triplet-triplet energy transfer. How fast can Dextermediated energy transfer be and to what extent do electron exchange-based electronic coupling matrix elements contribute to singlet energy transfer in these systems? In dyads 1 and 2 and several other carotenoid-containing

Phil. Trans. R. Soc. Lond. B (2002)

multichromophoric molecules, energy transfer from the triplet tetrapyrrole to populate the carotenoid triplet state is remarkably fast. Due to kinetic constraints inherent to these dyads, the actual rate cannot be determined (Macpherson *et al.* 2002). In these model systems, the rise time of the carotenoid triplet energy acceptor is the same as the singlet lifetime of the tetrapyrrole triplet energy donor. This indicates that intersystem crossing in the donor is the rate-limiting step in the flow of energy from the singlet of the donor to the triplet of the acceptor. In other words, the true rise time of the triplet carotenoid is much faster than intersystem crossing in the tetrapyrrole, so that the carotenoid triplet is observed to rise with the singlet lifetime of the tetrapyrrole.

The final answer to the second part of the question awaits detailed quantum chemical calculations. In the meantime, it has been addressed experimentally in one study of three isomeric carotenoporphyrins in which singlet– singlet energy-transfer efficiency (albeit at much lower efficiency than observed in dyads 1 and 2) and triplet– triplet energy-transfer rates were found to depend in the same way on the electronic structure of the interchromophore linkage group (Gust *et al.* 1992). Because the triplet energy-transfer process is mediated by the linkage group via the Dexter mechanism, the simplest interpretation is that in those dyads the singlet energy transfer is also electron-exchange mediated.

#### 4. EVOLUTION OF CAROTENOID ANTENNA/PHOTOPROTECTIVE FUNCTION IN PHOTOSYNTHESIS

One may assume that carotenoids were selected by the evolving photosynthetic apparatus because of their unique photophysical properties. Three properties can be identified that stand out in this regard: (i) a highly allowed absorption band in the 450 to 500 nm range, where the solar flux is high for efficient light harvesting; (ii) an  $S_1$ energy level that is highly forbidden (vide supra); and (iii) a lowest-excited triplet state that is well below the energy of singlet oxygen ( ${}^{1}\Delta g$ , 0.98 eV) so that equation (2.5) is irreversible. Notwithstanding the fact that the highly allowed absorption band near the solar irradiance maximum accounts for between 25 and 50% of the sunlight that drives photosynthesis on the earth, the crucial role of carotenoids in photosynthesis today is undoubtedly to protect photosynthetic organisms from the deleterious effects of singlet oxygen.

It is interesting to speculate about the evolutionary origin of photoprotection and the link between photoprotection and the highly forbidden carotenoid  $S_1$  level (Moore *et al.* 1990, 1994). From the earliest photosynthetic organisms until the evolution of oxygen production (2.5–*ca.* 3.5 billion years ago), atmospheric oxygen levels were extremely low; there would have been no need for protection from singlet oxygen under these conditions. However, it is almost certain that photosynthetic organisms evolving during this time used carotenoids as light-harvesting pigments. In order to do so, chlorophyll and carotenoid binding proteins evolved in which singlet energy transfer from carotenoids to chlorophylls was efficient. Because of the forbidden nature of the carotenoid  $S_1$  level, efficient energy transfer required extensive electronic coupling between the pigments. In fact, it appears that efficient energy transfer required electronic coupling sufficient to allow energy transfer from  $S_2$  with a rate constant of *ca*.  $10^{13}$  s<sup>-1</sup>. In order to achieve this coupling, the pigments had to be essentially in contact with one another. This, as it turns out, is exactly the requirement for triplet energy transfer by the Dexter mechanism. Thus, even before it was needed to suppress singlet oxygen sensitization, triplet-triplet energy transfer as shown in equation (2.4) was fast. In other words, because of the forbidden nature of  $S_1$  (and the short lifetime of  $S_2$ ), the evolutionary pressure to acquire efficient singlet energy transfer drove the system into a coupled state in which Dexter-based triplet energy transfer is facile.

#### 5. MIMICKING CHARGE SEPARATION IN PHOTOSYNTHESIS: ARTIFICIAL REACTION CENTRES

The conversion of solar energy to chemical potential in photosynthetic reaction centres begins with photoinduced electron transfer. A prototype reaction centre must contain pigments capable of absorbing visible light and either reducing an electron acceptor (A) or oxidizing an electron donor (D). In a dyad in which the pigment acts as the donor, excitation yields <sup>1</sup>D–A, which undergoes photoinduced electron transfer to give D'+–A<sup>--</sup>. We begin our discussion of artificial reaction centres with some 'unnatural' ones that we designed and synthesized in the mid 1990s.

#### (a) Fullerene-containing artificial reaction centres

The design of artificial reaction centres has not been restricted to components found in natural photosynthesis. Other pigments and redox-active compounds can give interesting new properties to the synthetic assemblies and expand their usefulness as potential solar energy conversion or molecular electronic devices. C<sub>60</sub>, with its extended absorption throughout the visible spectrum, is an example of such a compound. Studies of fullerenes as electron acceptors in photoinduced electron-transfer reactions in our laboratories have elucidated a number of characteristics of fullerenes that make them especially well suited to be components of artificial photosynthetic devices (Liddell et al. 1994, 1997; Imahori et al. 1995; Kuciauskas et al. 1996, 1998a,b, 1999; Gust et al. 1997, 1998, 2000; Carbonera et al. 1998). Some of these characteristics are listed below.

- (i) Fullerene derivatives are electron acceptors with first reduction potentials similar to those of benzoquinone.
- (ii) They can reversibly accept multiple electrons in cyclic voltammetric experiments, and in principle can act as electron accumulators.
- (iii) As chromophores, fullerenes absorb visible light, and can act as excited-state electron acceptors and singlet energy donors or acceptors.
- (iv) Fullerene-based electron donor-acceptor systems feature relatively rapid photoinduced charge separation and relatively slow charge recombination.
- (v) Donor-acceptor systems containing fullerenes can demonstrate photoinduced electron transfer in many



Figure 2. Energy level diagram showing relevant high-energy states and energy and electron transfer decay pathways for dyad, structure 9.

solvents, and in rigid glasses and plastics down to 8 K.

(vi) Donor-acceptor systems containing fullerenes can demonstrate charge recombination to yield triplet excited states, rather than the molecular ground state.

The last three characteristics are not in general shared by molecular systems that employ quinones as the primary electron acceptor. They are, however, characteristic of natural photosynthetic reaction centres. These three facets of fullerene behaviour are due to the small reorganization energies and low sensitivity to solvent stabilization of anions characteristic of  $C_{60}$ .

## (b) Simple artificial reaction centres: porphyrin fullerene dyads

Recent advances in synthetic methodology have permitted the functionalization of fullerenes and the possibility of linking them to other moieties. We reported the first example of a porphyrin–fullerene dyad (see Appendix A, structure 8) in 1994 (Liddell *et al.* 1994). Since then, many examples of dyads consisting of fullerenes linked to porphyrins or phthalocyanines have been prepared (Kuciauskas *et al.* 1996, 1998*a*). A number of dyads feature pyrrolidine-functionalized fullerenes that have been prepared by the widely used Prato reaction (Kuciauskas *et al.* 1996; Gust *et al.* 2000).

The general photophysical behaviour of such dyads can be illustrated by the energy-level diagram of dyad 9 (figure 2), which shows the relevant states and interconversion pathways (Kuciauskas *et al.* 1996). The energies of the excited singlet states in figure 2 were estimated from absorption and emission spectra. The fullerene absorbs throughout the visible spectrum with a threshold at *ca*. 710 nm; its extinction coefficients in the visible region are much lower than those of the porphyrin. The absorption spectra are essentially linear combinations of those of the component chromophores, which means that the spectra show no evidence of strong electronic interaction of the chromophores. Cyclic voltammetric studies on structure 9 (see Appendix A) and model compounds allow estimation of the energy of P<sup>++</sup> $-C_{60}^{--}$  as 1.58 eV above the ground state in polar solvents.

Analysis of time-resolved fluorescence and absorption data for the dyads and appropriate model compounds allows calculation of rate constants for various photochemical processes. In toluene,  ${}^{1}P-C_{60}$  of structure 9 decays by singlet-singlet energy transfer (step 1 in figure 2;  $k_1 = 4.5 \times 10^{10} \text{ s}^{-1}$ ) to yield P-<sup>1</sup>C<sub>60</sub>, which undergoes intersystem crossing to the triplet state. No photoinduced electron transfer is observed. In polar solvents such as benzonitrile, <sup>1</sup>P-C<sub>60</sub> decays by a combination of photoinduced electron transfer by step 2 ( $k_2 = 1.8 \times 10^{11} \text{ s}^{-1}$ ) to give  $P^{\cdot+} - C_{60}^{\cdot-}$  and singlet energy transfer by step 1  $(k_1 = 7.1 \times 10^{10} \text{ s}^{-1})$ . The P<sup>-1</sup>C<sub>60</sub> formed in this manner or by direct absorption of light decays by photoinduced electron transfer step 3 to also yield the  $P^{\cdot+}-C_{60}^{\cdot-}$  state  $(k_3 = 1.3 \times 10^{10} \text{ s}^{-1})$ . The charge-separated state is easily identified using transient absorption spectroscopy by the absorption of the porphyrin radical cation in the 600-700 nm region and the fullerene radical anion at ca. 1000 nm. The overall quantum yield of charge separation via both pathways is 0.99. The  $P^{\cdot+}-C_{60}^{\cdot-}$  state decays by charge recombination to the ground state with  $k_4 = 3.4 \times 10^9 \text{ s}^{-1}$ .

In general, the studies of photoinduced electron transfer in porphyrin-fullerene dyads have shown that chargeseparated states are produced in high yield, and have long lifetimes relative to those of similar porphyrin-quinone systems (Liddell et al. 1994; Kuciauskas et al. 1996). For example, a comparison of the photochemistry of a porphyrinfullerene dyad with that of a structurally related porphyrinquinone dyad shows that in tetrahydrofuran solution, photoinduced electron transfer in the C<sub>60</sub>-dyad occurs with a rate constant ca. six times larger than that in the quinone-based dyad, even though the latter one has a driving force that is larger by 0.28 eV (Imahori et al. 1996). But the most remarkable difference is in the charge recombination reaction. In the quinone-based dyad, this reaction occurs with a rate constant of ca. 25 times greater than in the C<sub>60</sub>-based dyad. The difference in rate constants has been ascribed to a significantly smaller reorganization energy for the large, diffuse fullerene radical anion than for the quinone radical anion, where the negative charge is mostly concentrated in the region of the oxygen atoms (Imahori et al. 1996; Guldi 2000; Kuciauskas et al. 2000). Because of the small  $\lambda$ , the maximum of the Marcus curve occurs at a numerically smaller  $-\Delta G^{\circ}$ , shifting the more exergonic recombination reaction well into the Marcus inverted region and slowing recombination.

#### (c) Triad-based artificial reaction centres

Dyad systems can generate charge separation with a quantum yield of essentially unity while preserving a large part of the photon energy as intramolecular redox poten-

Phil. Trans. R. Soc. Lond. B (2002)

tial. However, rapid charge recombination to the ground state, which usually occurs on the subnanosecond timescale, releases the stored energy as heat; it is difficult to devise intermolecular energy-conserving processes that can compete with this degradation.

In the early 1980s, we designed an artificial photosynthetic reaction centre that overcomes this problem by using a multistep electron-transfer strategy such as that found in natural reaction centres (Moore *et al.* 1984). Recently, we have designed a series of triad artificial reaction centres based on the dyads described above. These constructs illustrate that careful consideration of the thermodynamic and electronic factors controlling electron transfer can result in artificial reaction centres that generate charge-separated states that rival those from natural reaction centres in quantum yield, energetics and lifetime.

#### (d) Photochemistry of fullerene-based triad artificial reaction centres

As described above, photoinduced electron-transfer studies in porphyrin–fullerene dyads demonstrated that charge-separated states can be produced in high yield and that they have long lifetimes relative to quinone-containing dyads. These properties of fullerene systems indicate that they might be ideal components of more complex systems, where slow charge recombination would favour evolution of a charge-separated state into a longer-lived species via multistep electron transfers similar to the processes observed in the reaction centres of photosynthetic organisms.

In 1997, we reported a next step in the evolution of such molecules in the form of carotene (C) porphyrin (P) fullerene (C<sub>60</sub>) triad 10 (Gust et al. 1997; Liddell et al. 1997; Kuciauskas et al. 2000). Other triads based on C<sub>60</sub> have subsequently been reported (Luo et al. 2000). The photochemistry of this triad may be discussed with reference to figure 3, which shows the relevant high-energy states and decay pathways. Time-resolved spectroscopic studies have shown that at ambient temperatures in 2methyltetrahydrofuran solution, excitation of the porphyrin moiety yields  $C^{-1}P-C_{60}$ , which decays in 10 ps by photoinduced electron transfer to the fullerene to generate  $C-P^{+}-C_{60}^{-}$  (step 3 in figure 3;  $k_3 = 1.0 \times 10^{11} \text{ s}^{-1}$ ). Also, the fullerene excited singlet state accepts an electron from the porphyrin to yield  $C-P^{+}-C_{60}^{-}$   $(k_2 = 3.1 \times 10^{10} \text{ s}^{-1}).$ The overall quantum yield of  $C-P^{+}-C_{60}^{-}$  by these two pathways is essentially unity. Secondary electron transfer from the carotenoid (step 4) competes with charge recombination by step 7 to give the final  $C^{+}-P-C_{60}^{-}$  chargeseparated state. The rise time of this state is 80 ps and the overall yield is ca. 0.14, as determined by the comparative method. The C<sup>·+</sup>-P-C<sub>60</sub><sup>·-</sup> state decays in 170 ns exclusively by charge recombination to produce the carotenoid triplet state (step 9,  $\Phi_t = 0.13$ ). Decay of <sup>3</sup>C–P–C<sub>60</sub> occurs in 4.9 µs  $(k_{15} = 2.0 \times 10^5 \text{ s}^{-1})$ . Even in a glass at 77 K,  $C^{\cdot +}\!-\!P\!-\!C_{60}^{\cdot -}$  is formed from  $C\!-^1\!P\!-\!C_{60}$  with a quantum yield of *ca*. 0.10 and again decays to give  ${}^{3}C-P-C_{60}$ . The fullerene triplet, formed from  $C-P-^{1}C_{60}$  (step 10) through normal intersystem crossing, is also observed at 77 K.

Importantly, charge recombination to form a triplet species in triad 10 provides a spectroscopic label to follow the flow of triplet energy and the spin dynamics in model photosynthetic reaction centres. The spin-polarized EPR



Figure 3. Energy-level diagram showing relevant high-energy states and energy and electron-transfer decay pathways for triad 10. Triads 11 and 12 differ in the energies of certain charge-separated states as fully described in § 5d. The singlet radical-pair state  ${}^{1}(C^{+}-P-C_{60}^{-})$  is shown along with the three triplet radical-pair states  ${}^{3}(C^{+}-P-C_{60}^{-})$ . The splittings of the triplet radical-pair state energies are not to scale.

signal of the final charge-separated state, C<sup>+</sup>-P-C<sub>60</sub><sup>-</sup>, was observed and simulated (Hasharoni et al. 1990). The fact that both electron-transfer steps in structure 10 occur even in a glassy matrix at 77 K has made it possible to observe some unusual and biologically relevant triplettriplet energy-transfer phenomena in the triads (Gust et al. 1997). In triad 10, triplet energy is transferred from the fullerene moiety of C-P- ${}^{3}C_{60}$  (generated by intersystem crossing) to the carotenoid polyene (step 11 followed by step 12), yielding <sup>3</sup>C-P-C<sub>60</sub>. The transfer has been studied both in toluene at ambient temperatures and in 2methyltetrahydrofuran at lower temperatures. The energy transfer is an activated process (step 11), with  $E_{\rm a} = 0.17$  eV. This is consistent with transfer by a triplet energy-transfer relay, whereby energy first migrates from  $C-P-{}^{3}C_{60}$  to the porphyrin (step 11), yielding  $C-{}^{3}P-C_{60}$ in a slow, thermally activated step. Rapid energy transfer from the porphyrin triplet to the carotenoid (step 12) gives the final state. Triplet relays of this general kind have been observed in photosynthetic reaction centres and are part of the system that protects the organism from damage by singlet oxygen, whose production is sensitized by chlorophyll triplet states (Gust et al. 1993a; Krasnovsky et al. 1993).

As explained above, the  $C^{+}-P-C_{60}^{--}$  state decays at 77 K to give the carotenoid triplet. EPR investigation of the signal from this triplet state shows that the spin polarization of  ${}^{3}C-P-C_{60}$  is characteristic of a triplet formed by charge recombination of a singlet-derived radical pair. The kinetics of the decay of  ${}^{3}C-P-C_{60}$  to the ground state were also determined and the decay constants for the three triplet sublevels were measured. Similar spin states and dynamics, which are the signature of radical-pair recombination reactions, have been observed in photosynthetic reaction centres but are unique to structure 10 (and close relatives) in the area of photosynthetic model systems (Carbonera *et al.* 1998). To aid our interpretation of the EPR spectra of the triads, we carried out several other studies on the EPR properties of carotene–porphyrin dyads (Carbonera *et al.* 1997*a,b*). The generation in triad 10 of a long-lived charge-separated state by photoinduced electron transfer, the low-temperature electron-transfer behaviour and the formation of a triplet state by charge recombination are phenomena heretofore observed mostly in photosynthetic reaction centres, rather than in model systems.

Although triad 10 mimics some of the important features of photosynthetic electron transfer, the quantum yield of the final state is rather low (0.14). The initial  $C-P^{\cdot+}-C_{60}^{\cdot-}$  state is formed with a quantum yield approaching unity, but charge recombination to the ground state is more rapid than electron transfer from the carotenoid to yield the final state. In order to overcome this problem, triad 11 was designed. It differs from structure 10 mainly in the nature of the carotenoid-to-porphyrin linkage (Kuciauskas et al. 2000). A major effect of this structural change is to increase the electron-donating ability of the carotenoid by 0.18 eV, although it also affects the electronic coupling. In addition, the change raises the energy of  $C-P^{\cdot+}-C_{60}^{\cdot-}$  by 0.05 eV relative to structure 10. In accord with Marcus theory, these energetic changes increase the thermodynamic driving force and rate constant for formation of  $C^{\cdot+}-P-C_{60}^{\cdot-}$  from  $C-P^{\cdot+}-C_{60}^{\cdot-}$  (step 4). They also slightly increase the driving force for charge recombination of  $C-P^{\cdot+}-C_{60}^{\cdot-}$  (step 7). However, as this reaction occurs in the Marcus inverted region, the rate of recombination is slowed. The overall result is that the yield of  $C^{+}-P-C_{60}^{-}$  is 0.88 in structure 11 (see Appendix A).

In order to retard the rate of charge recombination of  $C-P^{\cdot+}-C_{60}^{\cdot-}$  even more, and thereby increase the yield of  $C^{\cdot+}-P-C_{60}^{\cdot-}$ , triad 12 was synthesized (Bahr *et al.* 2000). In structure 12 (see Appendix A), the octaalkylporphyrin serving as the primary donor was replaced with a tetra-

phenyl-based porphyrin that increases the energy of P<sup>·+</sup> by *ca.* 200 mV. Therefore, in structure 12, the charge recombination process (step 7) from  $C-P^{\cdot+}-C_{60}^{\cdot-}$  is even further in the Marcus inverted region and thereby slower, allowing the forward reaction yielding the final charge-separated species  $C^{\cdot+}-P-C_{60}^{\cdot-}$  to better compete with it. Moreover, this change increases the driving force for hole transfer from the porphyrin radical cation to the carotenoid (step 4), which is in the Marcus normal region and therefore is accelerated. The combination of a faster forward reaction combined with a slower recombination reaction from the intermediate  $C-P^{\cdot+}-C_{60}^{\cdot-}$  results in a measured quantum yield for the formation of  $C^{\cdot+}-P-C_{60}^{\cdot-}$  of *ca.* 100% in triad 12.

The availability of structures 10, 11 and model compounds has allowed the investigation of the effects of temperature and solvent on the various electron-transfer steps. For example, electron-transfer dynamics on a very short time-scale as a function of solvent were studied in triad 10 and in a related porphyrin-fullerene dyad using subpicosecond transient absorption techniques (Kuciauskas et al. 1998a). After porphyrin photoexcitation of the dyad, electron transfer to the fullerene was observed, yielding  $P^{+}-C_{60}^{-}$ . In toluene, this state recombines to give the fullerene first-excited singlet state, leading to fullerene singlet sensitization by an unusual two-step electron-transfer mechanism. This is somewhat analogous to the delayed fluorescence observed in natural reaction centres when secondary electron transfer is blocked. In polar solvents, structure 10 undergoes the electron-transfer process mentioned previously to produce  $C^{\cdot+}-P-C_{60}^{\cdot-}$ . The solvation dynamics of this state were investigated by monitoring carotenoid radical cation absorption band bathochromic spectral shifts. Picosecond solvation processes were observed in six solvents, with solvation lifetimes ranging from 0.4 to 35 ps. In all solvents, electron-transfer rates were slower than solvation rates, but the difference is not large. It was also observed that when solvation of the carotenoid radical cation is faster, electron transfer is more rapid.

An extensive comparison of the effects of temperature and solvent on triads 10 and 11 allowed several generalizations (Kuciauskas et al. 2000). Rate constants for photoinduced charge separation are much larger than those for charge recombination. In addition, the rate constants of the various electron-transfer reactions in structure 11 depend only weakly on temperature and solvent. The yield of  $C^{\cdot+}-P-C_{60}^{\cdot-}$  in structure 11 is essentially independent of temperature down to 8 K. The rate constants for photoinduced electron transfer and for charge recombination of  $C-P^{\cdot+}-C_{60}^{\cdot-}$  do not change drastically when the solvent changes from a fluid solution to a rigid glass. Charge recombination of C<sup>++</sup>-P-C<sup>-</sup><sub>60</sub> also has some unusual features. In non-polar solvents or frozen glasses down to 8 K, charge recombination in structures 10 and 11 yields only the carotenoid triplet state. However, in polar solvents, charge recombination in structure 11 yields only the singlet ground state (step 8). The rate of charge recombination of  $C^{+}-P-C_{60}^{-}$  in both structures 10 and 11 is temperaturedependent down to ca. 170 K, and temperature independent thereafter. The temperature-independent region is attributed to nuclear tunnelling.

#### (e) An artificial antenna-reaction centre complex: a molecular hexad

Antenna function in photosynthesis has been mimicked in artificial systems of covalently linked arrays. For example, metalated and free base porphyrins have been linked with alkvne units to form dimers, trimers, tetramers, pentamers and more complex supermolecules that absorb light and rapidly and efficiently transfer singlet excitation to energy sinks such as a free base porphyrin moiety (Seth et al. 1994, 1996; Bothner-By et al. 1996; Hsiao et al. 1996; Wagner et al. 1996a; Strachan et al. 1997; Lammi et al. 2000). Molecules of this type have been designed to act as photonic 'wires' and gates (Wagner & Lindsey 1994; Wagner et al. 1996b). Other porphyrin arrays, some of them including more than one hundred porphyrin units, with demonstrated or potential antenna function have been reported (Van Patten et al. 1998; Cho et al. 2000).

Because of the progress made in the mimicry of both photosynthetic antennas and reaction centres, a logical next step would be to link an artificial light-harvesting array with a synthetic reaction centre to produce a functional energy-conversion assembly. Indeed, a model photosynthetic antenna consisting of four covalently linked zinc tetraarylporphyrins,  $(P_{ZnP})_3$ - $P_{ZnC}$ , covalently joined to a free base porphyrin–fullerene artificial photosynthetic reaction centre, P–C<sub>60</sub>, to form  $(P_{ZnP})_3$ - $P_{ZnC}$ -P–C<sub>60</sub>, hexad 13, has been reported (Kuciauskas *et al.* 1999).

In order to evaluate the performance of hexad 13, rate constants were extracted from time-resolved spectroscopic data. The time-resolved fluorescence and absorption data yield a time constant of 700 ps that is associated with the zinc porphyrin moieties. The shortening of the zinc porphyrin singlet lifetime from 2.4 ns in tetrad  $(P_{ZnP})_3-P_{ZnC}$  to give a 700 ps component in a model pentad,  $(P_{ZnP})_3-P_{ZnC}-P$  and structure 13 (see Appendix A) is a result of singlet–singlet energy transfer from the zinc porphyrins to the free base porphyrin. In structure 13, fluorescence from the free base porphyrin is quenched from its usual lifetime of *ca*. 10 ns to such a degree that fluorescence is no longer observable. The picosecond transient absorbance results yield additional time constants of 3 ps, 12 ps and 1.3 ns.

The rate constant for photoinduced electron transfer and the charge recombination rate constant are directly observed experimentally. The reciprocal of the 3 ps timeconstant detected in the transient absorption experiments is the rate constant for photoinduced electron transfer. This assignment is verified by the results for a model P–  $C_{60}$  dyad, where the same value was obtained for the rate constant for photoinduced electron transfer. The charge recombination of  $(P_{ZnP})_3-P_{ZnC}-P^{++}-C_{60}^{--}$  is associated with the 1.3 ns decay component observed in transient absorption, as demonstrated by the spectral stamp of the fullerene radical anion with absorption in the 1000 nm region. This lifetime is within a factor of 2.5 of the lifetime observed for the P<sup>++</sup>-C\_{60}^{--} in a model dyad (480 ps).

The values for the singlet energy-transfer rate constants between zinc porphyrins and between the central zinc porphyrin and the free base porphyrin do not appear directly in the experimental decays. A kinetic model was developed to interpret the spectroscopic data and a solution of a set of rate differential equations using the experimentally measured lifetimes of 12 ps and 700 ps as constants yields values of 50 ps for the rate constant for energy transfer between metal porphyrins and 240 ps for the rate constant for energy transfer between the zinc and free base porphyrins. The value thus obtained for the rate constant for energy transfer between metal porphyrins is in excellent agreement with the results of Hsiao and coworkers who measured a rate constant of 53 ps for singlet energy transfer between two zinc porphyrins in a linear trimeric array of zinc porphyrins with diphenylethyne linkages very similar to those in structure 13 (Hsiao *et al.* 1996).

The singlet–singlet energy transfer between the central zinc porphyrin in the antenna of structure 13 and the free base porphyrin is slow (240 ps) compared with that between the zinc porphyrins (50 ps). Thus, the energy transfer between the central zinc porphyrin and the free base porphyrin creates a bottleneck that limits the quantum yield of the final charge-separated state  $(P_{ZnP})_3$ – $P_{ZnC}$ – $P^{-+}-C_{60}^{--}$ .

The quantum yield of  $(P_{ZnP})_3$ - $P_{ZnC}$ - $P^{++}$ - $C_{60}^{--}$  is wavelength dependent. Based on light absorbed by the free base porphyrin, the yield is unity, due to the very large rate constant for photoinduced electron transfer. The quantum yield based on excitation of the zinc porphyrins in the antennas is 0.70. In this process, the light-gathering power of the system is increased considerably at many wavelengths, as four zinc porphyrin moieties feed excitation energy to the reaction centre. In hexad 14 structural modifications to increase the electronic coupling between  $P_{ZnC}$ -P and the thermodynamic driving force for selected electron-transfer steps were carried out to improve the performance of this device in terms of lifetime and quantum yield of the final charge-separated state (Kodis *et al.* 2002).

Hexad 14 differs from structure 13 in several important ways. By changing the primary donor porphyrin from the free base octaalkylporphyrin of structure 13 to a free base tetraphenyl-type porphyrin, the energy of the corresponding radical cation is increased by ca. 200 mV. This provides an additional thermodynamic driving force for hole transfer from the primary donor to the Zn porphyrins acting as the antenna. Evidence for this is provided by the appearance in the transient spectra of the Zn porphyrin radical cation in 380 ps and greatly increased lifetime of the charge-separated state in structure 14 (see Appendix A) of up to 24  $\mu$ s (solvent dependent) versus 1.3 ns in structure 13. As mentioned above, this change of several orders of magnitude in lifetime is characteristic of multicomponent systems in which a secondary electron-transfer step results in formation of a neutral chromophore separating electron-hole pairs. Increasing the energy of the porphyrin radical cation also lowers the driving force for photoinduced electron transfer from the primary donor to the  $C_{60}$  and, indeed, charge separation is slower, 25 ps in structure 14 versus 3 ps in structure 13, because this step is in the Marcus normal region. Twenty-five picoseconds is still sufficiently fast so that the yield of the photoinduced electron-transfer process, competing against fluorescence and intersystem crossing, is not measurably changed from 100%.

A second consequence of the change to a tetraphenylbased porphyrin as the primary donor in hexad 14 is that there are no methyl groups at the beta positions. This

Phil. Trans. R. Soc. Lond. B (2002)

allows a more coplanar conformation between the central Zn porphyrin of the antenna and the primary donor, which increases the electronic coupling and results in much faster singlet energy transfer. The time for this step, which was 240 ps in structure 13 and limited the overall quantum yield of energy transfer, is reduced to 30 ps in structure 14, giving an energy-transfer yield of unity. Therefore, the quantum yield of photoinduced electron transfer from the primary donor to the  $C_{60}$  in structure 14 is essentially 100%, regardless of which porphyrin is initially excited.

In addition to the changes noted above in thermodynamics and conformation that are brought about by changing the primary donor from an octaalkylporphyrin to a tetraphenyl-based porphyrin, there are electronic effects arising from changes in the molecular orbital nodal pattern of the two porphyrins. Specifically relevant to hole transfer from the primary donor to the Zn porphyrin of the antenna, the HOMO of the octaalkylporphyrin has a node at the meso positions whereas the HOMO of the tetraphenyl-based porphyrin has amplitude at the meso positions. Because the meso position is the attachment point of the Zn porphyrin array to the primary donor porphyrin, and because the HOMOs are involved in hole transfer, this aspect of the electronic-coupling term should be larger in structure 14 than in structure 13. The result of improved thermodynamics for hole transfer and increased electronic coupling due to conformation and orbital effects is hole transfer from the radical cation of the primary donor to the antenna in 380 ps. This is sufficiently fast for this step to have a yield of close to unity so that the overall quantum yield of long-lived charge-separated species in hexad 14 is near 100%.

#### 6. ARTIFICIAL PHOTOSYNTHETIC MEMBRANES

Molecular triad 15, which consists of a porphyrin chromophore (P) bound covalently to a naphthoquinone derivative (NQ) and a carotenoid electron donor (C), was designed both to undergo multistep electron transfer and to organize itself in a bilayer lipid membrane. These two characteristics are discussed below.

Excitation of the porphyrin moiety of structure 15 (see Appendix A) (the photophysics of structure 15 is not strongly dependent on the ionization state of the carboxylate group) leads to C-1P-NQ, which decays by photoinduced electron transfer to give C-P'+-NQ'- with a quantum yield near unity. Competing with charge recombination, a second electron transfer from the carotenoid to the porphyrin radical cation occurs, yielding a C<sup>+</sup>-P-NQ<sup>--</sup> charge-separated state. In this state, the radicals are separated by the neutral porphyrin moiety and therefore charge recombination is slower by several orders of magnitude than that of a comparable dyad. We have used this strategy in a variety of other reaction-centre mimics (vide supra), some of which rival the natural systems in terms of quantum yield, fraction of energy stored and lifetime for charge separation (Gust & Moore 1991; Gust et al. 1993b).

It has been demonstrated that, under certain conditions, triad 15 inserts unidirectionally into a liposomal phospholipid bilayer membrane (Steinberg-Yfrach *et al.* 1997). How can this observation be rationalized? Triad 15 is highly amphiphilic due to the hydrophobic carotenoid pig-



Figure 4. Steps 1–7 illustrate a minimum set of proposed elementary processes involved in the redox loop-based proton translocation across a liposomal bilayer membrane.

ment distal to the carboxylate group that is attached to the naphthoquinone. When a small amount of structure 15 (dissolved in a suitable solvent such as tetrahydrofuran) is added to an aqueous solution of liposomes, the amphiphilic molecules associate with the membrane. It is probable that the most stable association features the hydrophobic carotenoid moiety in the low-dielectric interior of the bilayer and the hydrophilic carboxylate group at or near the aqueous interface. Because structure 15 is added from the bulk aqueous phase on the outside of the liposomal bilayer, the expected initial arrangement would be vectorial, with the majority of triads having their carboxylate-bearing naphthoquinones near the bulk aqueous phase as shown schematically in figure 4. The energetic cost of transporting the carboxylate across the bilayer would slow, but not prevent, flip-flop diffusion that would ultimately randomize the orientation.

#### (a) Artificial proton pump

The availability of artificial reaction centres that selfassemble vectorially into bilayer lipid membranes makes it possible to design a proton pump that will generate a transmembrane gradient in proton electrochemical potential, the next step in converting solar energy to a biologically useful form (Steinberg-Yfrach *et al.* 1997). In order to couple the vectorial redox potential in the species  $C^{+-}$ P–NQ<sup>--</sup> to proton translocation, a lipophilic shuttle quinone, Qs, was incorporated into the liposomal bilayer where it functions as a redox loop to shuttle protons across the bilayer. A proposed mechanism for this proton translocation is shown diagrammatically in figure 4.

To begin with, excitation of structure 15 generates the  $C^{+}-P-NQ^{-}$  charge-separated state via the multistep electron-transfer process described above. The shuttle quinone Qs is more easily reduced than the naphthoqui-

Phil. Trans. R. Soc. Lond. B (2002)

none of the triad and therefore accepts an electron from the charge-separated state to generate the Qs radical anion, which is basic enough to accept a proton from the nearby external aqueous phase. The resulting neutral semiguinone is lipid soluble and diffuses within the lipophilic part of the bilayer. When it encounters a carotenoid radical cation near the internal aqueous phase, it is readily oxidized, yielding a protonated quinone, QsH<sup>+</sup>. This strong acid releases a proton to the nearby interior aqueous volume, thereby lowering the pH inside the liposome and regenerating the components of the proton pump. In other words, the vectorial redox potential of C<sup>+</sup>-P-NQ<sup>+</sup> drives a redox loop in which the reduced (protonated) form of Qs carries protons inwards and the oxidized (unprotonated) form of Qs completes the loop by equilibrating across the membrane. The specific details of the proton-pumping photocycle may differ somewhat from this simple picture, but figure 4 describes the basic features of the system.

Proton import has been monitored by several methods. A pH-sensitive fluorescent dye, pyraninetrisulphonate, was trapped inside the liposomes, or 8-aminoacridine was used to measure  $\Delta pH$  directly, or 8-anilinonaphthalene-1sulfonic acid was used to measure the transmembrane electrical potential  $\Delta \psi$ . Illumination of the liposomes with light absorbed by the porphyrin moiety of structure 15 leads to a change in the fluorescence properties of these dyes that signals import of protons into the liposomes. A  $\Delta \mu H^+$  of ca. 4.4 kcal mole<sup>-1</sup> comprised of  $\Delta p H$  ca. 2.1 and  $\Delta \psi$  ca. 70 mV is attained after a few minutes of irradiation with less than 0.1 mW of red light. The pH and  $\Delta \psi$  gradients thus generated can be relaxed by addition of an ionophore such as FCCP. The photochemical import of protons is enhanced by the addition of potassium and valinomycin, which presumably reduce the uncompensated charge buildup  $(\Delta \psi)$  in the intraliposomal volume. Conversely, increasing the buffer concentration inside the liposomes decreases the magnitude of  $\Delta pH$  and results in the generation of a greater  $\Delta \psi$ . Thus, a photocyclic transmembrane proton pump has been assembled.

#### (b) ATP synthesis by the artificial membrane

Proton motive force is a basic form of biological energy that is used to drive most energy-requiring reactions in living organisms. Most importantly, it is used to power the synthesis of ATP. Having prepared a light-driven transmembrane proton-pumping system, we have used it to power ATP synthesis in a biomimetic fashion. In nature, ATP is synthesized by  $F_0F_1$ -ATP synthase, an enzymatic system that spans the lipid bilayer. Protons pass through the enzyme from one side of the membrane to the other in the direction of the pH gradient and the energy provided by relaxation of the electrochemical potential gradient is used to synthesize ATP from ADP and Pi.

Conditions for extraction of the  $CF_0F_1$ -ATP synthase from spinach chloroplast thylakoids and reconstitution of the functional enzyme into liposomes have been developed. Using this methodology, the enzyme has been reconstituted into liposomes containing the components of the light-driven proton pump discussed above (Steinberg-Yfrach *et al.* 1998).

The system is shown as a diagram in figure 5. The liposomes were formed from a 10 : 1 mixture of egg phosphat-



intraliposomal volume

Figure 5. Schematic diagram of  $CF_0$ - $F_1$  ATP synthase inserted into a liposomal membrane containing the components of the artificial reaction centre and redox loop-based proton pump. The enzyme is reconstituted into the membrane with the nucleotide binding sites in the external aqueous phase.

idylcholine and egg phosphatidic acid containing 20% cholesterol. The ATP synthase was inserted into the bilayer with the ATP-synthesizing portion of the enzyme extending out into the external aqueous solution. The constituents of the proton pump were incorporated as described above. Thioredoxin (10 µM) was added to activate the enzyme and ADP, Pi and an initial amount of ATP were added to the external phase. The aqueous phases were adjusted to a pH of 8 prior to illumination. The liposomes were illuminated for various periods of time with laser light at 633 nm, which was absorbed only by the triad. Illumination was terminated, and the ATP produced was measured using a calibrated luciferin/ luciferase assay, which is based on the luminescence of oxyluciferin. The amount of ATP produced during each period of illumination was determined; typical results are presented graphically in figure 6.

In order to establish that this increase was indeed due to ATP synthesis driven by light-induced proton motive force, several control experiments were performed. As shown in figure 6, no ATP was synthesized when 1  $\mu$ M FCCP, the proton ionophore mentioned above, was added to the system, making the membrane permeable to protons. Addition of 2  $\mu$ M tentoxin, which is a specific inhibitor of CF<sub>0</sub>F<sub>1</sub>–ATP synthase, halted ATP synthesis, as did omission of the shuttle quinone Qs or ADP. Likewise, omission of Pi, triad 15 or ATP synthase abolished the effect.

At low levels of illumination, where ATP synthesis was limited by light absorption, it was found that one ATP molecule was synthesized for every 14 photons incident on the sample. As *ca.* 50% of the light was absorbed by the scattering liposome solutions, this corresponds to a quantum yield of *ca.* 0.15. Assuming that four protons must be transported out of the liposome by ATP synthase per molecule of ATP produced, the yield of the proton pump must be *ca.* 0.6. It should be noted that the liposomes used for ATP synthesis were of a different size and lipid mix from those used in the proton translocation experiments discussed in the previous section (§ 6a), and that *ca.* 1000 triad 15 molecules could be incorporated into one proteoliposome. In the proton translocation



Figure 6. The synthesis of ATP as a function of illumination time at different ratios of [ATP]/[ADP] (triangles, [ATP] = [ADP] = 0.2 mM and [Pi] = 5 mM; filled circles, [ATP] = 0.2 mM, [ADP] = 0.02 mM and [Pi] = 5 mM. Also shown are results for control experiments showing that ATP was not synthesized (open circles, 1  $\mu$ M FCCP; filled triangles, 2  $\mu$ M tentoxin; diamonds, [Qs] = 0; open squares, [ADP] = 0.

experiments, only ca. 40 triad molecules were present in each liposome.

As illustrated in figure 6, the liposome system can synthesize ATP against a considerable ATP chemical potential. Under the conditions of 0.2 mM ATP, 0.02 mM ADP and 5 mM Pi shown in figure 6, ATP is synthesized against a chemical potential of *ca*. 12 kcal mol<sup>-1</sup>. Thus, the system is converting light energy to chemical potential, rather than simply catalyzing an exergonic reaction. Based on the ATP potential, the quantum yield and the energy of 633 nm photons, we estimate that *ca*. 4% of the absorbed light energy is conserved in the system. The limits of the system have not as yet been reached, nor has the system been optimized.

#### 7. CONCLUSION

Supramolecular structures that mimic the antenna, reaction centre and proton pumps of natural photosynthetic membranes can be designed and synthesized. Artificial reaction centres demonstrate that rapid charge separation and relatively slow charge recombination results in relatively long-lived charge-separated states that facilitate the use of the energy stored in these states in homogeneous solution, or even in interfacial reactions. The ability to carry out photoinduced electron transfer in media of low dielectric constant, in rigid media and/or at low temperatures indicates that fullerene-based systems would be good candidates for applications such as molecular scale (opto)electronics, sensors or bioenergetics where photochemistry would have to occur in selfassembled or Langmuir-Blodgett monolayers, in plastics or gels, in biological or artificial membranes, in micelles, etc. Large arrays of porphyrin-based antennas can be constructed in order to harvest light efficiently. If these arrays are coupled to reaction centres based on fullerenes such as in hexad 14, supermolecules can be assembled that can be effective transducers of light energy to potential energy in the form of charge-separated states. Furthermore, it is possible to mimic the basic features of energy conversion in bacterial photosynthetic membranes in a mostly artificial, 'bionic' construct that employs artificial reaction centres. Optimization of such systems could provide solar biological 'power packs' that might be used to drive the enzymatic synthesis of other high-energy or high-value compounds, or power natural or artificial nanoscale 'machines'. Both scientific and practical uses of such modules can be envisioned.

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#### **APPENDIX A**

Structures 1-15.



Structure 1. (Carotenopurpurin.)



Structure 2. (Purpurin.)



Structure 3. (Carotenoid of structure 1.)



Structure 4. (Carotenophthalocyanine.)





Structure 7.



Structure 8.





Structure 6. (Carotenoid of structure 2.)







Structure 10.



Structure 11.



Structure 12.



Structure 13.



Structure 14.



Structure 15.

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#### Discussion

J. Barber (Department of Biological Sciences, Imperial College, London, UK). Why have you used  $C_{60}$  as an electron acceptor?

T. A. Moore. Other than being beautiful molecules, they can also act as multiple electron acceptors. More importantly, they have low reorganization energies and it was for this reason that the use of  $C_{60}$  allowed us to generate recombination spin-polarized triplets similar to those observed in photosynthetic reaction centres.

R. van Grondelle (*Department of Biophysics, Free Univer*sity, Amsterdam, The Netherlands). In your artificial photosynthetic systems, the light-harvesting function of carotenoids is emphasized. In nature, the photoprotective role of carotenoids seems to be the optimized function. Please comment.

T. A. Moore. We have, indeed, in the past studied the protective effect of carotenoids, but in using them as a functional antenna, we automatically get the protective effect, bearing in mind that energy transfer from carotenoids requires that they be in van der Waals distance from the acceptor, which also favours triplet transfer. Moreover, when you think about it, carotenoids were probably used as functional antenna systems before oxygen appeared in the atmosphere. Therefore, only when an oxygenic atmosphere appeared did the protective nature of carotenoids become important. Of course, now carotenoids are vital for protecting photosynthetic organisms and, indeed, evolutionary pressure in the aerobic world could be brought about by further developments in carotenoid protective mechanisms, e.g. xanthophylls cycle.

L. Sun (Department of Organic Chemistry, Stockholm University, Stockholm, Sweden). What is the lifetime of the phthalocyanine excited state when it receives an electron from the carotenoid?

T. A. Moore. About 5 ns in non-polar solvents and quenched to *ca*. 6 ps in polar solvent by electron transfer from the carotenoid to the excited phthalocyanine.

L. Hammarström (Department of Physical Chemistry, Uppsala University, Uppsala, Sweden). In your porphorin– fullerene antenna-charge separation system, how fast did the 'hole' on the porphorin antenna hop between the Znporphorins? Did the charge recombination from  $C_{60}$  follow a single-exponential? Did you see the effect of holehopping?

T. A. Moore. We do not know how fast the 'hole' hops, although singlet energy transfer is in 50 ps. The decay, if I remember rightly, is single exponential.

#### GLOSSARY

ADP: adenosine diphosphate

ATP: adenosine triphosphate

EPR: electron paramagnetic resonance

FCCP: carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone

HOMO: highest occupied molecular orbital

Pi: inorganic phosphate

Qs: 2,5-diphenylbenzoquinone