Supplementary Information

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

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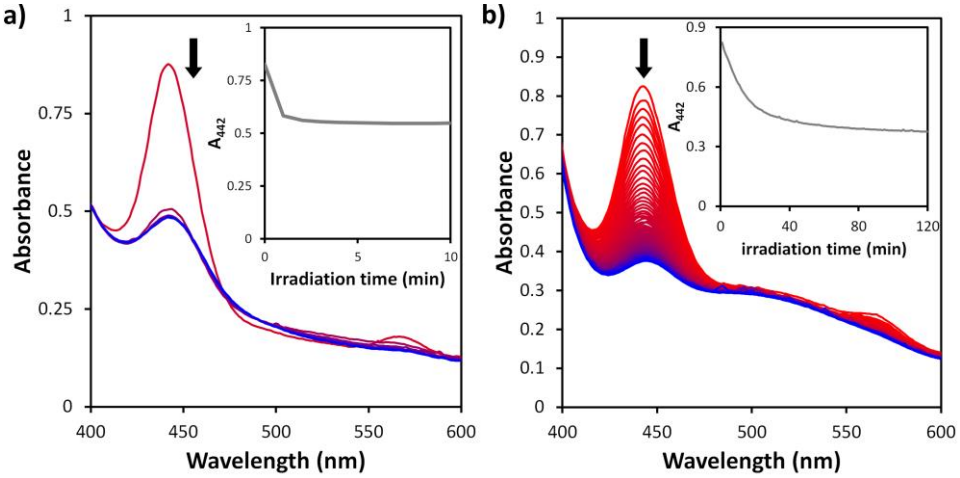


Figure S1. a) Evolution of the UV-vis spectrum of system $\mathbf{L_2A_2}$, *i.e.* $\mathbf{L_2A_1}$ without MMP^+, under blue light irradiation. One spectrum was taken every minute. Inset: evolution of the absorbance at λ_{max} = 442 nm versus irradiation time. b) Evolution of the UV-vis spectrum of system $\mathbf{L_2A_4}$, *i.e.*, non-deaerated $\mathbf{L_2A_1}$. Inset: evolution of the absorbance at λ_{max} = 442 nm versus irradiation time. Conditions for a and b: $WST1^-$ (0.33 mM), MMP^+ (42 µM), $ZnSO_4$ (5 mM), liposome sample **LA**: DPPC (~2.08 mM, the dilution factor of the liposomes due to extrusion / size exclusion chromatography is unknown), NaDSPE-PEG2K (1 mol% relative to DPPC), [**1**]Cl_4 (0.8 mol% relative to DPPC), in aqueous NH_4OAc (379 mOsm, pH = 7.0), T = 298 K, irradiation at λ_{irr} = 449 nm, $\Delta\lambda_{½}$ = 25 nm, photon flux $\Phi = 3.3 \cdot 10^{-8}$ einstein·s^{-1}.

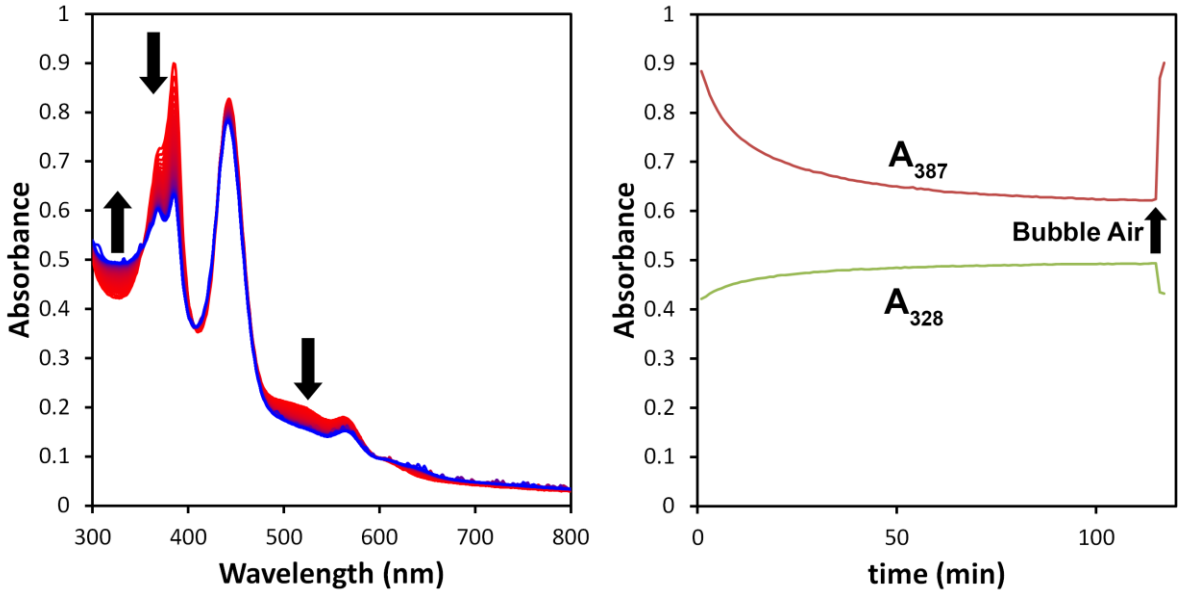
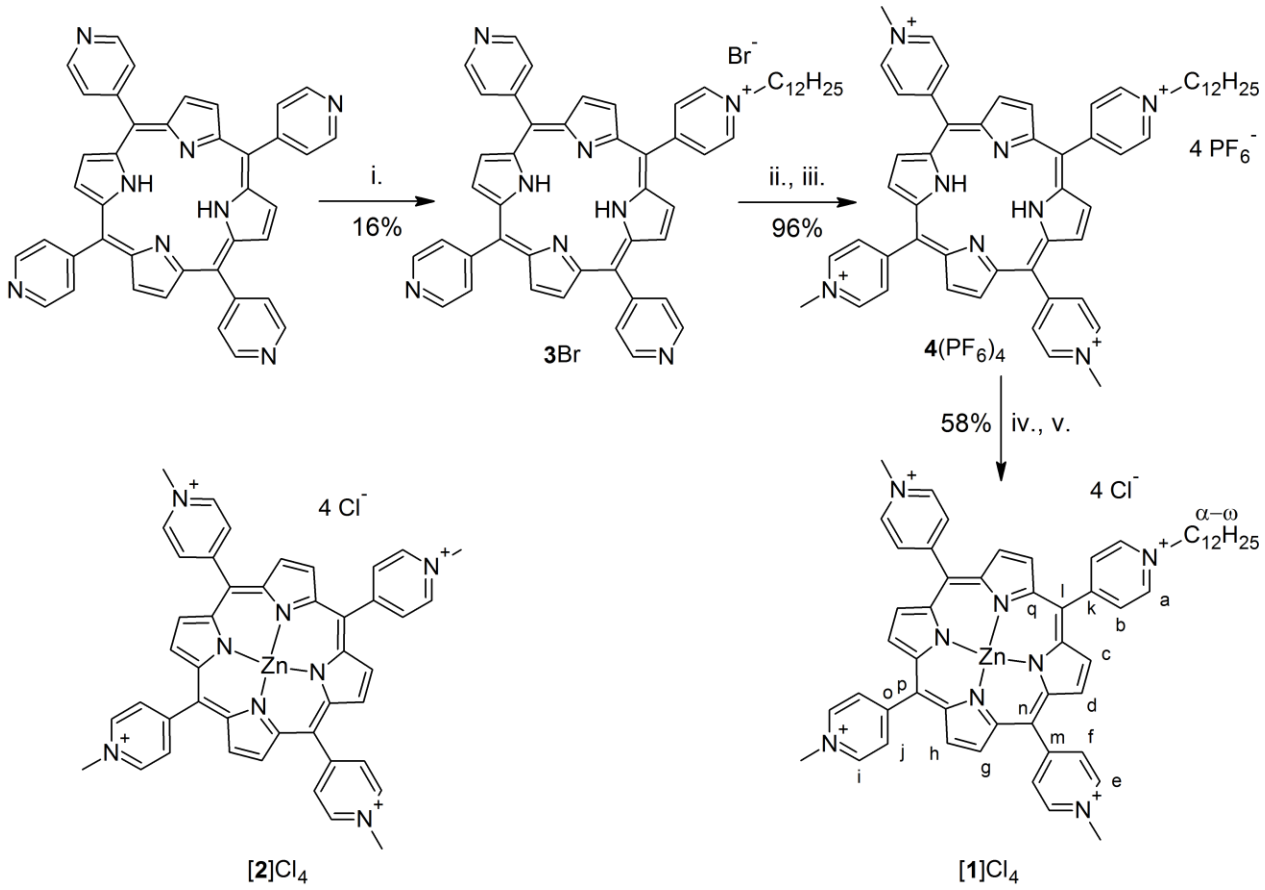


Figure S2. Irradiation of System **LA$_1$** without $WST1^{-}$. MMP^{+} (42 µM), in aqueous NH_4OAc (379 mOsm, pH = 7.0), T = 298 K, λ_{irr}= 449 nm, $\Delta\lambda_{½}$ = 25 nm, photon flux $\Phi = 3.3 \cdot 10^{-8}$ einstein·s^{-1}. Left: evolution of the UV-vis spectrum. Only the spectra before the bubbling of air are shown. Right: Traces of the absorbance at 387 nm (maximum of MMP^{+}) and 328 nm (maximum of MMPH). At *t*=117 min (indicated by the arrow), the irradiation was stopped and air was bubbled through the mixture. The full amount of MMP^{+} initially introduced was recovered, as indicated by the evolution of the absorbance at 387 nm.



Scheme S1. Synthesis of zinc porphyrin [**1**]Cl_4 and structure of the water-soluble zinc porphyrin [**2**]Cl_4. Conditions: *i.* 5.2 eq. n-$C_{12}H_{25}Br$, 3/1 $CHCl_3$ / EtOH. *ii.* 100 eq. MeI, DMF. *iii.* KPF_6, H_2O. *iv.* 1 eq. $Zn(OAc)_2$, DMF. *v.* Bu_4NCl, acetone.

Experimental

General. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer or a Bruker DMX 400 MHz spectrometer. Chemical shift values (δ) are reported in ppm relative to tetramethylsilane or the solvent. UV-vis absorbance spectra were recorded on a Varian Cary50 spectrophotometer. Dynamic light scattering (DLS) experiments were performed on a Malvern Instruments Zetasizer operated at 633 nm. All reagents were obtained from Sigma-Aldrich and used as received unless stated otherwise. 1,2-dipalmatoyl-*sn*-glycero-3-phosphocholine (DPPC) and sodium N-(carbonyl-methoxypolyethylene glycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (NaDSPE-PEG2K) were obtained from Lipoid and stored at –20 °C. *Meso*-tetra(4-pyridyl)porphine was obtained from Frontier Scientific. $K_3[Fe(C_2O_4)_3]\cdot 3H_2O$ was prepared following a literature procedure[59] and used within one week.

5-(N-dodecylpyridinium-4-yl)-10,15,20-tri(4-pyridyl)porphine bromide (3Br). *Meso*-tetra(4-pyridyl)porphine (500 mg, 0.81 mmol) was dissolved in 3:1 $CHCl_3$:EtOH (50 mL). To this mixture dodecyl bromide (1 mL, 1.04 g, 4.2 mmol) was added and the mixture was refluxed for 6 days. The solution was concentrated to 10 mL and poured into an excess of Et_2O (100 mL). The resulting precipitate was filtered and washed with Et_2O (50 mL). The residue was then purified by two consecutive columns (SiO_2, 90:10:1 $CHCl_3$:MeOH:H_2O). The second fraction was collected, concentrated to 10 mL and poured into Et_2O (100 mL). The resulting precipitate was filtered, washed with Et_2O (50 mL) and dried *in vacuo* to yield **3**Br (88 mg, 13%). ^{1}H NMR (300 MHz, $CDCl_3$, for numbering see Scheme S1) δ 9.80 (d, J = 6.0 Hz, 2H, a), 9.08 (d, J = 5.2 Hz, 2H, i), 9.04 – 8.91 (m, 6H, e+c), 8.91 – 8.65 (m, 8H, b+d+g+h), 8.15 (d, J = 5.3 Hz, 2H, j), 7.99 (d, J = 4.5 Hz, 4H, f), 5.36 (t, J = 7.2 Hz, 1H, α), 2.37 (dt, J = 14.9, 7.9 Hz, 2H, β), 1.60 (dt, J = 12.8, 6.8 Hz, 2H, γ), 1.42 (dt, J = 13.1, 6.9 Hz, 2H, δ), 1.34 – 1.04 (m, 14H, ε-ω_{-1}), 0.83 (t, J = 6.7 Hz, 3H, ω), –2.94 (s, 2H, N-H). ^{13}C NMR (75 MHz, $CDCl_3$, for numbering see Scheme S1) δ 159.30 (k), 149.51 (o), 149.37 (m), 148.58 (i), 148.48 (e), 143.39 (a), 133.23 (b), 129.35 (j), 129.29 (f), 119.43 (l), 118.76 (n), 112.70 (p), 62.50 (α), 32.06 (β), [31.96, 29.65, 29.60, 29.50, 29.38, 29.31] (δ-ω_{-2}), 26.59 (γ), 22.76 (ω_{-1}), 14.22 (ω). HRMS exp (calcd): 787.42236 (787.42312, $[M–Br]^+$), 394.21475 (394.21520, $[M–Br+H]^{2+}$).

5-(N-dodecylpyridinium-4-yl)-10,15,20-tri(N-methylpyridinium-4-yl)porphine hexafluorophosphate (4(PF_6)$_4$). **3**Br (100 mg, 0.12 mmol) was dissolved in DMF (5 mL). Methyl iodide (0.75 mL, 1.7 g, 12 mmol) was added and the mixture was refluxed for 10 minutes under Argon atmosphere. After cooling to room temperature, the mixture was poured into Et_2O (100 mL), and the resulting precipitate was filtered. The residue was dissolved in H_2O (20 mL), precipitated with a saturated solution of NH_4PF_6 (5 mL), and filtered. The resulting solid was washed with H_2O (20 mL) and Et_2O (20 mL), and dried *in vacuo* overnight to yield **4**(PF_6)$_4$ (157 mg, 0.11 mmol, 96%). ^{1}H NMR (300 MHz, acetone-d6, the same numbering scheme as for [**1**]Cl_4 was used, see Scheme S1) δ 9.65 (d, J = 6.5 Hz, 2H, a), 9.55 (d, J = 6.2 Hz, 6H, e+i), 9.30 – 9.16 (m, 8H, c+d+g+h), 9.13 (d, J = 6.5 Hz, 2H, b), 9.09 (d, J = 6.2 Hz, 6H, f+j), 5.16 (t, J = 7.8 Hz, 2H, α), 4.94 (s, 9H, N-Me), 2.46 (p, J = 7.8 Hz, 2H, β), 1.71 (p, J = 7.2 Hz, 2H, γ), 1.55 (dt, J = 14.1, 6.8 Hz, 2H, δ), 1.48 – 1.20 (m, 14H, ε-ω_{-1}), 0.87 (t, J = 6.0 Hz, 3H, ω), -2.97 (s, 2H, N-H). ^{13}C NMR (75 MHz, acetone-d6, the same numbering scheme as for [**1**]Cl_4 was used, see Scheme S1) δ 158.61 (k), 158.39 (m), 158.37 (o), 145.35 (e+i), 144.45 (a), 133.86 (c), 133.42 (f+j), 116.68 (l), 116.65 (n), 116.64 (p), 62.92 (α), 49.14 (N-Me), 32.56 (β), [32.37, 30.32, 30.29, 30.18, 30.12, 30.02, 29.89] (δ-ω_{-2}), 27.06 (γ), 23.26 (ω_{-1}), 14.33 (ω). HRMS exp (calcd): 325.81900 (325.81888, $[M–3\ PF_6]^{3+}$), 561.21041 (561.21068, $[M–2\ PF_6]^{2+}$), 488.22430 (488.22468, $[M–3\ PF_6–H]^{2+}$), 415.23847 (415.23867, $[M–4\ PF_6–2\ H]^{2+}$).

5-(N-dodecylpyridinium-4-yl)-10,15,20-tri(N-methylpyridinium-4-yl)porphinato zinc chloride ([1]Cl_4). **4**$(PF_6)_4$ (50 mg, 35 μmol) and $Zn(OAc)_2 \cdot 2\ H_2O$ (7.77 mg, 35 μmol) were dissolved in DMF (5 mL) and the solution was refluxed for 1 hour. After cooling to R.T., the solution was poured into Et_2O (100 mL) and the precipitate was filtered and washed with Et_2O (50 mL). The residue was dissolved in acetone (10 mL) and precipitated with a saturated solution of NBu_4Cl in acetone (5 mL). The precipitate was filtered and washed with acetone (20 mL) and Et_2O (50 mL). The residue was dried *in vacuo* to yield 21 mg [**1**]Cl_4 (20 μmol, 58%). ^{1}H NMR (400 MHz, MeOD, the same numbering scheme as for [**1**]Cl_4 was used, see Scheme S1) δ 9.45 (d, J = 6.5 Hz, 2H, a), 9.37 (d, J = 6.4 Hz, 6H, e+i), 9.21 – 9.08 (m, 8H, c+d+g+h), 9.00 – 8.87 (m, 8H, b+f+j), 5.02 (t, J = 7.6 Hz, 2H, α), 4.82 (s, 9H, N-Me), 2.41 (p, J = 8.1, 7.5 Hz, 2H, β), 1.71 (dt, J = 14.5, 7.1 Hz, 2H, γ), 1.60 (dt, J = 14.3, 6.7 Hz, 2H, δ), 1.53 – 1.26 (m, 14H, ε-ω_{-1}), 0.91 (t, J = 6.7 Hz, 3H, ω). ^{13}C NMR (100 MHz, MeOD, the same numbering scheme as for [**1**]Cl_4 was used, see Scheme S1) δ 161.23 (k), 160.99 (m+o), 150.41 (q), 144.98 (e+i), 144.07 (a), 134.38 (b), 134.02 (f+j), 133.80 (c+d+g+h), 117.36 (l+n+p), 63.17 (α), 48.89 (N-Me), 33.10 (β), [32.79, 30.83, 30.81, 30.78, 30.68, 30.52, 30.38] (δ-ω_{-2}), 27.61 (γ), 23.76 (ω_{-1}), 14.46 (ω). HRMS exp (calcd): 560.18888 (560.18829, $[M–4\ Cl+2\ TFA]^{2+}$), 335.79705 (335.79699, $[M–4\ Cl+TFA]^{3+}$), 521.18074 (521.18019, $[M–3\ Cl+TFA]^{2+}$).

Preparation of liposomes. *Liposome sample **LA**:* Aliquots of DPPC (5 mL of a 5 mM solution in $CHCl_3$), NaDSPE-PEG2K (0.5 mL of a 0.5 mM solution in $CHCl_3$) and [**1**]Cl_4 (4 mL of a 0.1 mM solution in MeOH) were mixed and rotary evaporated in reaction tubes to give a lipid film. The film was dried *in vacuo* for 1 hour, and hydrated with $HEDTA^{3-}$ buffer (2 mL, 0.125 M, 374 mOsm, pH = 8). The lipid film was repeatedly freeze-thawed and sonicated at 313 K for five seconds until homogeneously dispersed (a total of 5 times was required). The dispersion was then extruded trough 0.2 μm cellulose acetate filters and purified over Sephadex G-25 (GE Healthcare, illustra NAP, eluent: aqueous NH_4OAc, 379 mOsm, column size: h: 5 cm, d: 1 cm). The liposome fraction (3.2 mL to 4.4 mL) was then separated from the $HEDTA^{3-}$ fraction ($HEDTA^{3-}$ eluted from 4.4 mL and further, and was visualized by complexometry using murexide from a calibration run of only $HEDTA^{3-}$ buffer eluted with aqueous NH_4OAc, see Figure S3), stored at 277 K and used within one week (typical DLS: Z_{ave} 144 nm, PDI 0.15). The concentration of DPPC assuming no loss was 12.5 mM. The exact concentration after extrusion and size-exclusion chromatography was not measured.

*Liposome sample **LB**:* Aliquots of DPPC (5 mL of a 5 mM solution in $CHCl_3$), DSPE-PEG2K (0.5 mL of a 0.5 mM solution in $CHCl_3$) and [**1**]Cl_4 (4 mL of a 0.1 mM solution in MeOH) were mixed and rotary evaporated in reaction tubes to give a lipid film. The film was dried *in vacuo* for 1 hour, and hydrated with aqueous NH_4OAc (2 mL, 379 mOsm, pH = 7). The lipid film was repeatedly freeze-thawed and sonicated at 313 K for five seconds until homogeneously dispersed (a total of 5 times was required). The dispersion was then extruded trough 0.2 μm cellulose acetate filters, stored at 277 K and used within one week (DLS: Z_{ave} 159 nm, PDI 0.21). The concentration of DPPC assuming no loss was 12.5 mM. The exact concentration after extrusion was not measured.

The concentration of liposome components in samples **LA_1-LA_4** and **LB_1-LB_4** varies due to extrusion and size exclusion chromatography. For the preparation of samples **LA_1-LA_4** and **LB_1-LB_4** as listed in Table 1 (see manuscript), 0.5 mL of liposome sample **LA** or **LB** was used. For the samples with twice higher concentration, *i.e.*, **L_2A_{1-4}**, 1 mL of liposome sample **LA** was used. The samples were completed with stock solutions of the additional components (*e.g.,* $WST1^-$ and MMP^+) to a final volume of 3 mL. As the absorbance of the solution is such that >90% of the incoming photons are absorbed, the variations in the concentration of [**1**]Cl_4 are assumed not to influence the rate of the reaction.

Calibration run of the size-exclusion column. Sephadex G-25 size-exclusion columns (GE Healthcare, illustra NAP) were equilibrated with aqueous NH_4OAc (379 mOsm) by running a minimal amount of 30 mL through the column. Afterwards, 1 mL of an $HEDTA^{3-}$ solution (125 mM, pH = 8, 374 mOsm) was loaded on the column. The column was eluted with aqueous NH_4OAc (379 mOsm) and fractions of ~0.4 mL (10 drops from the column) were collected. To each fraction was added 0.2 mL of a $CuNO_3$:murexide solution ($Cu(NO_3)\cdot 2.5\ H_2O$ [2.33 mg] and murexide:K_2SO_4 1:250 [50 mg] in 10 mL aqueous NaOH [90 mM]) and each fraction was well-mixed. A yellow color indicated a concentration of $HEDTA^{3-}$ smaller than 0.1 mM, whereas a purple color indicated a $HEDTA^{3-}$ concentration greater than 0.1 mM. See Figure S1 for a visual representation of this calibration run. Fraction 8-11 was typically taken as liposome solution **LA**, *i.e.*, without $HEDTA^{3-}$ outside the liposomes. Non-encapsulated $HEDTA^{3-}$ eluted in fractions 12 and further, which were discarded.

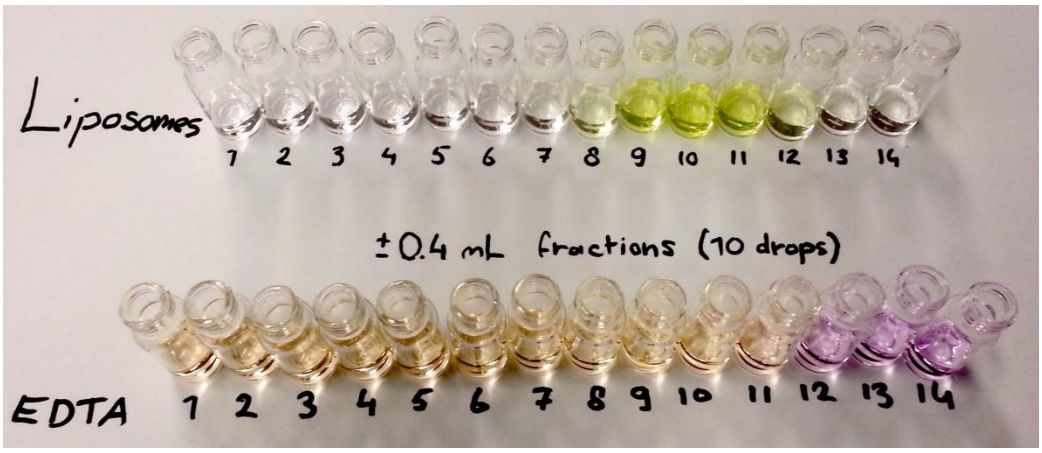


Figure S3. Visual representation of the fractions from the size exclusion column for the purification of $HEDTA^{3-}$-encapsulating liposomes. Top: the fractions from the liposome sample **LA** purification run (fractions 8-11 were pooled). Bottom: the fractions from the $HEDTA^{3-}$ calibration run. $HEDTA^{3-}$ elutes from the column from fraction 12 and further.

Titration to determine [$HEDTA^{3-}$] in liposome sample LA. The global concentration of $HEDTA^{3-}$ encapsulated in liposome sample **LA** was determined by complexometry. A solution of murexide (8.5 mg of 250:1 K_2SO_4:murexide) in 100 µL aqueous NaOH (0.1 M) was prepared. To this solution was added 200 µL of liposome **LA** solution, yielding a purple solution. The solution was diluted to 1.1 mL with aqueous NH_4OAc (379 mOsm). An aqueous solution of $Cu(NO_3)_2$ (10 mM) was titrated into the murexide/liposome solution 2 µL at a time, and after each addition a UV-vis spectrum was recorded in a 2 mm UV-vis cell. The solution turned orange almost instantaneously after the first addition, showing the low concentration of $HEDTA^{3-}$ outside of the liposomes. After addition of 10 µL of Cu^{2+} solution, 2 µL of a 10% w/v% triton-X100 solution was added. The solution turned purple again due to leakage of $HEDTA^{3-}$ from inside the destroyed liposomes, complexation to Cu^{2+}, and release of murexide. Finally, the solution of $Cu(NO_3)_2$ (10 mM) was titrated into the mixture again to form the Cu-murexide complex. At the equivalent point the solution turned orange; around 30 µL was required. The amount of Cu^{2+}/murexide complex was determined by the change in absorbance at 525 nm (Figure S4); from the amount of Cu^{2+} required before a change occurred, the concentration of $HEDTA^{3-}$ was determined. The concentration of $HEDTA^{3-}$ outside the liposomes was determined to be 60 µM. The bulk concentration of $HEDTA^{3-}$ after destruction of the liposomes was determined to be 1.4 mM. Under photocatalytic conditions the liposomes were diluted 12 times (see below), thus the bulk concentration of $HEDTA^{3-}$ after destruction of the liposomes was 0.11 mM.

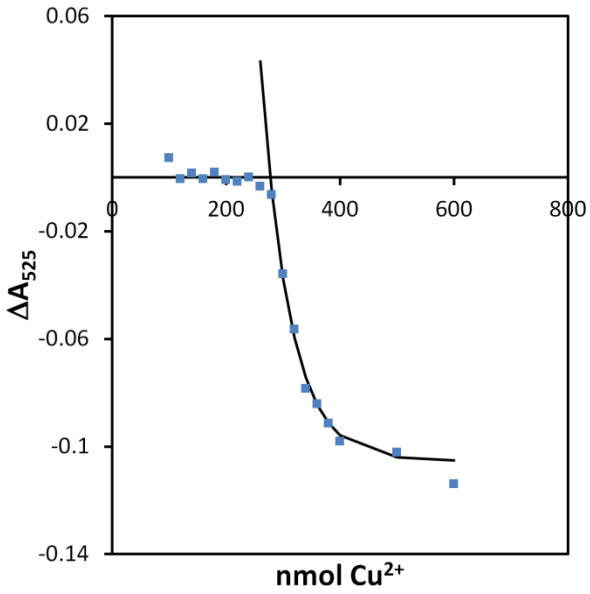


Figure S4. Titration to determine the amount of $HEDTA^{3-}$ inside liposome sample **LA**. The concentration of $HEDTA^{3-}$ was determined from the point at which the fit (black line) crosses the x-axis.

Photochemistry and quantum yield measurement. Irradiation experiments were performed using a quartz UV-vis cell irradiated from the top with a custom-built LED light source equipped with an OSRAM Opto Semiconductors LD W5SM LED (λ_{max} = 449 nm, $\Delta\lambda_{½}$ = 25 nm). The photon flux was determined using standard ferrioxalate actinometry to be $3.3 \cdot 10^{-8}$ Einstein·s^{-1}. The number of photons absorbed by the porphyrin was calculated from the probability of absorption, taking into account absorbance of other species at the wavelength of irradiation. Samples were prepared by mixing $WST1^-$ (1 mL, 1 mM), MMP^+ (125 µL, 1 mM) and/or $ZnSO_4$ (1.5 mL, 10 mM) in either in either aqueous NH_4OAc (379 mOsm, pH = 7.0) for liposome sample **LA**, or aqueous $HEDTA^{3-}$ buffer (125 mM, pH = 8) for liposome sample **LB**. The volume was completed to 2.75 mL with the corresponding buffer, and liposome sample **LA** or **LB** (0.25 mL) was added. The samples (3 mL) were introduced in a quartz UV-vis cell (l = 1 cm) placed inside the spectrometer, and deaerated for 30 minutes by slowly bubbling argon through the solution. Each sample was irradiated under thermostated conditions at 298 K and stirred under a constant flow of argon, also inside the spectrometer. The photoreaction was monitored with UV-vis spectroscopy measured perpendicular to the irradiation beam.