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# Supplementary Information

## Towards Compartmentalized Photocatalysis: Multiheme Proteins as Transmembrane Molecular Electron Conduits

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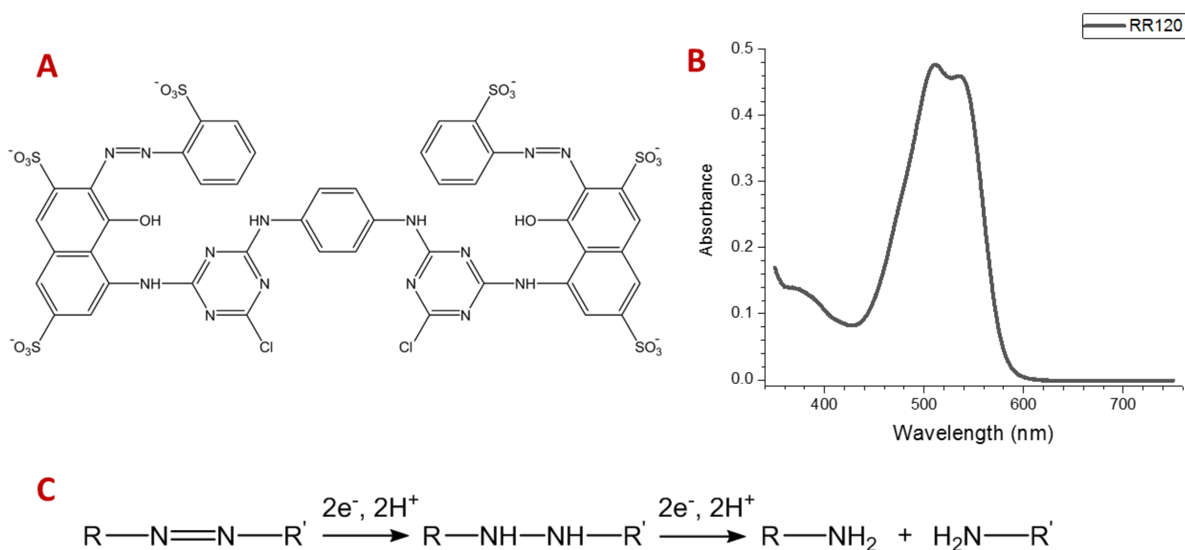


*Figure S1* The chemical structure (**a**) and absorbance spectrum of 15 µM RR120 (**b**). Buffer: 20 mM MOPS, 30 mM $Na_2SO_4$, pH 7.4. (**c**) Reduction reaction of azo groups.

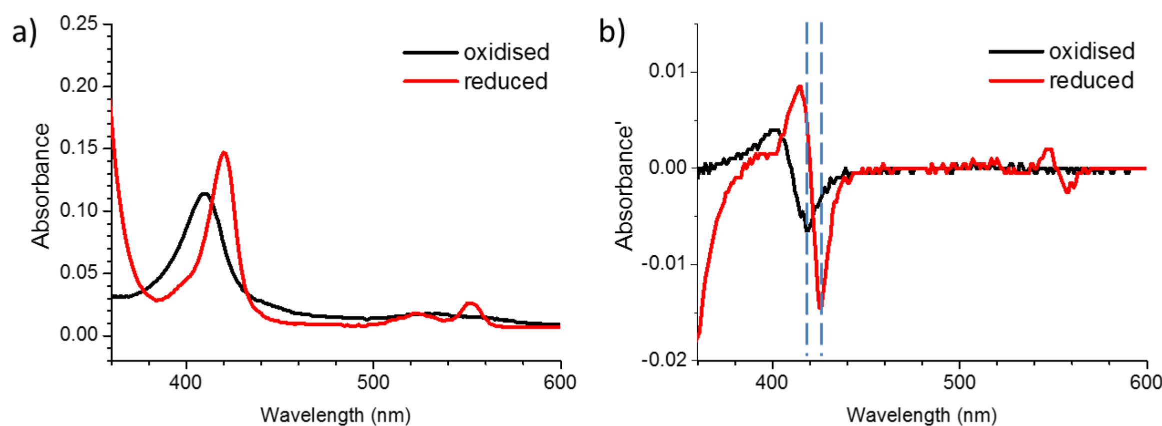


*Figure S2* MtrCAB absorbance (**a**) and the 1st derivative of MtrCAB absorbance (**b**). Black – oxidised MtrCAB, Red – MtrCAB reduced by dithionite. Dashed lines indicate wavelengths selected to assess haem reduction in Figure S4 by calculating the difference between them (i.e., ΔA' = A'418 - A'426)). In this case ΔA' is -0.0035 and 0.02 for oxidised and reduced MtrCAB, respectively.

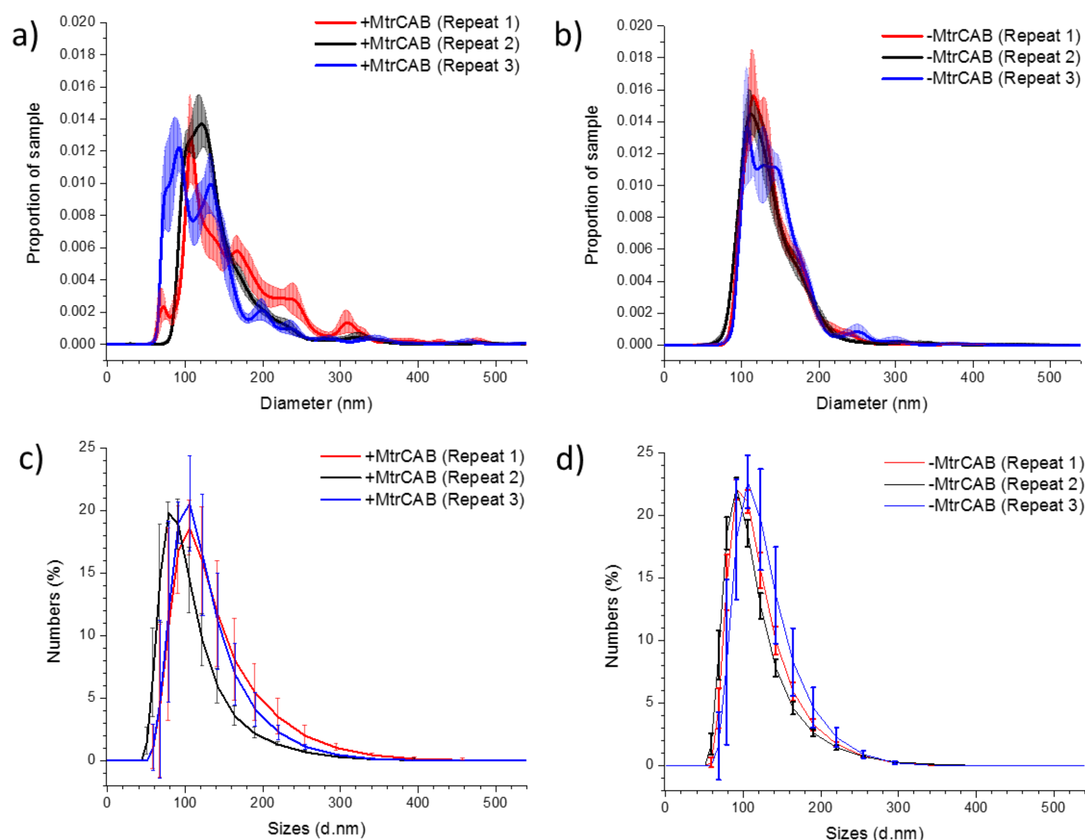


*Figure S3* Size distribution of liposomes with and without MtrCAB (**a, c** and **b, d**, respectively) determined from nanoparticle tracking analysis (NTA) (**a, b**) and dynamic light scattering (DLS) (**c, d**). Shaded area in NTA data represents the standard error, whereas the error bars in DLS represent the standard deviation (n=3). Bin size for NTA is 0.5 nm. Repeats 1 - 3 represent liposomes from three separate preparations.

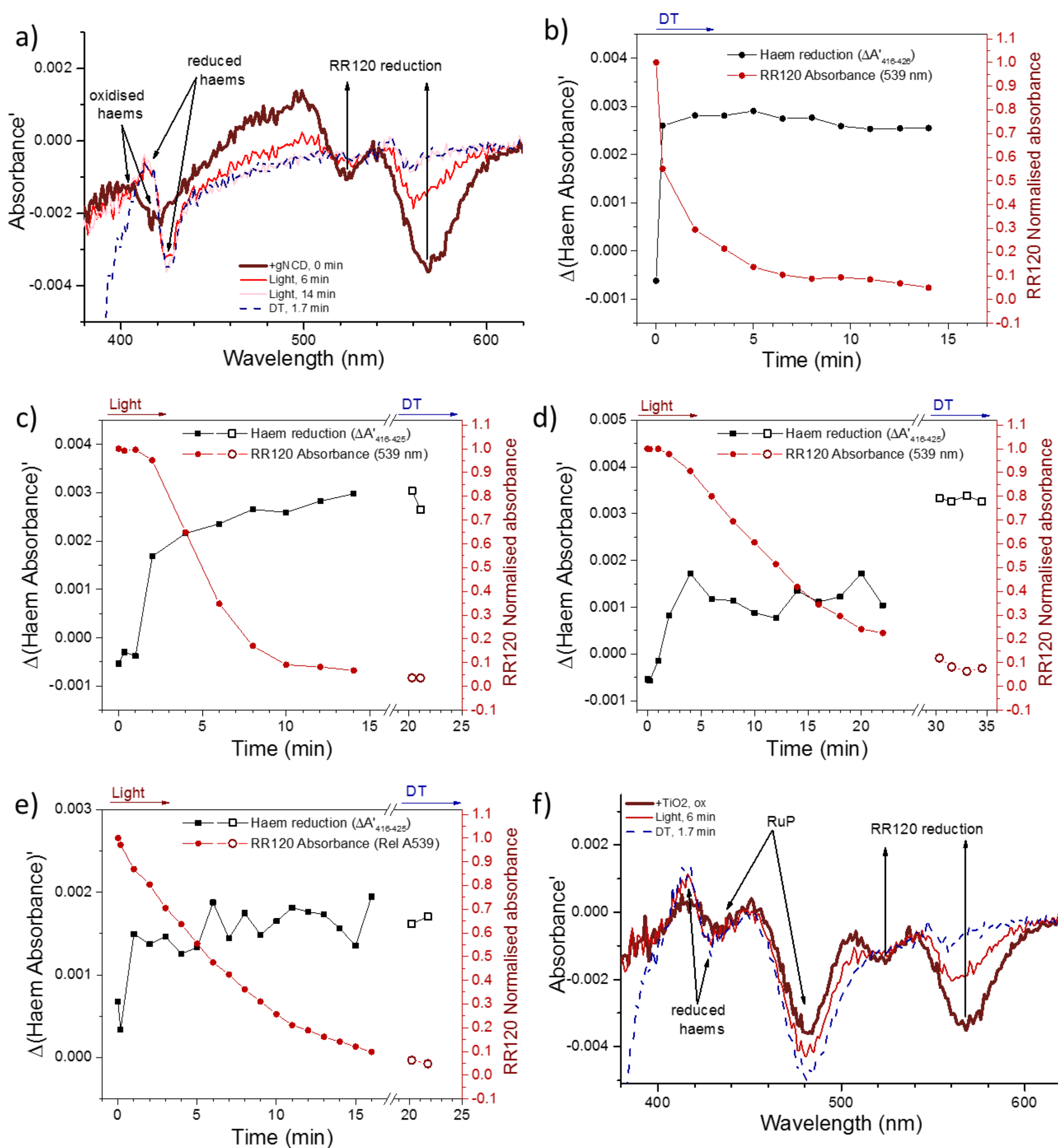


*Figure S4* Reduction of MtrCAB haems and RR120 by DT (**b**), *g*-N-CD (**c**), *a*-CD (**d**) and **RuP**-TiO2 (**e**). (**a**) Example of the 1st derivative taken of the absorbance spectra (A') from *g*-N-CDs photoreduction of MtrCAB proteoliposomes containing RR120. Oxidised and reduced haems have different signatures, and the haem reduction is estimated by calculating the A' difference at the wavelengths corresponding the oxidised and reduced haem minima (416 nm and 426 nm, respectively; see Figure S2). At the start of the experiment MtrCAB is oxidised (dark red line) and gets reduced during illumination (lighter lines); Dashed line – reduced sample after addition of DT. (**b - e**) Photoreduction of MtrCAB haems as observed by changes in the 1st derivative of haem absorbance (scale on the left, black) and photoreduction of RR120 encapsulated in MtrCAB proteoliposomes (scale on the right, red). Time indicates duration of total illumination. DT – dithionite is included as indicator of fully reduced haems. (**f**) Example of the 1st derivative taken of the absorbance spectra from **RuP**-$TiO_2$ photoreduction of MtrCAB proteoliposomes containing RR120. Although, **RuP** absorbance masks the signal of oxidised and reduced haems, the haem reduction was still estimated by calculating the A' difference at 416 nm and 426 nm.

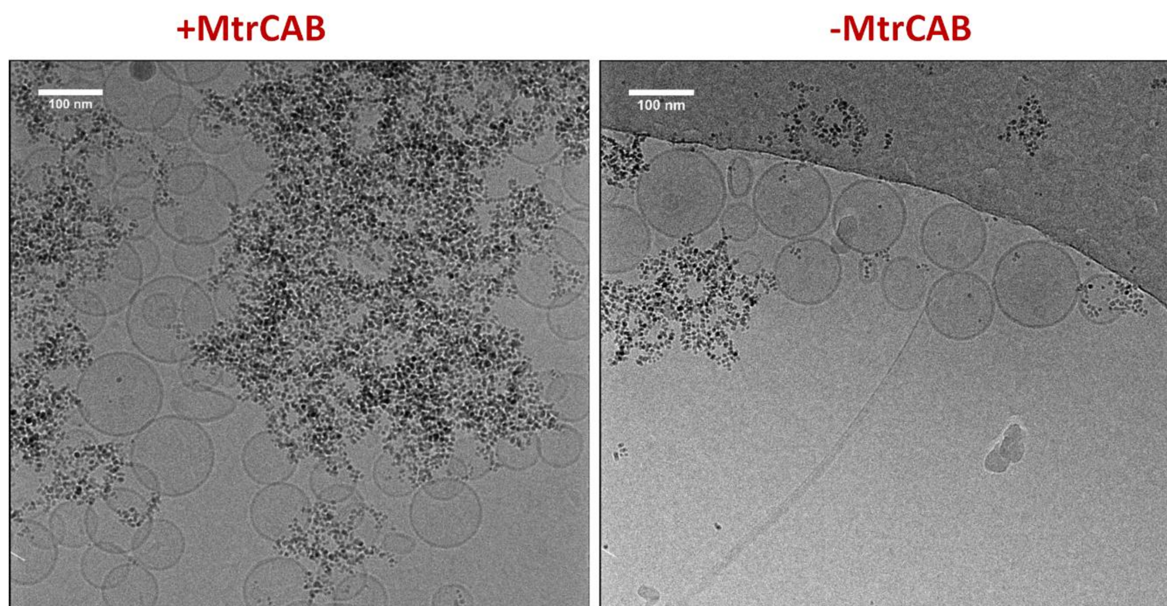


*Figure S5* Cryo-EM of E.coli liposomes with and without reconstituted MtrCAB mixed with **RuP**-$TiO_2$ NPs. **RuP**- $TiO_2$ agglomerates formed within 2-5 min after transfer from water to liposome sample.

## Supplementary Materials and Methods

### Cryo-Electron Microscopy (cryo-EM)

Liposome or **RuP**-$TiO_2$ sample or a sample containing both was placed onto lacey carbon grids with 200µm mesh (Agar Scientific), blotted, and plunged frozen into liquid ethane. Cryo-EM was performed as described elsewhere.[1] In short, cryo-EM was carried out at liquid nitrogen temperatures using an Oxford CT3500 holder and a FEI Tecnai-F20 electron microscope. Images were recorded at 50,000× magnification on a Gatan US4000 CCD camera under low-dose conditions (~20 $e^-/Å^2$).

### Observations of haem reduction state by spectroscopy (Soret band)

Observation of haem absorbance Soret peak is hindered by high background absorbance caused by absorbance and scattering of all three LHNPs and liposomes themselves. Haem difference spectra could not be used due to spectral overlap with changes in RR120 and DT absorbance. As the result, the first derivatives of all spectra were calculated. This allowed to observe different signatures of oxidised and reduced MtrCAB (Figure S2). The haem reduction was then estimated by calculating the A’ difference at the wavelengths corresponding the oxidised and reduced haem minima (416 nm and 426 nm, respectively; Figure S2 and S4).

## References

1 K. C. Dent, R. Thompson, A. M. Barker, J. A. Hiscox, J. N. Barr, P. G. Stockley and N. A. Ranson, *Structure*, 2013, **21**, 1225–1234.