Supplementary information

Self-assembled light-driven photosynthetic-respiratory electron transport

chain hybrid proton pump

David Hvasanov,^a Joshua R. Peterson^a and Pall Thordarson^{*a}

^aSchool of Chemistry, The University of New South Wales, Sydney, NSW 2052, Australia.

E-mail: p.thordarson@unsw.edu.au

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Experimental

General methods, instrumentation and the synthesis of light-harvesting bioconjugate **Ru-cyt***c* have been previously reported.¹

Preparation of proton pumping proteo-polymersomes. In a typical experiment, 33 μ l of a 1 mg mL⁻¹ solution of PS₁₄₀-*b*-PAA₄₈ in THF was injected into 200 μ L of a 7.5 μ M **Ru-cytc**, 0.75 μ M CcOx, 5 μ M HPTS or SNARF-5F solution in phosphate buffered saline (PBS, 150 mM, pH 7.5) at 25 °C. The buffered enzyme solution was allowed to equilibrate for 10 min prior to PS₁₄₀-*b*-PAA₄₈ in THF injection. After injection, the solution was allowed to equilibrate for at least 24 h and extensively dialysed into ultrapure water (2×1 L) using a 50 kDa molecular weight cut-off membrane over 24 h to remove non-encapsulated enzymes at 25 °C. This also removes both the external and internally encapsulated 150 mM PBS salt buffer from the proteo-polymersome sample. The concentration of **Ru-cytc** loaded proteo-polymersomes was estimated by UV-Vis absorbance ($\epsilon_{410} = 97.6 \text{ mM}^{-1} \text{ cm}^{-1}$).²

Photo-induced proton pumping studies. In a typical experiment, a solution of saltfree proteo-polymersomes (1.1±0.1 µM Ru-cytc, 113.6 µL) was adjusted to a final buffered condition of 5mM NaH₂PO₄ and 5 mM EDTA by addition of a stock solution of 94 mM NaH₂PO₄ and 94 mM EDTA (6.4 µL) at a desired pH. The sample was degassed for 20 min at 120 mbar in an ice bath and overlayed with nitrogen in the dark to remove oxygen quenching the excited state of Ru(II)-terpyridine complex as well as allowing equilibration of the buffer in the internal proteo-polymersome compartment due to porosity of the membrane. Photo-reactions were performed using a 465 nm (bandwidth $\approx \pm 10$ nm) 16 LED Blue Flashlight (LDP LLC). Power measurements of LED light source were made using a Newport Power Meter (Model 1918-C). A 20.0±2.3 mW/cm² of this 465 nm LEDlight source was placed 2.5 cm from the sample over a 1.0 cm \times 0.3 cm sample area and irradiated in a nitrogen purged fluorescence spectrometer. HPTS fluorescence emission measurements were monitored over 2 min intervals with an $\lambda_{ex} = 460$ nm and $\lambda_{em} = 510$ nm. HPTS fluorescence excitation measurements were made with an $\lambda_{em} = 510$ nm. Excitation and emission slit widths were 5 nm. After irradiation, a drop of aqueous sodium hydroxide (2 µL, 1 M) was added to determine the maximum emission intensity (pH > 9) to calibrate the HPTS pH titration curve (Fig. S2). Similarly, SNARF-5F fluorescence measurements were monitored

over 2 min intervals with an $\lambda_{ex} = 514$ nm and $\lambda_{em} = 580$ nm / 640 nm. Excitation and emission slit widths were 10 nm.

Cytochrome *c* **Oxidase-Dye.** A solution of Oregon Green® 488 carboxylic acid, succinimidyl ester *5-isomer* (0.12 mg, 0.236 μ mol) in N,N-dimethylformamide (50 μ L) was mixed with cytochrome *c* oxidase (4.04 mg, 0.0202 μ mol) in 50 mM sodium bicarbonate, pH 8.3 (950 μ L). The mixture was stirred at room temperature for 160 min and concentrated. The conjugate was then purified in 20 mM sodium dihydrogen phosphate buffer, pH 7.0 using size-exclusion chromatography (SEC, Sephadex G-25 superfine, 5 mL, GE Healthcare), concentrated and dialysed extensively into water (molecular weight cut-off 3 kDa) yielding cytochrome *c* oxidase-Dye.

Confocal laser scanning microscopy studies. Optical and confocal laser microscopy experiments were carried out with an Olympus Fluoview FV1000, fitted with monochromatic laser light sources for fluorescence measurements. Images were acquired on a confocal laser scanning microscope with a 40x 0.9NA water-immersion objective. Excitation was at 488 nm using an Ar⁺ laser. Detection was in the range 500-600 nm using internal PMTs (gains were set to 468 V). The confocal pinhole (aperture) was set to auto and images with a 640×640 pixel resolution were recorded at a scan rate of 40 μ s/pixel with a total acquisition time of \approx 16 s.

Transmission electron microscopy (TEM) studies. TEM micrographs were recorded on a JEOL 1400 (80 kV) instrument. The samples were prepared by placing 20 μ L of sample onto a formvar-coated copper grid and the excess water was blotted away after 2 min. with a filter paper. For statistical analysis, a population of 100 polymersomes was measured from TEM micrographs for determination of the average and standard deviation of diameters.

Determination of proton pumping rate (H⁺/s).

With the following representative calculation, proton pumping rate was determined.



Estimation of number of polymer molecules within membrane of one polymersome:

 $V_{membrane} = V_{out} - V_{in}$

 $V_{out} = \frac{4}{3} \pi d_{out}^{3} \text{ where } d_{out} = \text{diameter of vesicles } (d_{out} = 367 \pm 185 \text{ nm}) \text{ and membrane}$ thickness = 98±35 nm. $\therefore V_{out} = 2.06 \times 10^{-19} \text{ m}^{3}$ $V_{in} = \frac{4}{3} \pi d_{in}^{3}$ $\therefore V_{out} = 8.09 \times 10^{-20} \text{ m}^{3}$ Hence, $V_{membrane} = 1.25 \times 10^{-19} \text{ m}^{3}$ $V_{PS-b-PAA} \approx 46 \text{ nm}$ $V_{PS-b-PAA} = \pi (\frac{d}{2})^{2} l$ $V_{PS-b-PAA} = 2.63 \times 10^{-27} \text{ m}^{3}$

Therefore, one polymersome occupies total volume of $V_{\text{membrane}} = 1.25 \times 10^{-19} \text{ m}^3$. As a result, number of polymer molecules in one polymersome $n_{\text{PS-b-PAA}}$;

$$n_{\text{PS-b-PAA}} = \frac{V_{membrane}}{V_{PS-b-PAA}}$$

= 4.76 × 10⁷ molecules
= 7.90 × 10⁻¹⁷ mol
m = 1.42 × 10⁻¹² g (for one polymersome

 $m_{polymersome} = 1.42 \times 10^{-12} \text{ g}$ (for one polymersome).

Estimation of number of polymersomes in reaction sample:

Now, the mass of PS-*b*-PAA in 120 mL for a 1:6 THF:Buffer solution with 1 mg/mL polymer dissolved in THF and $MW_{PS-b-PAA} = 18054$ g/mol is 0.02 mg (6.67 × 10¹⁴ molecules).

Total number of polymersomes (N_{polymersomes}) in reaction sample is then; $N_{polymersomes} = 6.67 \times 10^{14}$ molecules/4.76 × 10⁷ molecules $= 1.40 \times 10^{7}$ polymersomes

Estimation of total encapsulated volume in reaction sample:

As a result, total encapsulated volume in a reaction solution (V_{total inside});

$$V_{\text{total inside}} = N_{\text{polymersomes}} \times V_{\text{in}}$$
$$= 1.13 \times 10^{-12} \text{ m}^3$$
$$= 1.13 \times 10^{-9} \text{ L}$$

Estimation of proton pumping rate:

Initial average internal pH for pH 7.2 buffered experiment was pH 7.28 and increased to pH 7.44 over 3360 s of irradiation (based on titration curve in Fig. S2).

Hence, $\Delta[H^+] = 1.63 \times 10^{-8}$ M protons pumped. Therefore, $n_{H^+} = \Delta[H^+] \times V_{\text{total inside}}$

 $= 1.85 \times 10^{-17} \,\mathrm{mol}$

= 1.12×10^7 H⁺ pumped across membrane over 3360 s.

Rate of proton pumping = $3.33 \times 10^3 \text{ H}^+/\text{s}$.

Calculation of pH using SNARF-5F.

The calibration and determination of pH using SNARF-5F can be measured at two different wavelengths and calculated using equation 1 as recommended by the supplier.³ R is the ratio $F_{\lambda 1}/F_{\lambda 2}$ of fluorescence intensities (F) at two wavelengths λ_1 and λ_2 and the subscripts A and B represent the limiting values at the acidic and basic endpoints of the titration, respectively. A number of fluorescence measurement artifacts are eliminated with this ratiometric method, including photo bleaching, cell thickness, instrument stability and leakage and nonuniform loading of the indicator.

$$[\mathrm{H}^{+}] = \mathrm{pK}_{\mathrm{a}} - \log \left[\frac{\mathrm{R} - \mathrm{R}_{\mathrm{B}}}{\mathrm{R}_{\mathrm{A}} - \mathrm{R}} \times \frac{\mathrm{F}_{\mathrm{B}(\lambda 2)}}{\mathrm{F}_{\mathrm{A}(\lambda 2)}} \right] \quad (1)$$

References

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Fig. S1 Structural characterisation of hybrid proteo-polymersomes (**Ru-cyt***c*:CcOx:HPTS) by transmission electron microscopy and confocal laser scanning microscopy of fluorescently labeled CcOx encapsulated in PS₁₄₀-*b*-PAA₄₈polymersome membrane. (a) Low magnification electron micrograph of proteo-polymersomes at time = 0 min and initial pH 7.2. (b) Electron micrograph of proteo-polymersomes at time 0 min and initial pH 7.2. (367±185 nm). (c) Electron micrograph after irradiation for 24 min using 465 nm light. (273±83 nm). (d) Electron micrograph after irradiation for 56 min using 465 nm light to a final light induced pH gradient of ≈ pH 7.4. (291±74 nm). (e) Transmission light micrograph of fluorescently labelled CcOx encapsulated proteo-polymersomes. (f) Confocal laser scanning micrograph excited at 488 nm (contrast enhanced). All sample measurements were made in a 5 mM sodium dihydrogen phosphate, 5 mM ethylenediaminetetraacetic acid buffer at an initial pH 7.2. For statistical analysis the standard deviation of the average value from *n* = 100 measurements from the TEM micrographs is shown.



Fig. S2. Representative histogram of PS_{140} -*b*-PAA₄₈ polymersome diameters for 7.5 μ M cyt *c*:0.75 μ M CcOx in PBS at 25 °C.



Fig. S3. pH titration curve of HPTS fluorescence intensity emission ($\lambda_{ex} = 460 \text{ nm}$, $\lambda_{em} = 510 \text{ nm}$). Bulk HPTS (**•**) and encapsulated HPTS (**•**) in PS₁₄₀-*b*-PAA₄₈ in 20 mM sodium dihydrogen phosphate. Bulk p $K_a = 7.56$ and encapsulated p $K_a = 7.62$.



Fig. S4. pH titration showing the changes in the excitation spectra ($\lambda_{em} = 510$ nm) of HPTS encapsulated in a diluted (0.003 mg/mL) PS₁₄₀-*b*-PAA₄₈ polymer micelles in 20 mM sodium dihydrogen phosphate with 20 mM ethylenediaminetetraacetic acid. Legend corresponds to pH values.



Fig. S5. Photoreduction scattering from EDTA by-products under various conditions. (a) Excitation spectra of HPTS encapsulated in a poly-L-lysine: PS₁₄₀-*b*-PAA₄₈ polymersome in 5mM sodium dihydrogen phosphate, 5mM EDTA at pH7.2. This experiment shows the characteristic (ratiometric) changes in the HPTS excitation spectra with the 405 nm maxima moving up and the 460 maxima moving down following slight acidification after irradiation of this system in the absence of Ru(II)-bisterpyridine chromophore and hence no EDTA by-product formation after irradiation. (b) Excitation spectra of **Ru-cytc**:CcOx:polymersome in 50mM unbuffered potassium chloride, 5 mM EDTA, pH 7.2. In this experiment, there is an overall increase in excitation spectra baseline due to scattering possibly caused by EDTA by-products from photoreduction resulting in the observed increase at both the 405 nm and 460 nm excitation maxima for HPTS. (c) UV-Vis spectra of bulk **Ru-cytc** after photoreduction in 5mM sodium dihydrogen phosphate, 5mM EDTA, pH 7.2 showing increased scattering due to EDTA by-products. (d) UV-Vis spectra of **Ru-cytc**:polymersome after photoreduction in 5 mM sodium dihydrogen phosphate, 5 mM EDTA, pH 7.2 showing increased scattering due to EDTA by-products.



Fig. S6. pH titration curve of SNARF-5F fluorescence intensity emission determined using a dual-emission ratio with $\lambda_1 = 580$ nm and $\lambda_2 = 640$ nm ($\lambda_{ex} = 514$ nm). Bulk HPTS (**■**) and encapsulated HPTS (**▲**) in PS₁₄₀-*b*-PAA₄₈ in 20 mM sodium dihydrogen phosphate. Bulk pK_a = 7.73 and encapsulated pK_a = 7.60.



Fig. S7. Light-induced transmembrane proton translocation in hybrid proteo-polymersomes. Internal pH monitored as a dual-emission ratio of internal SNARF-5F with λ_1 =580 nm and λ_2 = 640 nm (λ_{ex} = 514nm). (a) Irradiation at an initial pH of 6.3. **Ru-cytc**:CcOx:polymersome Δ [H⁺] = 1.1 × 10⁻⁷ M. Rate of proton pumping estimated to be 2.2±0.4 × 10⁴ H⁺/s.