Photosynthesis re-wired on the pico-second timescale

The photodynamics of Photosystems I and II have been extensively studied in vitro using ultrafast TA spectroscopy. In this technique, the sample’s absorbance spectrum is measured by a broadband probe laser after photo-excitation by a pump laser (that is, pump-probe spectroscopy), with access to photodynamics occurring on the subpicosecond timescale. Previously, it has been challenging to apply ultrafast TA spectroscopy to the photosystems in vivo, with plant or algal cells (typically 10 µm in size) being highly scattering. Recently, ultrafast TA spectroscopy was successfully applied to *Nannochloropsis* sp. algal cells (2–5 µm in size), whose small size lessened these scattering effects. Here, we studied *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) cyanobacterial cells (Fig. 1a–c), which is a model organism for studying photosynthesis because of the endosymbiotic origin of the chloroplast in eukaryotic plants and algae. The smaller size of *Synechocystis* cells (less than 2 µm) further reduced the scattering, and the photodynamics of the photosystems in vivo could be resolved using ultrafast TA spectroscopy.

Photo-excitation of *Synechocystis* cells with a 200 fs pump pulse centred at 450 nm revealed rich initial transient spectral features in the visible (550–800 nm). We identified a prominent negative feature centred at 450 nm revealed rich initial transient spectral features in the visible (550–800 nm). We identified a prominent negative feature at 685 nm (Fig. 1d), which decayed within roughly 20 ps to 10% of its initial value. We attribute this feature to the ground-state bleaching of the photoactive reaction centres of PSI and PSII. A lower-energy feature at 715 nm grew within roughly 2 ps after photo-excitation and subsequently decayed on a longer pico-second timescale, indicating rapid excited state relaxation processes. Similarly, we observed higher-energy positive transient features (less than 680 nm), which we assign to contributions from the photoinduced absorption of phycobilisomes (PBSs) and carotenoids, as these features resemble those of in vitro steady state absorption measurements and are absent from isolated photosystems (Supplementary Fig. 7). We verified that the photosystems remained intact without photodamage, consistent over several biological replicates, and were in a closed state throughout these measurements (Supplementary Figs. 1–4). We confirmed that the key spectral features arise from fully assembled photosystems (Supplementary Information section 1.5.4.).

To probe the effect of exogenous electron mediators on the photodynamics of cyanobacterial cells, we repeated the same measurements with the addition of DCBQ. As shown in Fig. 1e, we found that addition of DCBQ led to accelerated decay dynamics of the negative feature at 685 nm. Simultaneously, the signal rise observed at 715 nm was suppressed, with a noticeable effect evident in just 600 fs after photo-excitation. The effect was also observed when pumping at the
band edge of the reaction centres (Supplementary Figs. 5 and 6). These observations demonstrate that DCBQ can alter the excited state decay pathways of the photosynthetic reaction centres in *Synechocystis* cells on a subpicosecond timescale.

Previously, DCBQ has been thought to extract electrons only from the terminal electron acceptor site of PSII, the P680 pocket (Fig. 1c). However, we note that the mid-point potential of DCBQ (?0.315 V versus standard hydrogen electrode (SHE) at pH 7) makes it suitable for extracting electrons from both PSII (P700*/P700 = –1.290 V, Fd/Fd = –0.590 V) and PSII (P680*/P680 = –0.660 V). To rule out energy transfer from the reaction centres to DCBQ as the mechanism resulting in the observations shown in Fig. 1e, we characterized the optical properties of suspensions of cells in the presence of DCBQ. As reported in Fig. 2b, the absorption spectrum of cells includes prominent absorption bands at 450, 680 and 700 nm corresponding primarily to the chlorophylls in the cell’s PSII and PSI complexes, whereas DCBQ absorbs below 300 nm. The lack of overlap in the absorption spectra of DCBQ before reduction and fluorescence of the intact cells indicates that energy transfer mechanisms in the form of Förster resonances are not active. After considering possible quenching mechanisms, we conclude that the effect caused by DCBQ must stem from an electron transfer mechanism (more detail in Supplementary Information section 1.5.5.).

It is known that all chlorophylls embedded within PSII and PSI are energetically degenerate at room temperature. Photo-excitation thus results in a highly delocalized excited state shared across several chlorophylls. Previous studies on isolated reaction centres from the photosynthetic purple non-sulfur bacterium *Rhodobacter sphaeroides* at 77 K showed that the initially excited state shows charge-transfer character and can form intermediate charge-transfer states within 200 fs (ref. 19). Similarly, recent femtosecond crystallography results of the photosynthetic reaction centre of another purple non-sulfur bacterium *Blastochloris viridis* (formerly known as *Rhodopseudomonas viridis*) demonstrated electron transfer reactivity within 3 ps (ref. 19).

In the light of these observations, we postulate that photo-excitation of the chlorophyll pigments within the photosystems of *Synechocystis* at room temperature can also form highly delocalized intermediate charge-transfer states within the time resolution of our measurement (200 fs), followed by electron transfer kinetics. Given that the cell absorption spectra for all DCBQ concentrations remained unchanged (Supplementary Fig. 22), we conclude that DCBQ does not tightly bind to the chlorophylls at the core of the reaction centre. Instead, DCBQ most likely interacts with peripheral chlorophyll pigments protruding from the protein scaffold of the photosystems, thereby interacting with the highly delocalized charge-transfer state formed after the initial photo-excitation (Fig. 2a, also see Supplementary Information for in-depth discussion).

On the basis of this assessment, we constructed a simple kinetic model (Supplementary Fig. 14) and applied global analysis techniques to time-resolved TA data from cells to extract the relevant lifetimes. We then used this model to determine how efficiently DCBQ diverts electrons away from the native electron transfer chain (Supplementary Information section 1.5.3.). As highlighted in Fig. 2c, our analysis yielded declining electron transfer lifetimes for increased DCBQ concentration. On the basis of our model and these values, we can estimate that
1 mM DCBQ diverts 17 ± 6% of the initial photoexcited reaction centre population in wild-type *Synechocystis* cells. Critically, this electron transfer occurs as early as 600 fs (Fig. 1d,e), which is consistent with our hypothesis that electrons can be extracted from rapidly formed, delocalized chlorophyll charge-transfer states.

Further support for this mechanistic picture stems from further control experiments with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which binds to the Q_b pocket in PSII (ref. 21), competing against DCBQ docking and reduction thereafter. Here, the TA measurements with DCBQ and DCMU yielded similar effects to those with only DCBQ, indicating that DCBQ acts at the initial stages of the photosynthetic pathway, rather than only at the Q_b site (Fig. 2d and Supplementary Fig. 15). This trend is also supported by analogous photo-electrochemistry measurements, in which DCBQ was observed to extract electrons at a site that is alternative to the Q_b site (roughly 30% of the photocurrent, Supplementary Fig. 26).

The ability of DCBQ to interact with the photosynthetic electron transport chain at longer timescales was also probed. Photoluminescence decay measurements revealed a reduction in luminescence lifetime with increasing DCBQ concentration, consistent with pulse amplitude modulation fluorometry studies of eukaryotic algal cells in the presence of benzoquinones (Supplementary Fig. 28)3,14,22. This is consistent with DCBQ interfering with the photosynthetic electron transport chain beyond the pico-second timescales explored in TA. Oxygen evolution measurements confirmed that PSII continues to perform oxidation of water in vivo in the presence of DCBQ at all concentrations tested in the TA experiments, suggesting the pathway for holes generated by photo-excitation remains active on the addition of DCBQ (Supplementary Fig. 30). However, a long-term cytotoxicity assay revealed that all concentrations of DCBQ greater than 200 µM are cytotoxic to the cells after 12 h, but there was no correlation between the cytotoxicity of benzoquinones and their ability to re-direct electrons from the electron transport chain (Supplementary Fig. 31).

We tested two other common benzoquinone mediators, phenyl-1,4-quione (PPBQ) and 2,6-dimethyl-1,4-benzoquinone (DMBQ), which have comparable mid-point potentials to DCBQ14,23. Figure 2d outlines that the early onset decay in the TA spectrum found for DCBQ was not observed in PPBQ- and DMBQ-treated cells (Supplementary Information section 1.6., Supplementary Fig. 15). Similarly, complementary photo-electrochemistry experiments highlighted that DCBQ was the only benzoquinone screened that was reduced earlier than Q_b in PSII in vivo, as it mediated electron transfer to electrodes in the presence of DCMU (Supplementary Fig. 26). The observation that PPBQ and DMBQ did not demonstrate early interactions with the reaction centres may originate from poor protein–molecule association effects or their lower solubilities in aqueous solutions, which may lead to the quinones being sequestered in intracellular membranes and precipitating in aqueous compartments as they entered the cell4.
Fig. 3 | Action of DCBQ on cells genetically modified to have only one type of photosystem. a. Schematic representation of photosystem-less mutants analysed (top, PSI-less and bottom, PSII-less). b. TA spectra between ~2 and 20 ps of cell mutants excited at 450 nm under the same conditions as wild-type cells in their TA spectrum in Fig. 1d (top, PSI-less; bottom, PSII-less). The PSII-less spectrum (bottom) closely resembles that of the wild-type cells. Maps are of one sample, representative of several biological replicates.

We then studied the effect of DCBQ addition on these mutant cells, as they most closely resemble our in vivo studies (full details in Supplementary Information section 1.7.). On the addition of 1 mM DCBQ to the PSI-less cells, we found a reduction from 74 to 53 ps in the lifetime of the short component of the signal (Fig. 3c, top panel). This suggests that electron extraction by DCBQ treatment from PSI in PSI-less cells occurs over a much longer timescale than the sub-20 ps quenching dynamics observed in the wild-type cells. By contrast, on the addition of 1 mM DCBQ to PSII-less cells, we found a reduction in the lifetimes that closely resembled the behaviour in wild-type cells.

Measurements of living cells are advantageous compared to the those in vitro systems due to the robustness of the cells and the ability to study altered pathways produced by means of genetic engineering. However, in vitro measurements are useful in identifying spectral features due to their relative simplicity. Measurements of isolated PSI and PSII showed similar spectral features (Supplementary Information section 1.7.) and lifetime reductions compared to mutant cells on the addition of DCBQ, further supporting our assignments and proposed mechanism. Considering the in vitro and in vivo measurements together, we conclude that in the experimental arrangement described here, we primarily resolve electron transfer from PSI to DCBQ. This is in line with the more accessible nature of PSI compared to PSII. Although PSI is also weakly accessible to DCBQ in the PSI-less cells, no corresponding long-lived signal is observed in the wild-type cells that can be directly assigned to PSI with sufficient statistical certainty.

Whereas the fast kinetics of DCBQ reduction by PSI in vivo is desirable, the positive mid-point potentials of benzoquinones limit the power densities they can generate or redox reactions they can catalyse in semi-artificial photosynthesis. To test whether alternative classes of mediators can also extract charge through this non-classical pathway, methyl viologen (MV\textsuperscript{2+}/MV\textsuperscript{-} = −0.325 V versus SHE at pH 7)\textsuperscript{31} was investigated. Methyl viologen is the most common synthetic mediator used in reductive catalysis and could unlock greater energy densities compared to DCBQ while bridging the gap between natural and artificial
photosynthesis. Our results are consistent with methyl viologen also being able to extract charge from PSI chlorophylls at the pico-second timescale in isolated PSI (Supplementary Fig. 20). This demonstrates that ultrafast electron extraction from photosystems is not limited to any one class of mediator molecules and opens new opportunities in mediator design and chemical generation.

Conclusion and outlook

Taken together, our results show that in vivo electron transfer from photosynthesis to various exogenous electron mediators is possible directly from the initially photoexcited states of the photosystems, that is, from the earliest possible step in the photosynthetic electron transport chain. This opens new possibilities for re-wiring biological photosynthesis and creates a link between biological and artificial photosynthesis. For example, manipulation of this non-classical pathway could mitigate the losses associated with light stress in biotechnological and agricultural applications, or help to channel charges out for alternative uses in power and chemical generation. Furthermore, these results call for a re-examination of mediated electron transfer strategies, which have long been used to study photosynthesis as well as for enhancing the performance of bioelectrochemical devices such as biophotovoltaics.

We also show that the pico-second charge-extraction pathway is not limited to one class of mediators, suggesting that the bioengineering of endogenous mediators, cascades and other biohybrid approaches could be used in the future to expand on and optimize this pathway. These results also yield new insights into photosynthesis. Contrary to the current understanding that the excited reaction centres are insulated within the photosystem protein scaffold, the action of DCBQ and methyl viologen demonstrate that the scaffold is leaky. This is a potentially significant route in the cell for loss of electrons that might otherwise be used for photosynthesis and may have a role in photo-protection or be a key cause of cell damage from production of reactive oxygen species. More generally, our work highlights that in vivo ultrafast TA spectroscopic measurements are feasible and shows rich information on photoexcited dynamics of living systems. This could be a powerful new tool for understanding photosynthesis bioenergetics and its regulation, especially under conditions of light stress.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information, details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-023-05763-9.
The PSII stock had a chlorophyll concentration of 2.4 mg ml$^{-1}$ in solid medium. Culture growth was measured by attenuation at 750 nm (optical density, OD$_{750}$). Culture chlorophyll concentration (nmol Chl a ml$^{-1}$) was calculated from absorbances at 680 and 750 nm: $(A_{680} - A_{750}) \times 10.814$ (ref. 40). All measurements were taken using a UV-1800 Spectrophotometer (Shimadzu).

Photosystem-less mutants. The modifications to the culturing protocol for the Synechocystis photosystem-less mutants are as follows. Wild-type cells were cultured in BG1I with no additives at 30 °C with 40 μmol$_{photon}$ m$^{-2}$ s$^{-1}$ light. PSI-less (psaAB$^-$), with 10 μg ml$^{-1}$ chloramphenicol, were cultured in BG1I plus 5 mM glucose, at 30 °C and 5 μmol$_{photon}$ m$^{-2}$ s$^{-1}$ light. PSI-less cells (psbDIC/psbDII$^-$) with 10 μg ml$^{-1}$ chloramphenicol and 10 μg ml$^{-1}$ spectinomycin, were cultured in BG1I plus 5 mM glucose at 30 °C with 40 μmol$_{photon}$ m$^{-2}$ s$^{-1}$ light$^4$. Olive mutant. The Synechocystis Olive mutant was generated previously by markerless disruption of the cpcBAC122 genes$^4$. As reported previously, the Olive mutant has no phycocyanin discs in the PBS. The Olive mutant still has the allophycocyanin core of the PBS. Deletion of the entire PBS results in extremely poor growth$^{41}$, so this mutant was not analysed in this study.

Isolated photosystems. In this study, whole cells of cyanobacterium Synechocystis were compared against PSI and PSII protein complexes isolated from the cyanobacterium Thermosynechococcus elongatus. PSII isolated from mesophilic Synechocystis is significantly more unstable than those extracted from T. elongatus. Previous studies have compared the photo-electrochemistry of PSI in vitro versus in vivo from T. elongatus and Synechocystis, respectively, for similar stability reasons. The purified T. elongatus PSI was provided by the group of A.W. Rutherford (Imperial College London, UK). PSI dimers were isolated from T. elongatus using methods previously reported$^{43}$. The PSI11 stock had a chlorophyll concentration of 2.4 mg ml$^{-1}$ in 2-(N-morpholino)ethanesulphonic acid (MES) storage buffer (10% glycerol, 15 mM MgCl$_2$, 15 mM CaCl$_2$, 1 M betaine, 40 mM MES (pH 6.5)). Purified PSI trimers were isolated from T. elongatus using methods previously reported$^{41}$. The PSI11 stock had a chlorophyll concentration of 9.07 mg ml$^{-1}$ in MES storage buffer. Isolated photosystems were stored in small aliquots in a liquid N$_2$ Dewar flask, and a new aliquot was thawed on each day of experiments. Photo-electrochemistry and spectroscopy measurements on isolated photosystems were conducted in MES buffer (50 mM KCl, 15 mM CaCl$_2$, 15 mM MgCl$_2$, 40 mM MES (pH 6.5)). The T. elongatus was cultured at 60 °C, the spectroscopy suite was maintained at less than 21 °C and the photo-electrochemistry set-up was controlled at 25 °C. Preparation methods for spectroscopy has been reported previously$^{41}$.

Chemicals

Unless otherwise stated, all materials used throughout this research were purchased from commercial suppliers and used without further purification. The exogenous electron mediators used in this study were (Supplementary Fig. 32): DCBQ (98%, Sigma-Aldrich), PBQ (95%, ACROS Organics), DMBQ (97% Sigma-Aldrich) and MV$^{2+}$ (Sigma-Aldrich). Benzoinone candidates were selected from previous studies with Chlamydomonas reinhardtii by the group of F. Lemaître$^{13,22,46}$. The photosynthetic electron transport chain inhibitor used in this study was DCMU (98%, Alfa Aesar). DCMU was used as an inhibitor of PSII at the Q$_e$ site$^{21}$. Stocks of 100 mM DCBQ, 100 mM MV$^{2+}$ and 100 mM DCMU were made in dimethylsulfoxide (DMSO) for addition to biological samples. Stocks of 10 mM DMBQ and PBQ were made in ethanol for addition to biological samples, with 8.2 mM SDS where specified.

Ultrafast TA spectroscopy

Pico-second TA measurements were performed as previously reported$^{24,48}$. In brief, we used an Yb-based amplified system (PHAROS, Light Conversion) providing 14.5 W at 1,030 nm and a 38 kHz repetition rate. The probe beam is generated by focusing a portion of the fundamental in a 4 mm YAG substrate to generate a white light that spans 520 to 900 nm. The pump beam is generated by seedling a portion of the fundamental to a narrow band optical parametric oscillator (ORPHEUS-LYRA, Light Conversion). The pump pulse was set to 450 nm, unless otherwise indicated. The sample solutions were placed in 1 mm path length cuvettes (Hellma). The pump and probe beams were focused to sizes of 280 × 240 and 55 × 67 μm, respectively. The probe is delayed relative to the pump using a computer-controlled translation stage (Newport), and a chopper wheel (Thorlabs) modulated the pump beam to gain access to differential TA spectra. The probe pulses transmitted through the sample were detected on a single-shot basis by a line camera (Stemmer Imaging). All measurements presented in our work were acquired within 15 min of loading the sample. Further control experiments exposed the cells to the laser for 120 min (eightfold longer).

Time-correlated single-photon counting

To record the time-resolved photoluminescence decay of the samples, time-correlated single-photon counting (TCSPC) was performed. Samples were excited with a pulsed laser (PicOQuant LDH-400-B and LDH-470-B (at up to 40 MHz, typically operated at 2.5 MHz)) at either 407 or 470 nm, with the resulting photoluminescence decay collected on a 680 ± 10 nm. Diode lasers were controlled by a trigger box/pump supply unit (PDL 800-B, PicOQuant). TCSPC used an emission spectrometer (Lifescpec-ps unit, VTC900 PCI card, Edinburgh Instruments) with a multi-channel plate detector (R3809U-50, Hamamatsu). The instrument response was determined by scattering excitation light into the detector using a piece of frosted glass; a value of 265 ps was obtained. Pulse energy was 2 pJ. Pulse width of the diode lasers was measured to be 80 ps (full-width at half-maximum).

Spectroelectrochemistry

Solutions of DCBQ (1 mM) in BG1I medium and MES buffer were prepared. The DCBQ solution (1 ml) was loaded into an optically transparent thin-layer electrochemical cuvette with a pseudo reference electrode (Ag wire). Cyclic voltammetry was performed (applied potential 0.5 to −0.2 V versus pseudo reference Ag wire, scan rate: 0.05 V s$^{-1}$) to determine that the working electrode poised at −0.1 V would reduce the DCBQ. The ultraviolet-visible light spectrometer was baselined with a solution of 1% DMSO (v/v) in BG1I medium or MES buffer as appropriate. The spectrum of the DCBQ solution was measured without applying a potential (neutral form). Chronoamperometry experiments were performed at −0.1 V. At 30 min into the chronoamperometry, the spectrum of the DCBQ (doubly reduced form) solution was measured.

Photo-electrochemistry

All photoelectrochemical measurements were performed at 25 °C under atmospheric conditions using an Ivium Technologies Compact-Stat, with an Ag/AgCl (saturated KCl) reference electrode (corrected by +0.197 V for SHE) and a platinum mesh counter electrode. Chronoamperometry experiments were performed at an applied potential 0.5 V versus SHE. Chronoamperometry experiments were performed at a sampling rate of 1 s$^{-1}$, under light and dark cycles using a collimated
LED light source (50 µmol photons m⁻² s⁻¹, roughly 1 mW cm⁻² equivalent, 680 nm, Thorlabs).

Protein-film photo-electrochemistry. Inverse opal indium-tin oxide (IO-ITO) electrodes with 750 nm pore sizes were prepared using a previously reported method. Protein-film photo-electrochemistry was performed as previously reported. In brief, a one-in-three dilution of the 2.4 mg ml⁻¹ (77 µM) stock solution of isolated PSII was made immediately before adsorption on the electrodes to give a final concentration of 25.6 µM. A small aliquot (1 µl) of the new solution was drop-cast onto 750 nm IO-ITO electrodes and left to stand for 15 min in a closed Petri dish in the dark before being used in photoelectrochemical experiments. The electrolyte was MES buffer (pH 6.5). Light/dark cycles (15 s on/15 s off) were used.

Cell photo-electrochemistry. IO-ITO electrodes with 10 µm macropores and 3 µm interconnecting channels at a thickness of 40 µm were prepared using the method previously reported. Photo-electrochemistry of cyanobacterial cells was performed as previously reported. In brief, planktonic cultures of early stationary phase cyanobacterial cells at an attenuation at OD₅₇₀nm of around 1 were concentrated by centrifugation at 5,000 x g for 10 min, the supernatant removed, and the pellet resuspended in fresh BG11 medium to a concentration of 150 nmoI Chl a ml⁻¹. This solution (250 µl) was drop-cast onto the IO-ITO electrodes and left overnight at room temperature in a humid chamber in the dark to allow cell penetration and adhesion, yielding cell-loaded electrodes that were used for analysis 16 h later. The electrolyte was BG11 medium (pH 8.5). Light/dark cycles (60 s on/90 s off) were used.

Oxygen evolution measurements

Oxygen evolution measurements were made using a Clark electrode consisting of an Oxygraph Plus Electrode Control Unit, S1 Oxygen Electrode Disc, DW2/2 Electrode Chamber and a LED1 High Intensity LED Light Source (Hansatech Instruments). Measurements were performed on 1.5 ml samples of wild-type Synechocystis cells containing 10 µg ml⁻¹ of Chl a in BG11 supplemented with different concentrations of DCBQ. Measurements were collected at 25 °C with 1 min of darkness, followed by 1 min of 1,500 µmol photons m⁻² s⁻¹ light at 627 nm (equivalent to 28.65 mW cm⁻²). The rate of oxygen production in the dark was subtracted from that in the light and normalized to Chl a content. Data were collected from five biological replicates, each with three technical replicates.

Cytotoxicity assays

Wild-type Synechocystis cells (5 nmoI Chl a) were incubated with different concentrations of exogenous mediator for 24 h. Synechocystis cells incubated in BG11 with no electron mediator or in BG11 with 5% (v/v) DMSO solvent under the same conditions were used as controls. Following incubation, the cells were resuspended in fresh BG11 and their concentration was standardized to an OD₅₇₀nm of 0.5. Aliquots (10 µl) of three serial dilutions (x1, x10⁻³, x10⁻⁶) were spotted on BG11 agar plates, which were then incubated for 1 week at 30 °C and 50 µmol photons m⁻² s⁻¹ light. The growth of the cells pre-incubated with mediator was compared to the controls to assess the cytotoxicity of the mediators.

Statistics

The ratios of the lifetime of samples on the addition of benzoquinones and DCMU compared to when nothing was added were compared using Student’s t-tests.

Data availability

The data underlying all figures in the main text are publicly available from the University of Cambridge repository at https://doi.org/10.17863/CAM.92167.

Code availability

All code used in this work is available from the corresponding authors upon reasonable request.

3. Stanier, R. Y., Kunisawa, R., Mandel, M. & Cohen-Bazire, G. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35, 171–205 (1971).

4. Lea-Smith, D. J. et al. Thylakoid terminal oxidases are essential for the cyanobacterium Synechocystis sp. PCC 6803 to survive rapidly changing light intensities. Plant Physiol. 162, 484–495 (2013).

5. Lea-Smith, D. J. et al. Phycobilisome-deficient strains of Synechocystis sp PCC 6803 have reduced size and require carbon-limiting conditions to exhibit enhanced productivity. Plant Physiol. 165, 705–714 (2014).

6. Zhang, J. Z. et al. Photoelectrochemistry of photosystem II in vitro vs in vivo. J. Am. Chem. Soc. 140, 6–9 (2018).

7. Mersch, D. et al. Wiring of photosystem II to hydrogenase for photoelectrocatalytic water splitting. J. Am. Chem. Soc. 137, 8541–8549 (2015).

8. El-Mohsnawy, E. et al. Structure and function of intact photosystem 1 monomers from the cyanobacterium Thermosynechococcus elongatus. Biochemistry 49, 4740–4751 (2010).

9. Paul, N. Intermolecular Photophysics of Photosystems II Core Complexes at Protein-Nanomaterial Interfaces. PhD thesis, Univ. Cambridge (2015).

10. Longatte, G., Rappaport, F., Wollman, F.-A., Guille-Collignon, M. & Lemaître, F. Mechanism and analyses for extracting photosynthetic electrons using exogenous quinones—what makes a good extraction pathway? Photochem. Photobiol. Sci. 15, 969–979 (2016).

11. Pandya, R., MacQueen, R. W., Rao, A. & Davies, N. J. I. K. Simple and robust parsimonious light harvesting antennacomposites via FRET engineering in solid state host matrices. J. Phys. Chem. C. 122, 223330–223338 (2018).

12. Hinrichsen, T. F. et al. Long-lived and disorder-free charge transfer states enable endothermic charge separation in efficient non-fullerene organic solar cells. Nat. Commun. 11, 5617 (2020).

13. Wey, L. T. et al. A biophotocatalytic approach to unravelling the role of cyanobacterial cell structures in exoelectrogenesis. Electrochem. Acta 395, 138214 (2021).

Acknowledgements

We acknowledge W. Vermaas (Arizona State University, USA) for the gift of the photosystem-less mutants used in this study and W. Rutherford (Imperial College London) for the gift of isolated PSII as well as valuable discussions on this project. We thank X. Chen for provision of porous electrodes. We acknowledge F. Lemaître (École Normale Supérieure, France) and P. Rich (University College of London, UK) for helpful discussions about exogenous benzoquinones and photosynthetic microorganisms. We thank K. Redding for helpful discussions on photocysted states of reaction centre proteins. C.S. and T.K.B. thank V. Gray for insightful discussion at the start of the project. We acknowledge N. Paul for his PhD work, which contributed ideas to this study. T.K.B. gives thanks to the Centre for Doctoral Training in New and Sustainable Photovoltaics (grant no. EP/L01551X/2) and the NanoDTC (grant no. EP/L015978/1) for financial support. T.W. acknowledges financial support from the Cambridge Trust. C.S. acknowledges financial support from the Royal Commission of the Exhibition of 1851. We acknowledge financial support from the EPSRC (grant no. EP/R001982/1 to J.Z.Z.), the BBSRC (grant no. BB/R011923/1 to J.Z.Z.) and the NanoDTC (grant no. EP/L015978/1 to J.Z.Z.) and the University of Cambridge repository at https://doi.org/10.17863/CAM.92167.

Author contributions

T.K.B. and L.T.W. contributed equally to the work and initially developed the application of ultrafast techniques to examine cyanobacteria. C.S. and T.K.B. supervised the cell work, J.Z.Z. supervised the research question. T.K.B. performed the TA and TCSPC experiments and the analysis, and prepared the figures. L.T.W. chose and prepared the samples for TA and TCSPC, performed the photo-electrochemistry, oxygen evolution, cytotoxicity and microscopy experiments, and did protein crystal structure analysis. J.M.L. prepared samples for the MV⁺ study. H.M. prepared isolated PSI. T.K.B., L.T.W., C.S., M.M.N., R.H.F., E.R., C.S., J.Z.Z., C.J.H. and A.R. contributed to discussions, analysis and writing of the manuscript.

Competing interests

The authors declare no competing interests.