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Catalytic photoinduced electron transport across a lipid bilayer mediated by a membrane-soluble electron relay†

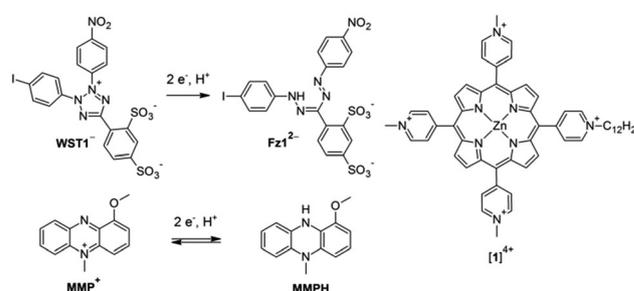B. Limburg, E. Bouwman and S. Bonnet*

Unidirectional photocatalytic electron transfer from a hydrophilic electron donor encapsulated in the interior of a liposome, to a hydrophilic electron acceptor on the other side of the membrane, has been achieved using the simple membrane-soluble electron relay 1-methoxy-*N*-methylphenazinium (MMP⁺). The total amount of photoproduct (>140 nmol) exceeds the number of moles of MMP⁺ present (125 nmol), thus showing that the transport of electrons is catalytic.

To accomplish efficient artificial photosynthesis, two complimentary redox reactions must be fuelled by visible light irradiation. In both photoreactions the generation of a long-lived charge-separated species is required, and the greatest challenge in photocatalysis is to overcome charge recombination. In order to do so, the reduced electron acceptor and the oxidized electron donor must be physically separated. In green plants for example such separation is realized by large protein molecules embedded in the thylakoid membrane of chloroplasts. The dissymmetry of biological membranes allows for unidirectional electron transfer to occur from the lumen to the stroma. However, such photocatalytically active protein assemblies are highly complex and fragile, and artificial systems based on simpler, more robust molecules are required for building artificial devices capable of solar fuel production.

Among the numerous tools offered by supramolecular photocatalysis liposomes holds great promise.[1] They offer a simple way to organize molecular components in space and mimic biological photosynthesis. By segregating electron donors and acceptors on each side of a lipid membrane, it should be possible to create long-lived charge-separated states that, in combination with two different catalysts, would allow for producing solar fuels.[1] Electron transfer across the liposomal membrane to

electron acceptors such as quinones,[2] Co(bpy)₃³⁺,[3] or viologens[4] has been investigated intensively in the past. However, especially with viologens some ambiguity about the true nature of the charge separation was later revealed.[5] It was shown that in those systems electron transport occurred due to diffusion of the reduced electron acceptor through the membrane, after which it remained occluded in the interior of the liposome. Thus, because the electron-transporting molecule never returned to its original location, it was unable to turn over for producing a true catalytic reaction. Up to now, there are only few systems known in the literature that catalytically transport electrons across membranes.[2a,d–f,6] However, all of them contain a complicated molecular structure to realize the charge separation across the membrane.

Herein, we report the truly catalytic unidirectional photo-induced electron transfer from the interior of a liposome to its exterior, based largely on commercially available compounds. This study is inspired by the widely used "WST-1" cell-counting assay, in which a water-soluble electron acceptor, the 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium anion (WST1⁻, Scheme 1), is reduced to the corresponding formazan dye (Fz1²⁻, Scheme 1) by addition of two electrons

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Scheme 1 Chemical structures of the membrane-insoluble electron acceptor 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium anion (WST1⁻), the membrane-soluble electron relay 1-methoxy-*N*-methylphenazinium cation (MMP⁺), their reduction products Fz1²⁻ and MMPH, respectively, and the lipophilic zinc porphyrin photosensitizer [**1**]⁴⁺.

and one proton. Usually the electrons and protons are generated inside living cells in the form of nicotinamide adenine dinucleotide (NADH), which is too hydrophilic to cross the cell membrane. The WST-1 assay requires the membrane-soluble relay 1-methoxy-N-methylphenazinium cation (MMP$^+$, Scheme 1) to transport the biological reducing equivalents from inside the cell to the WST1$^-$ acceptor situated on the other side of the membrane.[7] By analogy, we envisioned that biomimetic liposomes containing a water-soluble electron donor in their interior (ethylenediaminetetra-acetic acid, H$_4$EDTA) and a photosensitizer in the membrane would be able to unidirectionally transport the photoelectrons to the WST1$^-$ molecules located outside the liposomes, provided that MMP$^+$ is added to the system.[7] We describe below photo-catalytic studies showing that the lipid membrane diminishes product inhibition, while the use of membrane-insoluble electron donors and acceptors allows for avoiding the mechanistic ambiguities generally associated with this type of studies.

Dye-functionalized liposomes were prepared in which the amphiphilic photosensitizer [1]$^{4+}$ (Scheme 1) was added in the membrane, the electron donor HEDTA^{3-} was placed in the inner water pool of the liposome, and the electron acceptor WST1$^-$ was added into the bulk solution, $i.e.$, on the outside of the liposome. The liposome bilayer composition consisted of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1.6 mol% of the alkyl-functionalized zinc porphyrin [1]Cl$_4$, and 1 mol% of the pegylated lipid sodium N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (NaDSPE-PEG2K) to stabilize the liposome dispersion. The liposomes were prepared by standard extrusion methods in an HEDTA^{3-} buffer at pH = 8, and were subsequently purified by size-exclusion chromatography to remove non-encapsulated HEDTA^{3-} (see ESI†). To this liposomal solution (called here-after liposome sample LA) was then added an isotonic solution containing the electron acceptor WST1$^-$ (as the sodium salt), the electron relay MMP$^+$ (as methylsulfate salt), and ZnSO$_4$. The addition of Zn^{2+} ions allowed for binding and thus inactivating traces of HEDTA^{3-} that may remain in the bulk aqueous phase after size-exclusion chromatography.[4g,h] The resulting mixtures, called system LA$_1$ or L$_2$A$_1$ (see compositions in Table 1), were de-aerated and irradiated in the Soret band of [1]$^{4+}$ (λ_{irr} = 449 nm). The excited photosensitizer was assumed to not differ significantly from the well-characterized methyl analogue [ZnTMPyP]$^{4+}$, $i.e.$, to be of triplet character and to have an oxidation potential of $\sim$ +0.8 V $vs.$ NHE and a reduction potential of $\sim$ −0.45 V $vs.$ NHE.[8]

A typical evolution of the UV-vis spectrum of the irradiated dispersions as a function of irradiation time is shown in Fig. 1a. Clearly, a band ascribed to the Fz1^{2-} dye arises at λ_{max} = 438 nm as a result of the photoinduced reduction of WST^{1-}.[7] The amount of photoproduct, n_{Fz1}, determined by the absorbance due to Fz1^{2-} (ε_{438} = 37 000 M^{-1} cm^{-1}),[7] was plotted as a function of the number of photons $Q(t)$ absorbed by system since t = 0 (Fig. 1b, L$_2$A$_1$). The slope of this curve at t = 0 was determined to be 1.2(1)% in both LA$_1$ and L$_2$A$_1$. However, as only half of the photosensitizers are assumed to participate in the reaction, only half of the photons are effectively used. The quantum yield for Fz1^{2-} formation, φ_0, is thus twice higher

Table 1 Bulk concentrations and initial quantum yields for the different photocatalytic liposomes described in this report

System[a]	MMP^{+}[b] (μM)	Triton X-100 (μM)	O$_2$	EDTA (mM)	ZnSO$_4$ (mM)	φ_0 (%)
LA$_1$	42	—	—	[c]	5	2.4
LA$_2$	—	—	—	[c]	5	—
L$_2$A$_1$[d]	42	—	—	[c]	5	2.4
L$_2$A$_2$[d]	—	—	—	[c]	5	—
LA$_3$	42	0.3	—	[c]	5	—
L$_2$A$_4$[d]	42	—	Yes	[c]	5	—
LB$_1$	42	—	—	125	—	0.90
LB$_2$	—	—	—	125	—	0.72
LB$_3$	—	0.3	—	125	—	3.8
LB$_4$	—	—	Yes	125	—	0.84

[a] For the details on the preparation of liposome samples, see ESI. Bulk concentrations before extrusion and/or size exclusion: DPPC (1.04 mM), NaDSPE-PEG2K (10.4 μM), [1]Cl4 (16.7 μM). Final concentration of [1]Cl4 $\sim$2.1 μM, as determined by UV-vis. [b] MMP$^+$ added as methylsulfate salt. [c] For these samples, Na$_3$HEDTA was encapsulated inside the liposomes. To obtain the same osmolality inside and outside the liposomes, all solutions were prepared using isotonic NH$_4$OAc (379 mOsm, pH = 7). [d] These samples contained twice more liposomes per unit volume than LA$_1$ (DPPC: 2.08 mM, NaDSPE-PEG2K: 20.8 μM, [1]Cl$_4$: 33 μM).
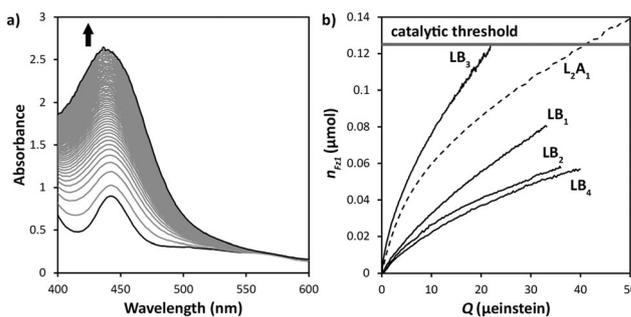

Fig. 1 (a) Evolution of the UV-vis spectrum of L$_2$A$_1$ (see Table 1) under blue light irradiation. Spectra were recorded every minute. (b) Photocatalytic formation of Fz1^{2-} (n_{Fz1}) as a function of the amount of photons $Q(t)$ absorbed by the porphyrin since t = 0. See Table 1 for the composition of systems L$_2$A$_1$ and LB$_{1-4}$. Conditions: T = 298 K, λ_{irr} = 449 nm, $\Delta\lambda_{\frac{1}{2}}$ = 25 nm, photon flux Φ = 3.3 × 10^{-8} einstein s^{-1}. Total irradiation time is 30 minutes, or shorter if the absorbance of the solution became higher than A = 3 at 438 nm.

(2.4(2)%). Dynamic light scattering analysis of the mixture before and after 60 min irradiation gave essentially the same results (Z_{ave} = 166 nm, PDI = 0.13 before; Z_{ave} = 162 nm, PDI = 0.16 after), indicating that the liposomes stayed intact during photocatalysis. Sample LA$_1$ is thus able to photocatalytically transport the reducing equivalents from inside the liposomes (HEDTA^{3-}) to the outside (WST1$^-$), thus realizing trans-membrane charge separation. In L$_2$A$_1$, the twice-higher lipo-some concentration allowed for observing that more Fz1^{2-} was produced during the reaction (141 nmol after 1 hour of irradiation) than the number of moles of MMP$^+$ present (125 nmol) in the system (Fig. 1b), indicating that MMP$^+$ functioned as a true catalyst in this system.

When the electron relay MMP$^+$ was omitted from LA$_1$ or L$_2$A$_1$ (systems LA$_2$ or L$_2$A$_2$, respectively, see Table 1), formation of Fz1^{2-} was not observed. Instead the Soret band of the porphyrin bleached to around half of its initial intensity

(Fig. S1a, ESI†). It was thus clear that systems **LA₁** and **L₂A₁** require MMP⁺ to perform charge separation, and that the electrons cannot be transferred directly from HEDTA³⁻ to WST1⁻ because they are positioned in two different aqueous phases separated by an impermeable lipid membrane. Furthermore, in **LA₂** and **L₂A₂** only half the porphyrin molecules were bleached, probably those on the inside of the membrane that were photoreduced by HEDTA³⁻. In system **LA₃**, triton X-100 was added to **LA₁** prior to the reaction, resulting in disruption of the liposomes and release of HEDTA³⁻ into the bulk solution. In such conditions neither formation of Fz1²⁻, nor bleaching of the porphyrin was observed upon light irradiation. Since the bulk concentration of HEDTA³⁻ was 0.11 mM (see ESI†), *i.e.* ~50 times lower than the bulk concentration of Zn²⁺, the released HEDTA³⁻ was fully bound to the Zn²⁺ ions thereby losing its ability to donate electrons in system **LA₃**.

In order to investigate in which way physical separation of the electron donor and acceptor by the lipid membrane influences the rate of the photocatalytic reaction, the photocatalytic system **LB₁** was developed, which contained both the electron donor and acceptor on the outside of the liposome membrane (Fig. 2, bottom). The liposomes used for these systems, called liposome sample **LB**, were prepared by standard extrusion methods from a lipid film of DPPC, 1 mol% NaDSPE-PEG2K and 1.6 mol% [**1**]Cl₄ hydrated in aqueous NH₄OAc (pH = 7.0). To this liposomal solution were added an isotonic HEDTA³⁻ buffer at pH = 8.0, WST1⁻, and the electron relay MMP⁺, to form system **LB₁** (see Table 1). System **LB₁** was deaerated and irradiated as described above, which also led to the formation of Fz1²⁻. A much lower initial photoelectron transfer quantum yield $\varphi_0 = 0.90(9)\%$ was found for this system compared to **LA₁**, indicating that the physical separation of the electron donor and acceptor in **LA₁** leads to a more stable separation of charges (Fig. 1b). In system **LB₁**, the photoreduced electron acceptor Fz1²⁻ is in the same aqueous phase as the photosensitizer, so that it may also serve as an electron donor and quench the porphyrin excited state, or react with porphyrin cation radicals. Such reactions diminish the overall quantum yield of the photoreaction. When MMP⁺ is left out from this mixture (system **LB₂**), the reaction is only slightly inhibited ($\varphi_0 = 0.72\%$) compared to **LB₁** (Fig. 1b), confirming that electron transfer from HEDTA³⁻ to WST1⁻ in **LB₁** occurs within a single aqueous phase where no membrane needs to be crossed. The addition of triton X-100 to system **LB₁** (*i.e.*, system **LB₃**) leads to an increase in the efficiency of the photoreaction, confirming that in **LA₃** triton X-100 quenches the photoreaction solely by disrupting the liposomes.

Many photocatalytic systems are not active in the presence of dioxygen, which would pose a problem in a photocatalytic fuel production system based on water oxidation. Thus, the sensitivity of systems **LA₁** and **LB₁** to O₂ was investigated. When system **LB₁** was not deaerated (system **LB₄**) the presence of O₂ did not significantly influence the rate of the photoreaction, nor did it cause any induction period common to this type of systems due to the initial competition between WST1⁻ and O₂ to accept the first photoelectrons.[4g,9] In contrast, when system
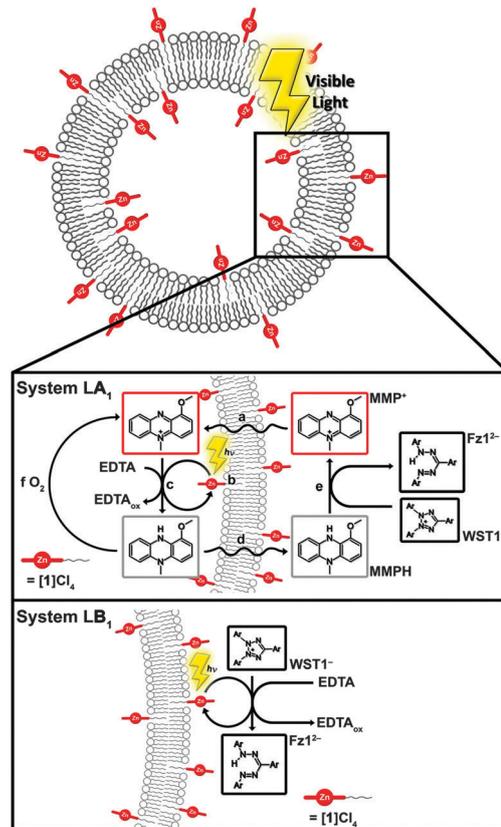

Fig. 2 Top: scheme of the photocatalytic reduction of WST1⁻ by HEDTA³⁻ with dye-functionalized liposomes. Middle: system **LA₁**, with EDTA and WST1⁻ on opposite sides of the membrane. (a) Passive diffusion of MMP⁺ through the membrane into the liposome. (b) Absorption of light by [**1**]⁴⁺. (c) Photocatalytic oxidation of EDTA (in the form of HEDTA³⁻ at pH 8) and reduction of MMP⁺ to MMPH. (d) Passive diffusion of MMPH through the membrane. (e) Chemical (dark) reduction of WST1⁻ to Fz1²⁻ by MMPH. (f) Undesired re-oxidation of MMPH to MMP⁺ by molecular oxygen in system **LA₄**. Bottom: system **LB₁**, with HEDTA³⁻ and WST1⁻ on the same side of the membrane. MMP⁺ has been left out for clarity as it does not influence the system significantly.

L₂A₁ was not deaerated (system **L₂A₄**) complete bleaching of the Soret band of the porphyrin was observed and no Fz1²⁻ was produced (Fig. S1b, ESI†). The striking difference between systems **L₂A₄** and **L₂A₁** shows that one of the catalytic intermediates in **LA₁** and **L₂A₁** is unstable in the presence of O₂. We hypothesized that such an intermediate would be MMPH, as it plays a key role in the photocatalytic mechanism of system **LA₁**. In a control experiment, system **LA₁** without WST1⁻ was irradiated in the absence of O₂; in this reaction MMPH was produced, which could be detected by UV-vis spectroscopy (Fig. S2, ESI† left). After bubbling air through the solution the reaction was fully reversed: all MMPH was oxidized back to MMP⁺ (Fig. S2, ESI† right). Thus, photocatalytic electron transfer through the membrane such as that observed in system **L₂A₁** is sensitive to dioxygen because the reduced electron relay is rapidly oxidized by air.

Based on the observations described above the mechanism depicted in Fig. 2 (middle) is proposed for system **LA₁** and **L₂A₁**.

First MMP$^+$, introduced in the bulk aqueous phase, diffuses through the membrane into the liposomes (a),[7] where it is photoreduced with HEDTA^{3-} (b). This reaction presumably occurs via reductive quenching of *[**1**]$^{4+}$ by HEDTA^{3-} (high local concentration, 125 mM), followed by reduction of MMP$^+$ by [**1**]$^{3+}$. Oxidative quenching would be thermodynamically feasible (the redox potential of the MMP$^+$/MMPH couple is +0.06 V vs. NHE),[10] however kinetically unlikely due to the low concentration of MMP$^+$ (42 µM). MMPH produced inside the liposomes can diffuse through the membrane in absence of O$_2$, thus transporting the electrons and proton out of the liposome (d). Reduction of WST1$^-$ by MMPH outside of the liposomes forms Fz1^{2-} (e) and regenerates MMP$^+$, as reported for the WST-1 cell counting assay. MMP$^+$ can then diffuse through the membrane again and turn-over further. Since the amount of Fz1^{2-} produced (e), monitored by UV-vis, exceeds the amount of MMP$^+$ present at the start of the reaction (Fig. 1a), MMP$^+$ must be concomitantly regenerated in the bulk aqueous phase, i.e., MMP$^+$ must be catalytic.

In summary, unlike previous studies in which electron transfer across lipid bilayers occurred concomitantly with the stoichiometric transport and occlusion of the electron acceptor into the membrane, directional electron transfer across a DPPC bilayer is unambiguously demonstrated here by combining a membrane-soluble electron relay, hydrophilic electron donors and acceptors situated inside and outside liposomes, respectively, and a membrane-bound photosensitizer. In this system the MMP$^+$ electron relay truly acts catalytically in absence of oxygen, but under air it is oxidized and cannot fulfill its electron-transporting role. Under inert atmosphere, the actual transport of electrons across the membrane does not limit the reaction rate, as the initial quantum efficiency of the reaction through the membrane (**LA$_1$**) is three times higher than when the donor and acceptor are both positioned on the same side of the membrane (**LB$_1$**). Keeping the photoreduced electron acceptor physically separated from the electron donor allows for preventing product inhibition, i.e., quenching of the porphyrin excited states by Fz1^{2-}, which would otherwise diminish the quantum efficiency of the photoreduction. Finding a membrane-soluble electron relay that is stable in the presence of dioxygen will be required for the future development of supramolecular photocatalytic systems in which water oxidation and proton reduction catalysts are combined.

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