Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Information

Phenazines as model low-midpoint potential electron shuttles for photosynthetic bioelectrochemical systems

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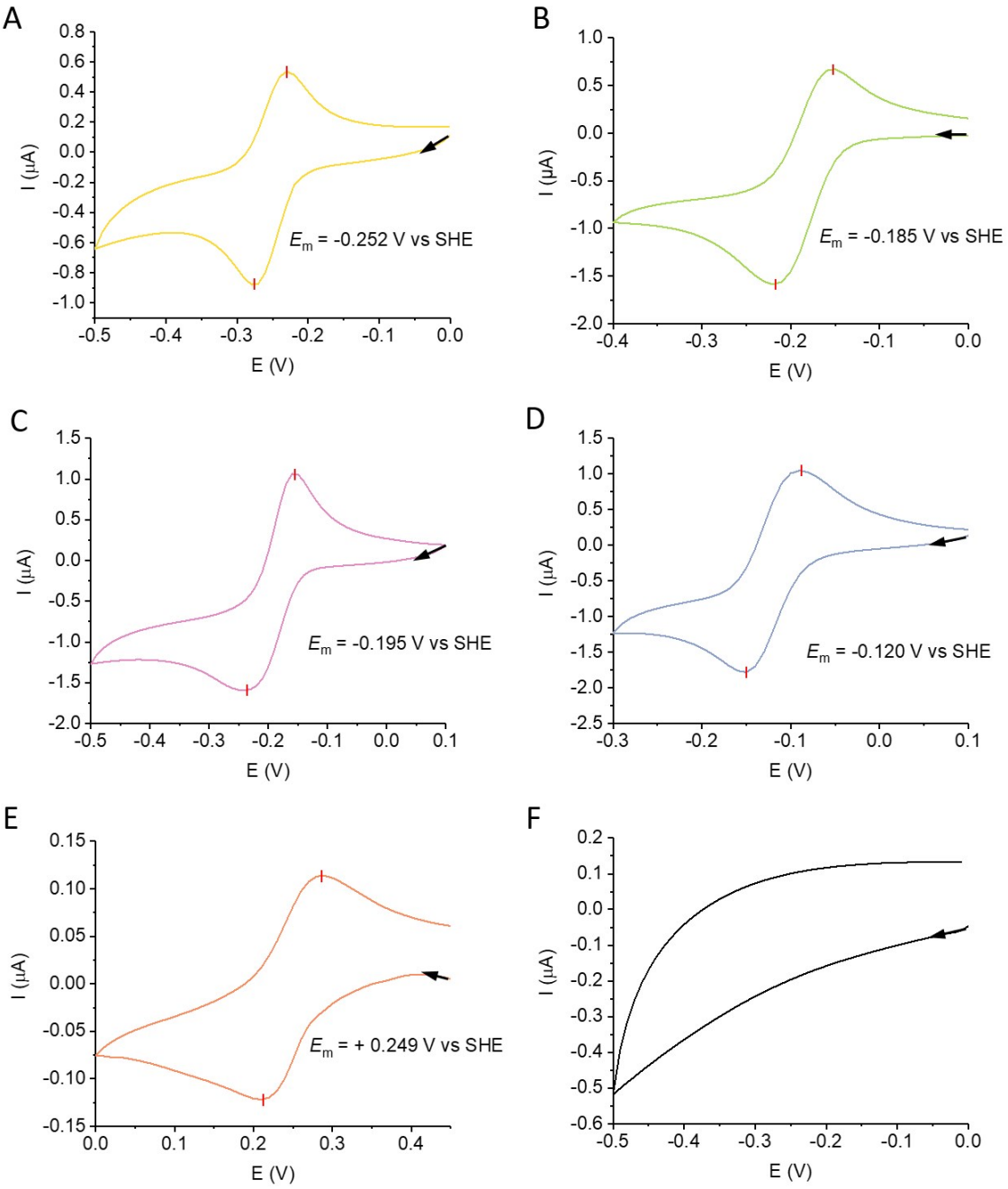

Figure S1. Cyclic voltammogram (CV) of **A)** phenazine (PHZ), **B)** 1-hydroxyphenazine (1-OHP), **C)** phenazine-1-carboxylic acid (PCA), **D)** pyocyanin (PYO), **E)** 2,6-dichloro-1,4-benzoquinone (DCBQ), and **F)** BG11 electrolyte only. CVs were recorded with a mediator concentration of 200 µM, in BG11 (~pH 8.5) at 25 °C, using a glassy carbon working electrode (diameter = 3 mm), and a scan rate of 10 mV s^{-1}. In all cases, the electrolyte was bubbled with N_2 for 20 min before the measurements were taken, and a stream of N_2 was maintained in the headspace of the electrochemical cell during measurements.

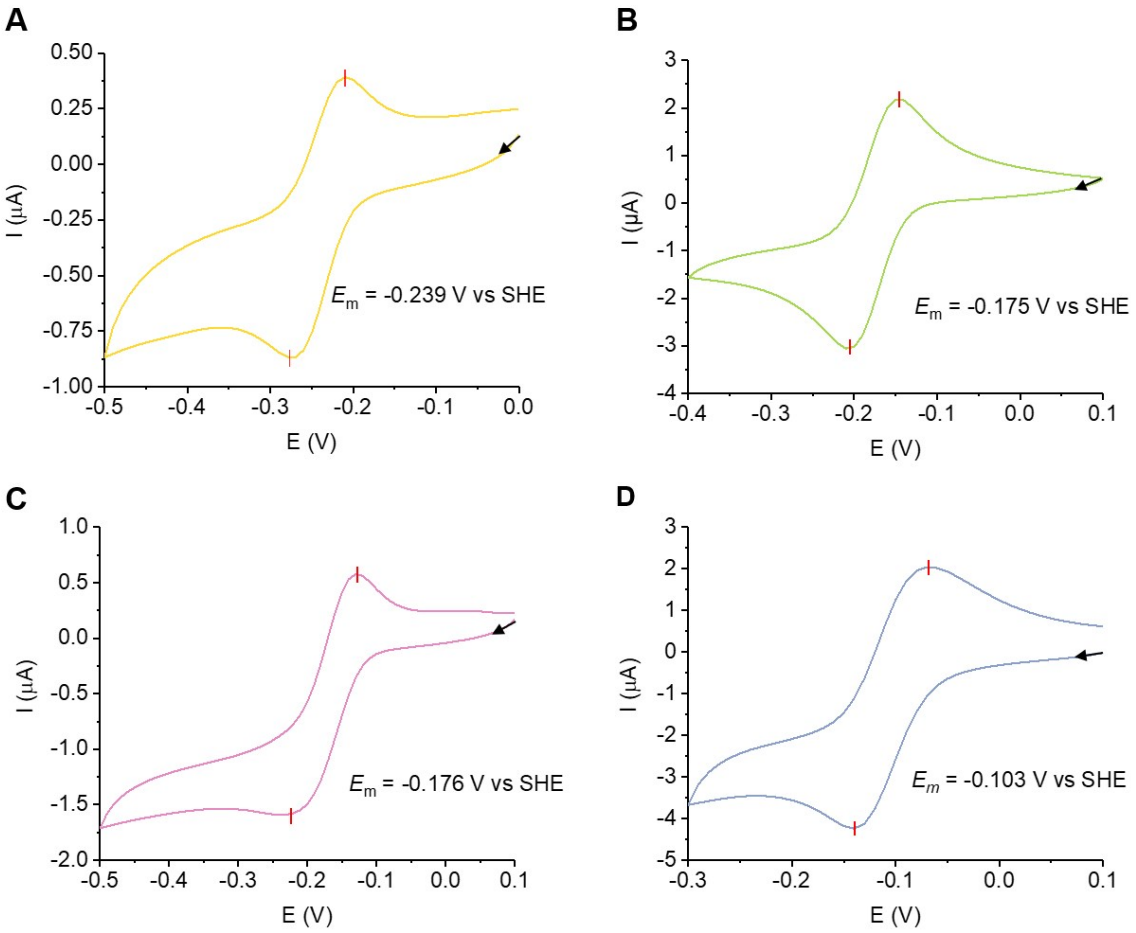

Figure S2. Cyclic voltammogram (CV) of **A)** phenazine (PHZ), **B)** 1-hydroxyphenazine (1-OHP), **C)** phenazine-1-carboxylic acid (PCA), **D)** pyocyanin (PYO). CVs were recorded with a mediator concentration of 200 µM, in pH 7 BG11 at 25 °C, using a glassy carbon working electrode (diameter = 3 mm), and a scan rate of 10 mV s^{-1}. In all cases, the electrolyte was bubbled with N_2 for 20 min before the measurements were taken, and a stream of N_2 was maintained in the headspace of the electrochemical cell during measurements.

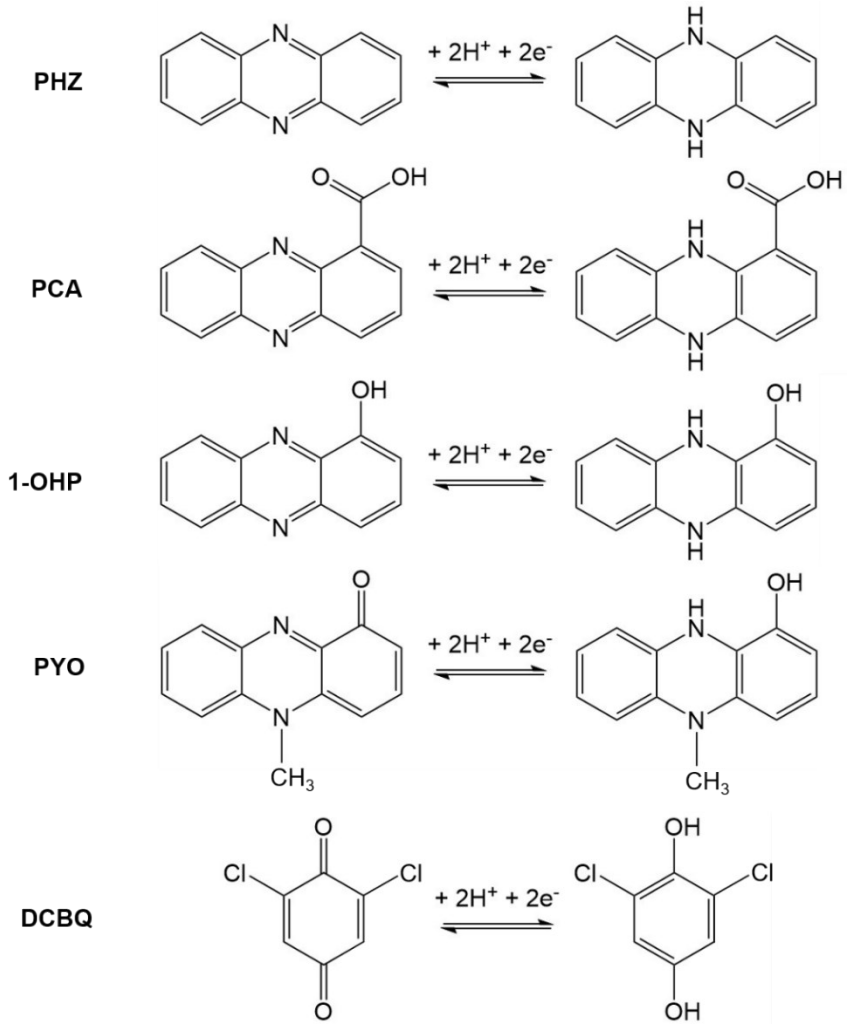

Figure S3. Schematic of the electrochemical reactions of PHZ, PCA, 1-OHP, PYO and DCBQ.

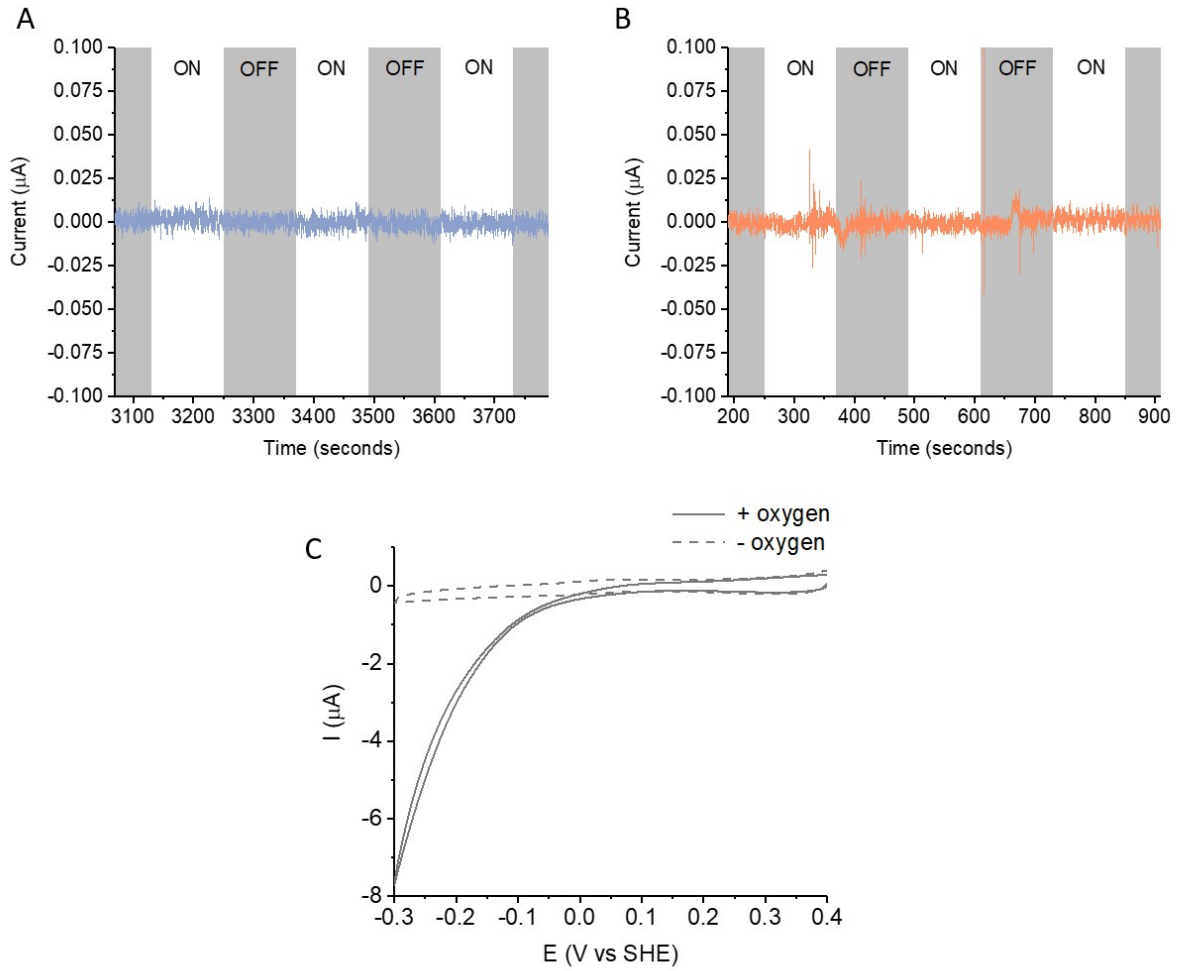

Figure S4. Chronoamperometry of a blank IO-ITO electrode in **A)** 200 µM PYO and **B)** 200 µM DCBQ. Chronoamperometry measurements were recorded at applied potential of 0.3 V vs SHE for PYO and 0.5 V vs SHE DCBQ, with dark/light cycles (1 mW cm^{-2} (50 µE m^{-2} s^{-1}), 680 nm), at 25˚C. Currents are shown with background correction. **C)** Cyclic voltammogram of BG11 electrolyte (~pH 8.5) with and without O_2. CVs were recorded at 25 °C, using an ITO working electrode and a scan rate of 10 mV s^{-1}. To remove oxygen from the system, the electrolyte was bubbled with N_2 for 20 min before the measurements were taken and a stream of N_2 was maintained in the headspace of the electrochemical cell during measurements.

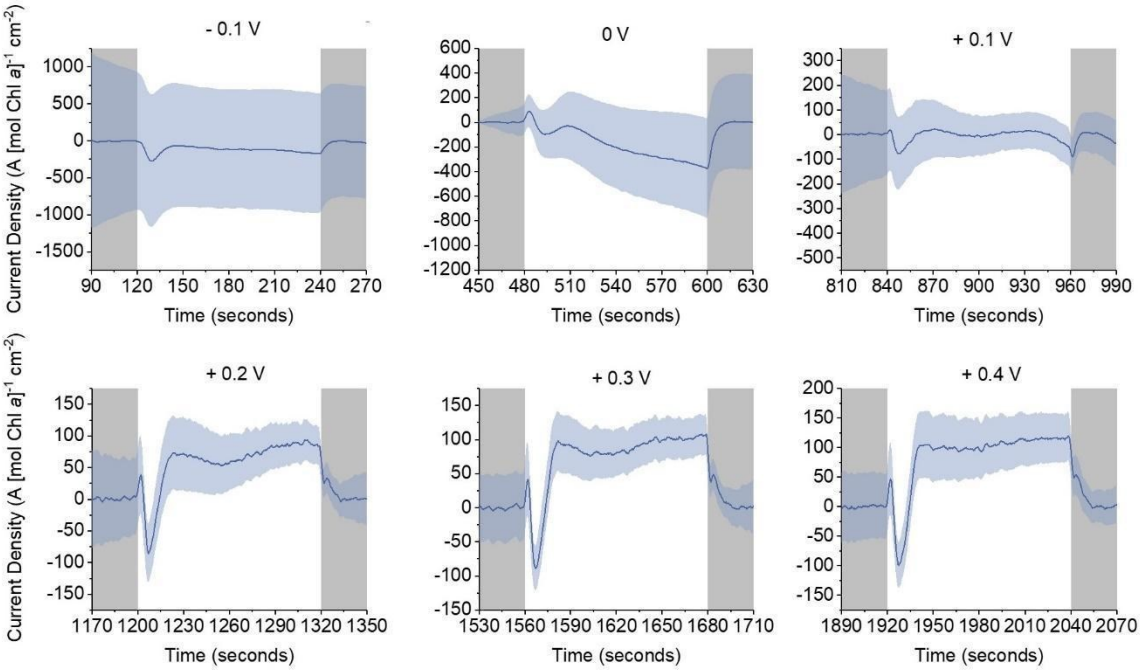

Figure S5. Background corrected chronoamperometry profiles obtained under dark/light cycles at stepped applied potentials (V vs SHE) recorded using *Synechocystis*-loaded IO-ITO electrodes in 200 µM PYO in BG11 electrolyte. Prior to the experiment the electrolyte was bubbled with N_2 gas for 20 minutes to purge the system of O_2, and a stream of N_2 was maintained in the airspace during the experiment. Data were recorded in the dark with 1 mW cm^{-2} (50 µE m^{-2} s^{-1}) of 685 nm wavelength light. Light/dark cycles of 2 minutes off, 2 minutes on, 2 minutes off at each potential. Data was collected from 3 biological replicates and error bars represent standard error of the mean (n=3).

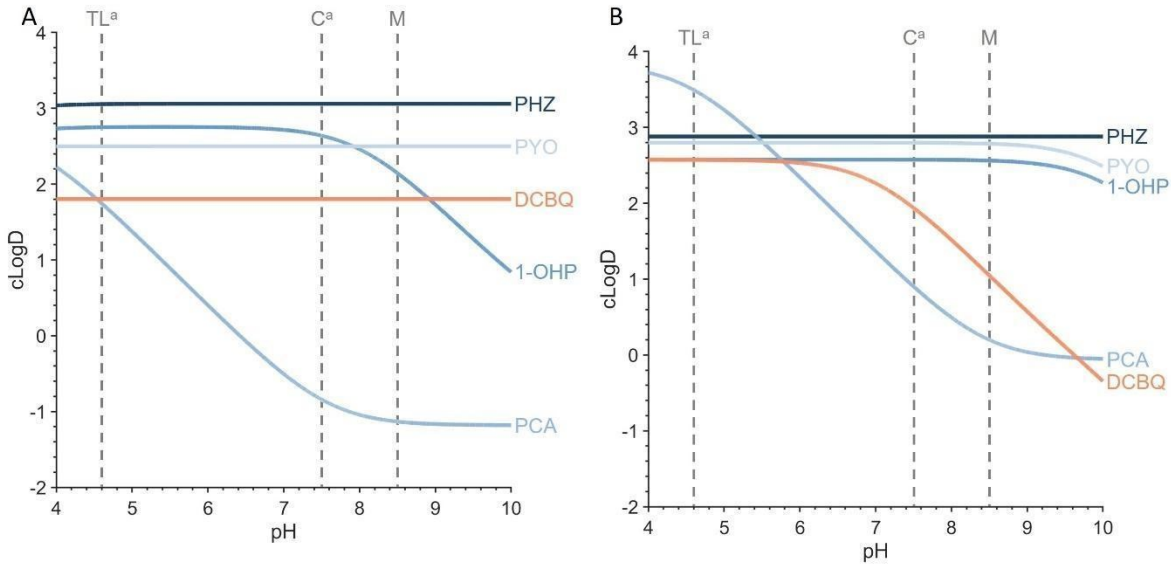

Figure S6. Predicted membrane permeability of mediators in their **A)** oxidised and **B)** reduced state at different pH values. LogD values were calculated in ChemAxon Marvin. pH of the thylakoid lumen (TL), cytoplasm (C) and BG-11 media (M) are labelled. [a]Value from Falkner et al.[1]

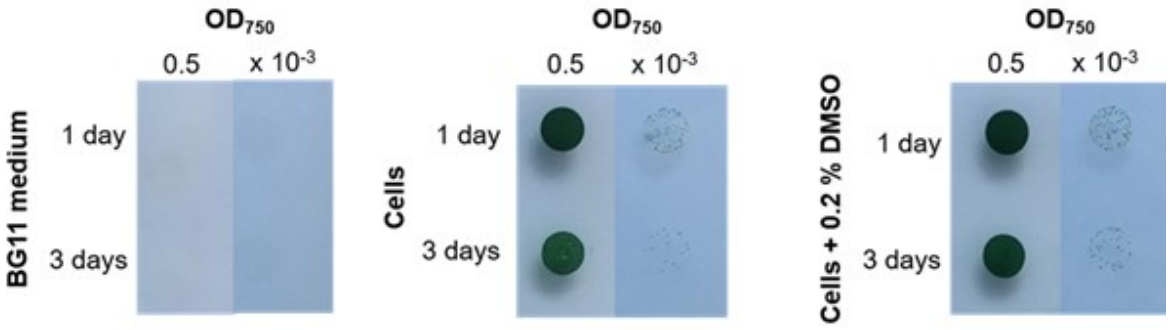

Figure S7. Cytotoxicity spot assay controls for Fig, 3B: BG11 only (no cells), *Synechocystis* cells in BG11 (no mediator), and cells in BG11 with 0.2 % (v/v) DMSO (no mediator) were incubated for 1 or 3 days under 1 mW m^{-2} (50 µE m^{-2} s^{-1}) white light. The cells were resuspended in BG11 following incubation and their concentration standardized to an OD_{750} of 0.5. Serial dilutions were prepared, spotted on BG11 agar and incubated for 1 week. Data from a single representative plate from 3 replicates is shown.

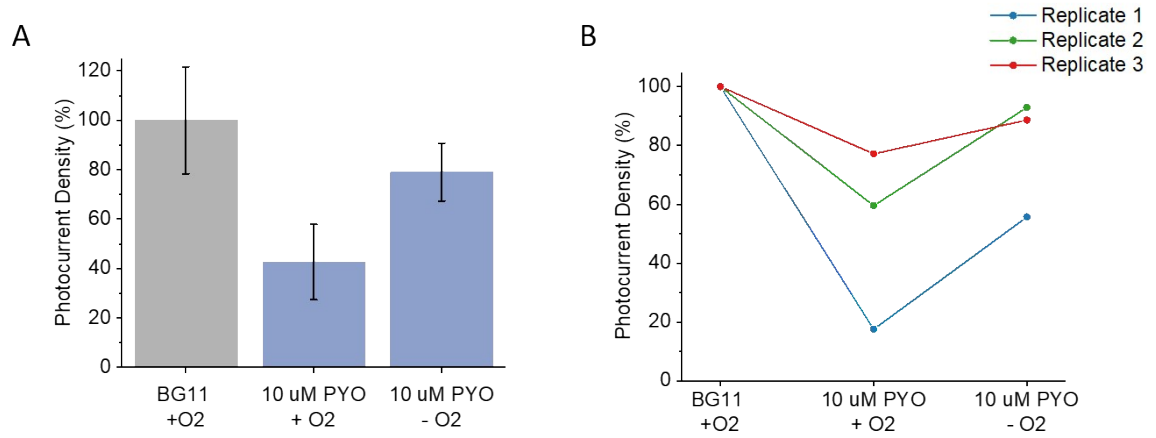

Figure S8. Photocurrent densities stemming from *Synechocystis* in 10 µM PYO in atmospheric conditions (+ O_2) or with nitrogen bubbling (− O_2), expressed as a percentage of the BG11 only photocurrent. To purge the system of O_2 a stream of N_2 gas was bubbled through the electrolyte for at least 20 minutes prior to the experiment and a stream of N_2 was maintained in the headspace during the experiment. Chronoamperometry measurements were recorded at an applied potential of 0.3 V SHE and with dark/light cycles (1 mW cm-2 (50 µE m^{-2} s^{-1}), 680 nm), at 25˚C. **A)** Mean photocurrent densities. For 10 µM PYO + O_2, the mean photocurrent density from 4 biological replicates is shown and error bars represent standard error of the mean. For 10 µM PYO + N_2, the mean photocurrent density from 3 biological replicates is shown and error bars represent standard error of the mean. **B)** Photocurrent densities of 3 biological replicates, shown individually. Each data point represents the mean photocurrent density of 3 technical replicates. Note that the photocurrent magnitude is highly dependent on O_2 concentration in the electrolyte, which is difficult to keep constant when oxygenic photosynthetic microorganisms are present. This caused the large variance in the data.

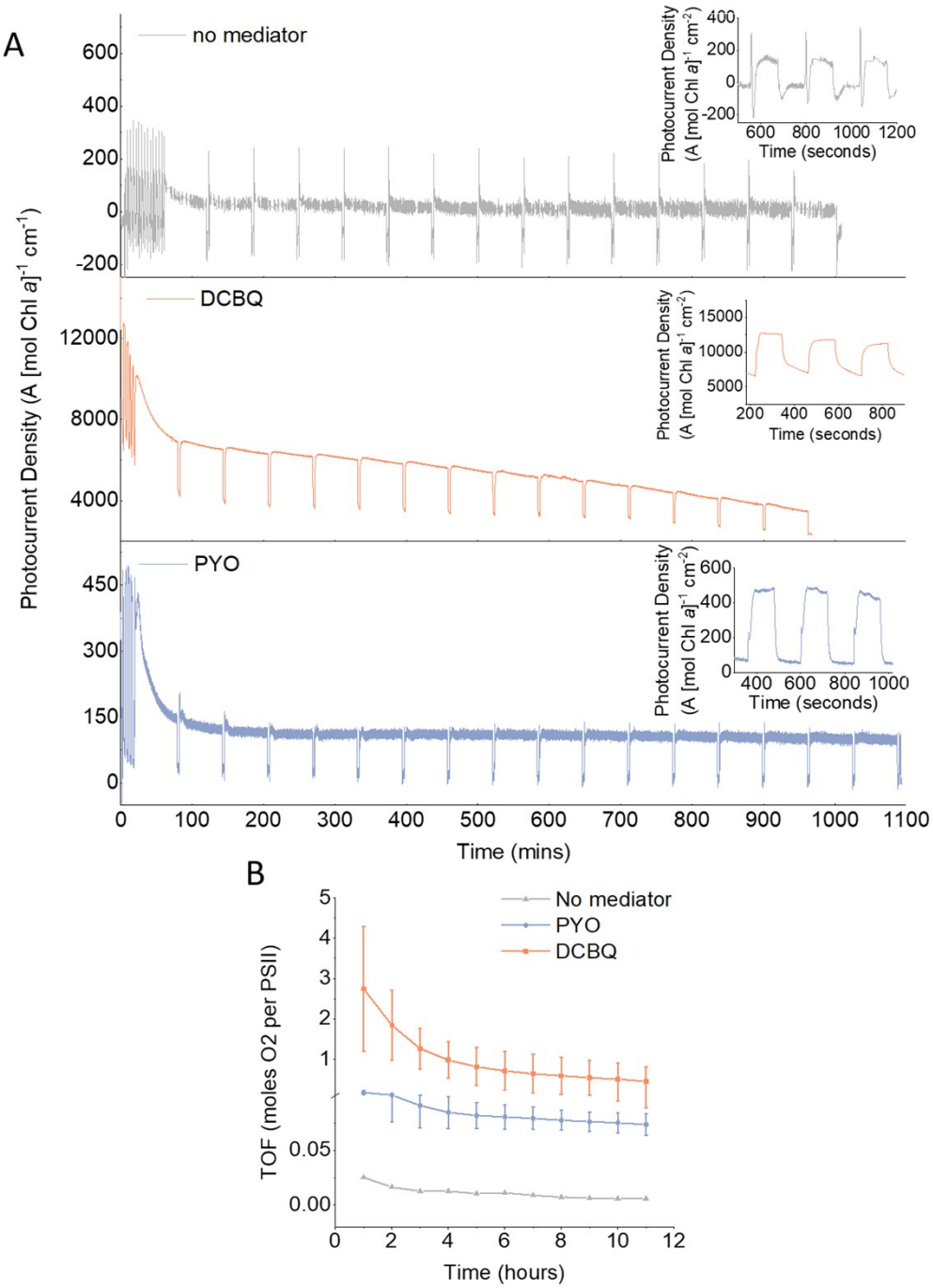

Figure S9. A) Chronoamperometry scans of *Synechocystis*-loaded IO-ITO electrodes in BG11 only, in 200 µM PYO and in 200 µM DCBQ over 15 hours of light exposure. Light conditions used: 1 mW cm^{-2} (50 µE m^{-2} s^{-1}) of 680 nm chopped light. Light was turned off for 1 minute each hour to accurately determine the baseline. Each plot shows a single representative trace. **Inset:** Initial Chl-normalised photocurrent densities of *Synechocystis*-loaded IO-ITO electrodes before 15 hours of light exposure. Each plot shows a single representative trace. **C)** Turn-over frequencies (TOF) of *Synechocystis*-loaded IO-ITO electrodes per PSII in BG11 only, in 200 µM PYO and in 200 µM DCBQ over 15 h of light exposure. The mean was taken from three biological replicates and the error bars represent standard error of the mean (n=3).

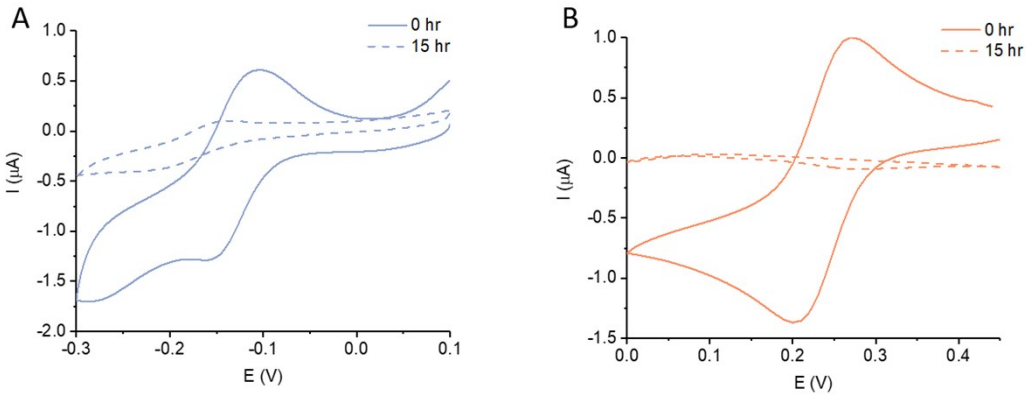

Figure S10. Cyclic voltammograms (CVs) of **A)** PYO and **B)** DCBQ in BG11 before and after 15 hours of redox cycling under light illumination. CVs were recorded with a mediator concentration of 200 µM, in BG11 (~pH 8.5) at 25 °C, using a glassy carbon working electrode (diameter = 3 mm), and a scan rate of 10 mV s^{-1}. In all cases, the electrolyte was bubbled with N_2 for 20 min before the measurements were taken, and a stream of N_2 was maintained in the headspace of the electrochemical cell during measurements. The 15-hour redox cycling was carried out using cyclic voltammetry under 1 mW cm^{-2} (50 µE m^{-2} s^{-1}) of 680 nm light with an ITO working electrode, under atmospheric conditions and with continuous stirring.

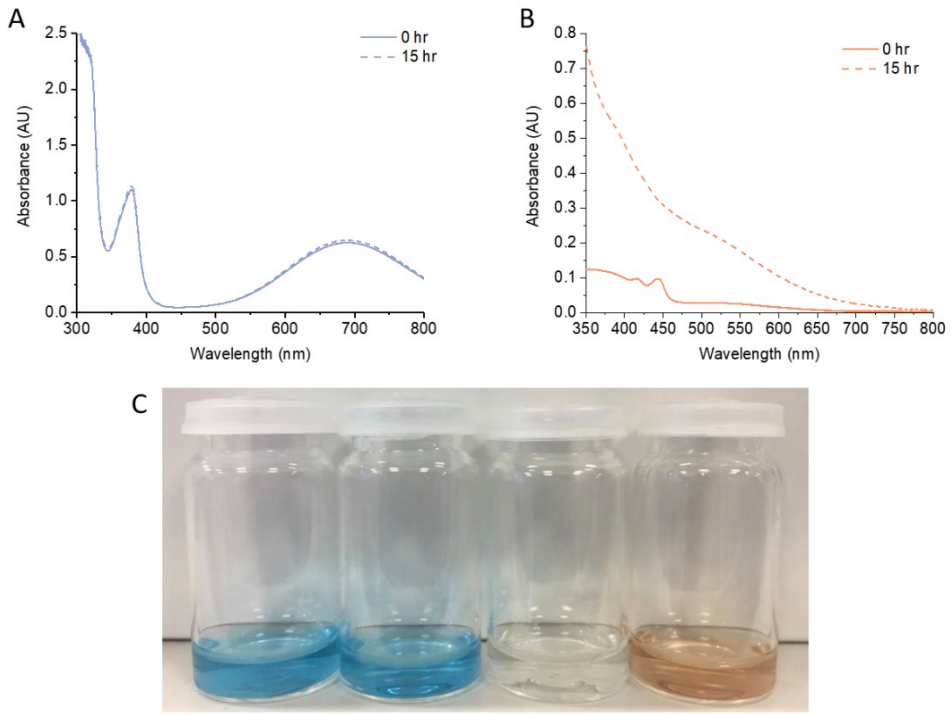

Figure S11. UV-vis spectra of **A)** PYO and **B)** DCBQ before and after 15 hours light illumination with 1 mW cm^{-2} (50 µE m^{-2} s^{-1}) of 680 nm light. UV-vis spectra were recorded with a mediator concentration of 200 µM, in BG11 (~pH 8.5), with BG11 taken as background. **C)** Photograph showing (from left to right): PYO before 15-hour light exposure, PYO after 15-hour light exposure, DCBQ before 15-hour light exposure and DCBQ after 15-hour light exposure. In all cases, mediator is present at a concentration of 200 µM in BG11, and 1 mW cm^{-2} (50 µE m^{-2} s^{-1}) of 680 nm light was used for illumination.

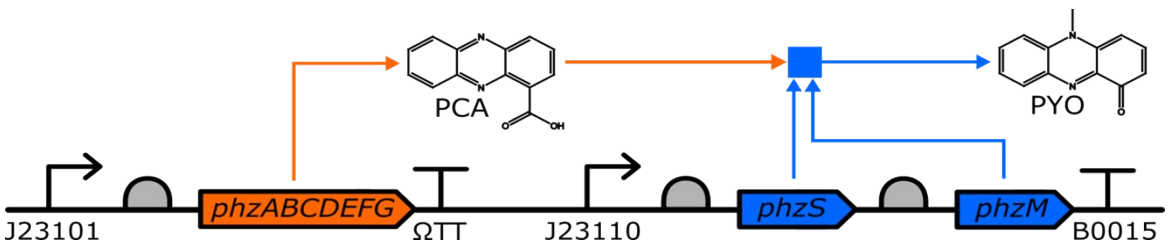

Figure S12. Genetic construct for pyocyanin expression. The *phzA-G* genes and *phzSM* genes are expressed as separate transcriptional units from constitutive promoters. The *phzA-G* operon contains seven open reading frames, each with its own ribosome binding site (RBS), but is represented as a single open reading frame for simplicity. All RBSs are the native *P. aeruginosa* PAO1 sequences. ΩTT is the Omega transcription terminator. Further details can be found in **Supplementary methods** and **Table S2**.

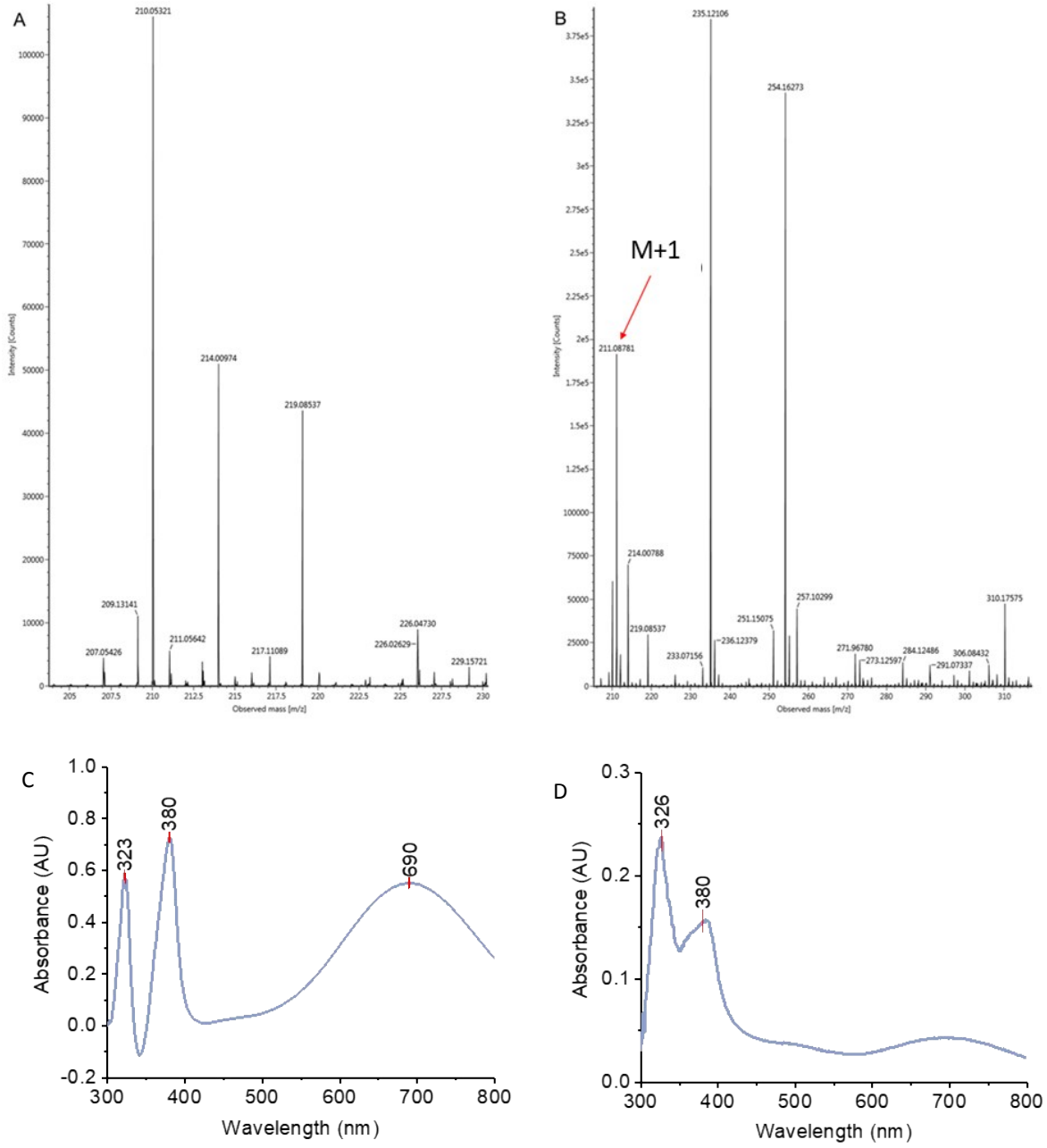

Figure S13. Mass spectra of spent medium from the 4-day cultures of *E. coli* strains transformed with **A)** an 'empty' plasmid (pSB3K3-BBaJ04450) and **B)** a plasmid with PYO biosynthesis synthesis genes (pSB3K3-PYO). The peak with mass 211.08781 corresponds to PYO (M_r = 210.23 g mol^{-1}). No peak for PCA is observed (M_r = 224.21 g mol^{-1}), indicating no PCA production from either *E. coli* strain. **C)** UV-Vis spectrum of 125 µM PYO in LB medium. Recorded from 200 to 800 nm with LB media taken as the background. **D)** UV-Vis spectrum of spent medium from a 4-day culture of *E. coli* transformed with pSB3K3-PYO. Spectra were recorded from 300 to 800 nm with spent medium from the strain with pSB3K3-BBaJ04450 taken as the background. A single representative trace of three biological replicates is shown.

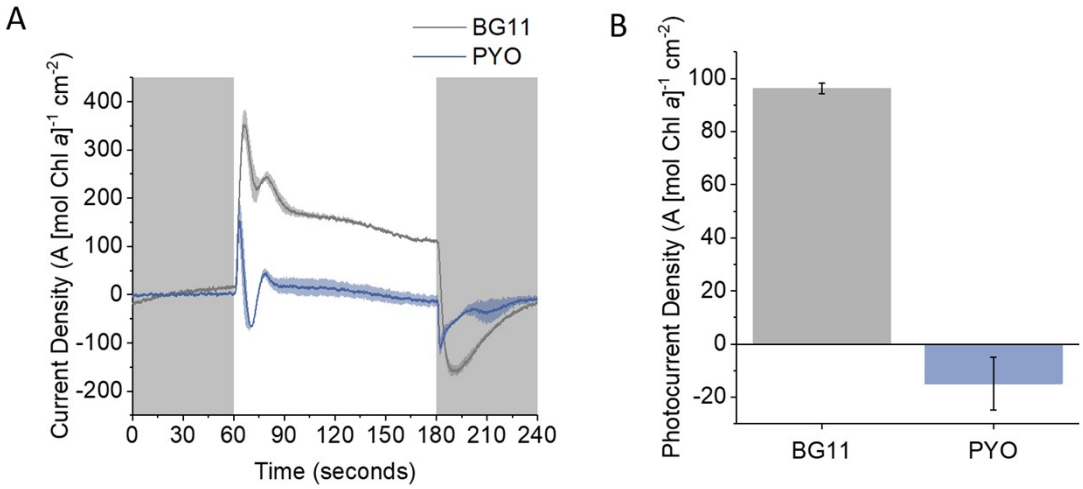

Figure S14. A) Chl-normalised photocurrents of the triple RTO knockout strain of *Synechocystis* in BG11 and in 200 µM PYO. The mean current trace of 3 biological replicates is shown and error bars represent standard error of the mean (n=3). Photocurrents are shown with background correction. **B)** Chl-normalised photocurrent densities stemming from the RTO knockout strain of *Synechocystis* in BG11 and in 200 µM PYO. Data was collected from 3 biological replicates and error bars represent standard error of the mean (n=3). All chronoamperometry measurements were recorded at an applied potential of 0.3 V SHE, under atmospheric conditions and with dark/light cycles (1 mW cm^{-2} (50 µE m^{-2} s^{-1}), 680 nm), at 25˚C.

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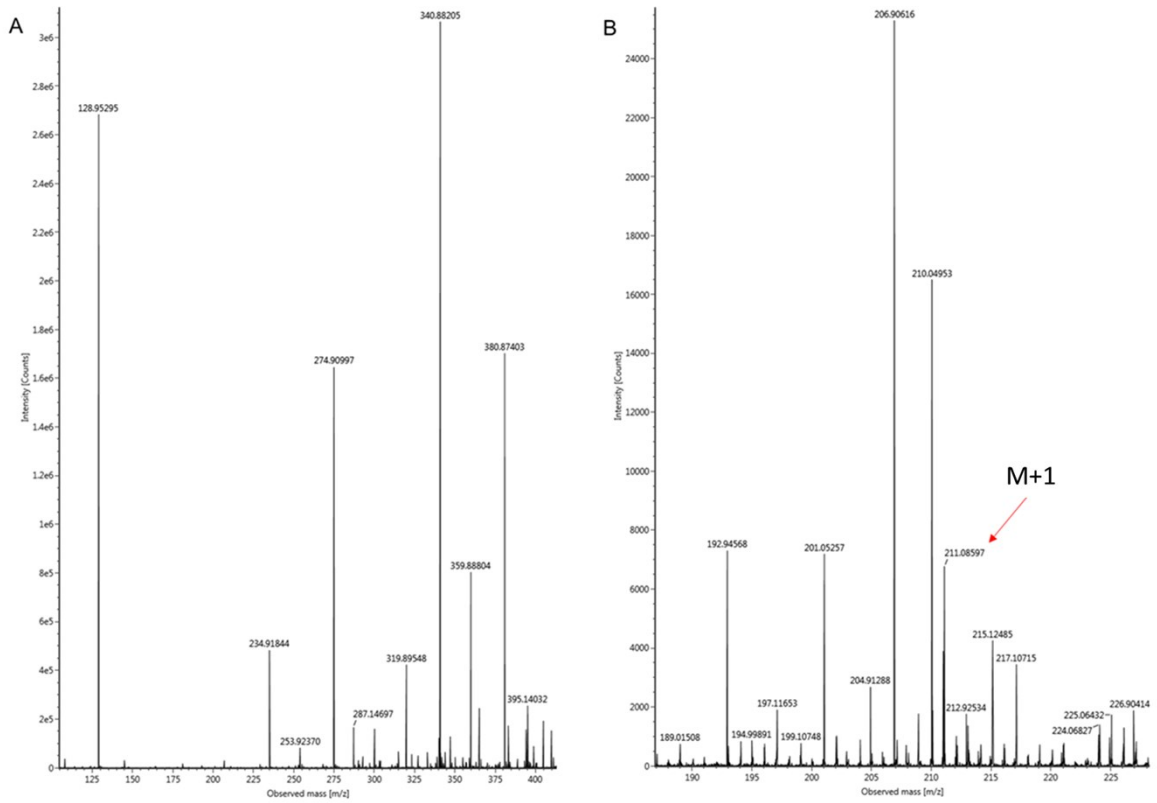

Figure S15. Mass spectra of spent medium from late stationary phase culture of *Synechocystis* strains transformed with **A)** an 'empty' plasmid (pDF-lac) and **B)** a plasmid with PYO biosynthesis genes (pDF-PYO). The peak with mass 211.08781 corresponds to PYO (M_r = 210.23 g mol^{-1}). No peak for PCA is observed (M_r = 224.21 g mol^{-1}), indicating no PCA production from either *Synechocystis* strain.

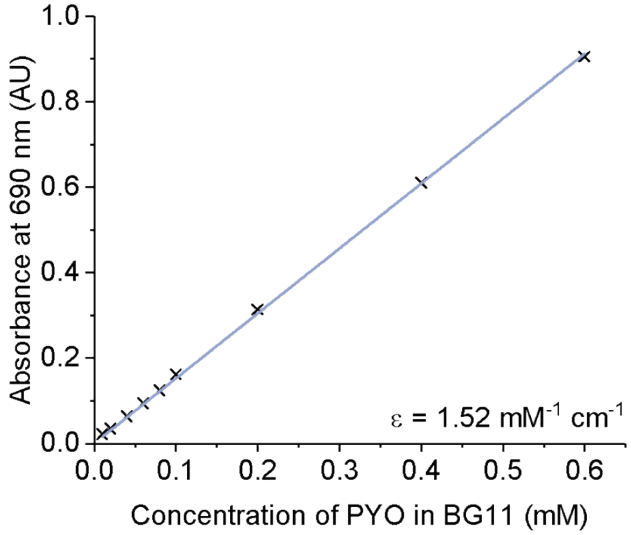

Figure S16. Calibration curve for PYO in BG11 medium using absorbance at 690 nm. Each UV-Vis spectrum was recorded from 200 to 800 nm with BG11 medium taken as the background.

Supplementary methods

Materials

Phenazine (PHZ), 1-hydroxyphenazine (1-OHP), pyocyanin (PYO), 2,5-dichloro-1,4-benzoquinone (DCBQ), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and methyl viologen (MV) used in this study were purchased from Sigma Aldrich. Phenazine-1-carboxylic acid (PCA) used in this study was purchased from Apollo Scientific. All chemicals were used without further purification.

Strains and culturing conditions

Escherichia coli TOP10 was used for cloning and expression of phenazine genes. All *E. coli* strains were cultured at 37°C and with 200 rpm shaking in LB-Lennox medium supplemented with 30 μg mL^{-1} kanamycin, 100 μg mL^{-1} spectinomycin, 25 μg mL^{-1} chloramphenicol, or 100 μg mL^{-1} ampicillin where appropriate.

Wild-type, triple RTO knockout and plasmid-bearing derivatives of *Synechocystis* sp. PCC 6803 (GT-I) were cultured photoautotrophically under 1 mW cm^{-2} (equivalent to 50 μmol photons m^{-2} s^{-1}) of continuous white light at 30°C with 120 rpm shaking in BG11 medium,[2] supplemented with 2 μg mL^{-1} (for solid media) or 10 μg mL^{-1} (for liquid media) spectinomycin where appropriate. 1.5% (w/v) agar was used in all solid media.

Spent medium from the cultures was obtained by centrifugation at 5000 *g* for 10 minutes and microfiltration of the supernatant (pore size = 0.2 μm, Whatman®).

Replicative plasmids were transformed into *Synechocystis* by tri-parental mating[3,4] using *E. coli* HB101 as the donor strain and *E. coli* ED8654 with plasmid pRL443 as the helper strain. Plasmid pRL443 (Addgene plasmid #70261) was a gift from Peter Wolk.[5]

Cloning

The *phzA-G* operon including native RBS sequences was PCR amplified (primers P1/P2) from *P. aeruginosa* PAO1 genomic DNA and cloned (*XbaI/NsiI*) into pSB3K3 (*XbaI/PstI*). Two rounds of Gibson assembly were employed to remove *EcoRI* (primers P3/P5, P6/P7, P8/P4) and then *PstI* (primers P3/P9, P10/P11, P12/P13, P14/P4) restriction enzyme sites, replacing nucleotides to produce synonymous mutations, with choices guided by E. coli codon usage. Finally, the entire operon was PCR amplified as a BioBrick part (primers P15/P16), cloned, and verified by sequencing.

The *phzS* and *phzM* open reading frames were obtained as synthetic DNA from GeneArt, based on the *P. aeruginosa* UCBPP-PA14 sequence with synonymous mutations to remove BioBrick restriction sites. To include native RBSs, gene sequences including the RBS were amplified from *P. aeruginosa* PAO1 genomic DNA (primers P17/P18 for *phzS* and P19/P20 for *phzM*) and spliced with the synthetic DNA, using the unique *ScaI* site for *phzS* and *BglII* site for *phzM*, so that the 5'-UTR and start of each gene are from PAO1.

Expression units were constructed by BioBrick cloning, using standard sequences from the Registry of Standard Biological Parts (http://parts.igem.org/). The non-standard part of the Omega terminator sequence[6,7] was produced by annealing oligos P21/P22.

For PCA production the *phzA-G* operon was combined with the constitutive minimal sigma-70 J23101 promoter and Omega terminator. For PYO production *phzS* was cloned upstream of *phzM* to create a synthetic bicistronic operon, which was then combined with the J23110

promoter and BBa_B0015 terminator, and the entire *phzSM* expression unit was cloned downstream of the *phzA-G* expression unit. Different terminator sequences were used due to the high propensity for homologous recombination in *Synechocystis*. The phenazine biosynthesis genes were expressed from pSB3K3 (p15A origin, kanamycin resistance) in *E. coli*. For conjugation and expression in *Synechocystis,* phenazine expression BioBricks were cut with *XbaI/PstI* and cloned between the *NheI/NsiI* sites of pDF-lac[8] (a gift from Patrik Jones), an RSF1010-based cargo plasmid with spectinomycin resistance. The presence of the plasmid in the bacterial strains was confirmed by colony PCR [data not shown]. Attempts were also made to integrate phenazine expression genes into the chromosome of *Synechocystis* but none was successful, possibly due to the large size of the insert.

Table S1 Oligonucleotide sequences

Oligo	**Sequence 5'-3'**	**Notes**
P1	CGTCTAGAGGAGCCCTCTCGGAGGCGGC GCATGAACGGTCAGCGGTACAGGGAAAC	phzA-G native RBS FWD (XbaI)
P2	GGGGTACCATGCATCGGCCGCTACTAGT AGTGTCGTCACGGTTGCAGGTAGCGGTG CTTC	phzA-G REV (SpeI/NsiI/KpnI)
P3	GACATTATCGCGAGCCCATTT	pSB3K3 vector Gibson assembly kanR FWD
P4	GGGTATAAATGGGCTCGCGA	pSB3K3 vector Gibson assembly kanR REV
P5	CGCAATTGCTGGAAGACGTTCATAAATTC GCGGTTGCGCTTG	phzA-G Gibson assembly EcoRI site 1 REV
P6	AAGATCAAGCGCAACCGCGAATTTATGAA CGTCTTCCAGCAATTGC	phzA-G Gibson assembly EcoRI site 1 FWD
P7	TCGGCTTCCTTGCGGTTGTCGAGAAATTC CATGACCTCGGCCAG	phzA-G Gibson assembly EcoRI site 2 REV
P8	AACCTGGCCGAGGTCATGGAATTTCTCGA CAACCGCAAGGAAGC	phzA-G Gibson assembly EcoRI site 2 FWD
P9	ATCGCCCCATTCGGGTTGCTGCAACGCTT CGCAGCGCCG	phzA-G Gibson assembly PstI site 1 REV
P10	AACGCGTACGGCGCTGCGAAGCGTTGCA GCAACCCGAATGGG	phzA-G Gibson assembly PstI site 1 FWD
P11	GGGGATCTCGATGCGGTCCTGCAAGGCG CGCGCAGCGAA	phzA-G Gibson assembly PstI site 2 REV
P12	ACAACACCTTCGCTGCGCGCGCCTTGCA GGACCGCATCGAG	phzA-G Gibson assembly PstI site 2 FWD
P13	GTGGGGATCGGCTGCTGCATGCTACAGG CCACCACCTTGCC	phzA-G Gibson assembly PstI site 3 REV
P14	CAGGACGGCAAGGTGGTGGCCTGTAGCA	phzA-G Gibson assembly PstI site 3

	TGCAGCAGCCGATC	FWD
P15	AGCTGAATTCGCGGCCGCTTCTAGAGGAGCCCTCTCGGAGGCGGCGCATGAACGGTCAGCGGTACAGGGAAAC	phzA-G native RBS BioBrick FWD
P16	AGCTCTGCAGCGGCCGCTACTAGTATTATCACGGTTGCAGGTAGCGGTGCTTC	phzA-G BioBrick REV
P17	AGCTGAATTCGCGGCCGCTTCTAGAGCCATCGATTCGAACACTCGAGA	phzS native RBS BioBrick FWD
P18	AGCTCTGCAGCGGCCGCTACTAGTATTAGCGTGGCCGTTCCAC	phzS BioBrick REV
P19	AGCTGAATTCGCGGCCGCTTCTAGAGTGGGTACTTCTCGGGTTACG	phzM native RBS BioBrick FWD
P20	AGCTCTGCAGCGGCCGCTACTAGTATTAGGCCCTGGCAGCGA	phzM BioBrick REV
P21	CTAGAGTTAATTAATTAAGGGGACCCTAGAGGTCCCCTTTTTTTATTTTTACTAGTAGCGGCCGCTGCA	Omega transcription terminator FWD
P22	GCGGCCGCTACTAGTAAAAATAAAAAAAGGGGACCTCTAGGGTCCCCTTAATTAATTAACT	Omega transcription terminator REV

Table S2 Standard BioBrick parts

Part designation	**Part type**	**Use**
BBa_B0015	Transcription terminator	Terminator for *phzSM* operon
BBa_J23101	Promoter	Promoter for *phzA-G* operon
BBa_J23110	Promoter	Promoter for *phzSM* operon
pSB3K3	Plasmid backbone	Expression and cloning vector
pSB3C5	Plasmid backbone	Cloning vector

Table S3 Plasmids used in this study

Plasmid	**Description**	**Reference**
pUC19-phzAG	Vector carrying the phzA-G operon including native RBSs; deposited with Addgene #141106	This study
pSB3C5-phzSM	Vector carrying the phzSM synthetic operon including native RBSs; deposited with Addgene	This study

	#141105	
pSB3K3-BBaJ04450	*E. coli* vector, p15A origin, kanamycin resistance, RFP cargo. Used as an empty vector control for phenazine expression in *E. coli*.	Standard BioBrick part
pDF-lac	Broad host range vector, RSF1010 origin, spectinomycin resistance. Used as an empty vector control for phenazine expression in *Synechocystis*.	Guerrero et al.[8]
pSB3K3-PYO	pSB3K3 backbone with cargo of J23101-phzA_G-ΩTT-J23110-phzSM-B0015 for PYO expression in *E. coli*.	This study
pDF-PYO	pDF backbone with cargo of J23101-phzA_G-ΩTT-J23110-phzSM-B0015 for PYO expression in *Synechocystis*.	This study
pRL443	Helper plasmid for RP4-based conjugation into cyanobacteria	Elhai et al.[5]

cLogD Determination

cLogD determination was performed in ChemAxon Marvin using the LogD plugin.[9] cLogD values were calculated to 3 decimal places between pH 4-10 for all mediators used in this study. The consensus LogP method was used and tautomerization and resonance of the structure was considered. A Na^+ concentration of 17.6 mM was used, which is the concentration of $NaNO_3$ in BG-11 mediu

Toxicity Assays

Wild-type *Synechocystis* cells (5 nmol Chl *a*) were incubated with different concentrations of PCA, PYO and DCBQ for 1 or 3 days, as stated, under 1 mW cm^{-2} (50 µE m^{-2} s^{-1}) white light at 30°C with 120 rpm shaking. *Synechocystis* cells incubated in BG11 with no electron mediator or in BG11 with 0.2 % (v/v) DMSO solvent under the same conditions were used as controls. Following incubation, the cells were resuspended in fresh BG11 and their concentration standardized to an optical density at 750 nm (OD_{750}) of 0.5. Aliquots (10 µL) of three serial dilutions (x 1, x 10^{-3}, x 10^{-6}) were spotted on BG11 agar. Serial dilutions were used to allow the number of colony forming units (CFUs) to be determined and to better compare growth to the controls. The plates were incubated for a week at 30˚C under 1 mW cm^{-2} (50 µE m^{-2} s^{-1}) white light. The plates were photographed and growth of the cells pre-incubated with mediator was compared to the controls by eye to assess the cytotoxicity of the mediators.

Detection of PYO in Spent Medium

To detect PYO production from *E. coli* and *Synechocystis*, UV-Visible spectroscopy (Varian Cary 50 Bio UV–vis spectrometer) was performed between 200 and 800 nm on the spent medium from the negative control strains (‘empty’ plasmid) and the PYO-producing strains of *E. coli* and *Synechocystis*. *E. coli* was grown from mid-log phase for a further 4 days before taking the spent medium; *Synechocystis* was grown from mid-log phase to early stationary phase at an OD_{750} of ca. 1 before taking the spent medium. Spectra of solid PYO dissolved in LB medium (125 µM) and BG11 medium (80 µM) were also obtained to help validate the

results. Mass spectrometry (MS) was performed using a Waters Vion IMS Qtof Mass Spectrometer on spent medium from the negative control strains and the PYO producing strains of *E. coli* and *Synechocystis* to detect the presence of PYO.

Synechocystis-electrode Preparation

Inverse opal mesoporous ITO (IO-ITO) electrodes with 10 μm pore size and 3 μm channels were prepared by Dr Xiaolong Chen using the method reported in Zhang et al.[10] Planktonic cultures of early stationary phase *Synechocystis* at an OD_{750} of ca. 1 were concentrated by centrifugation at 5000 g for 10 minutes, the supernatant removed and the pellet resuspended in fresh BG11 medium to a concentration of 150 nmol Chl *a* mL^{-1}. 250 μL of this was dropcast onto the IO-ITO electrodes and left overnight at room temperature in a covered humid chamber in the dark to allow cell penetration and adhesion, yielding *Synechocystis*-loaded electrodes the following day which were used immediately for analysis.

Photoelectrochemical Measurements

All (photo)electrochemical measurements were performed at 25°C using an Ivium Technologies CompactStat, an Ag/AgCl (saturated) reference electrode (corrected by + 0.197 V for SHE), a platinum mesh counter electrode and a glassy carbon (diameter = 3 mm), IO-ITO or *Synechocystis*-loaded IO-ITO working electrode. Experiments were carried out with light/dark cycles, using a collimated LED light source (1 mW cm^{-2} (50 μE m^{-2} s^{-1}), 680 nm, Thorlabs). All (photo)electrochemical measurements were performed in 4 mL of BG11 electrolyte at pH 8.5 unless otherwise stated, supplemented with phenazines, DCBQ or inhibitors as stated. 500 μM stock solutions of phenazines were prepared in BG11 electrolyte. The stock was diluted using BG11 electrolyte to make up the final concentrations stated. 100 mM stock solutions of DCBQ in DMSO were prepared. A fresh DCBQ stock was prepared prior to each experiment to avoid the photodegradation of DCBQ. A volume of the DCBQ stock of 0.2 % (v/v) of the final volume was added to the electrolyte to make up the 200 μM solution used. 200 mM stock solutions of DCMU and MV were prepared in DMSO and water respectively. A volume of the DCMU/MV stock of 0.5 % (v/v) of the final volume was added to the electrolyte to make up the 1 mM concentration used.

Chronoamperometry experiments were performed with a dark/light cycle of two minutes off, two minutes on for all experiments except for the longevity study in which a dark/light cycle of 3 minutes off, 1 hour on was used. In cases where mediators were screened using photoelectrochemistry, around 6 dark/light cycles were performed first in BG11 alone until the photocurrent profile reached steady state. Then the mediator was added to the electrolyte and, for the phenazine mediators, the experiment run until the photocurrent profile reached steady state and three steady-state photocurrent profiles were taken for analysis. For the DCBQ experiments, following mediator addition the first three initial photocurrent cycles were taken for analysis.

Photocurrents were normalised against the geometric area of the electrode (0.75 cm^2) to obtain photocurrent densities. The Chl *a* content of the *Synechocystis*-loaded electrode was determined by scraping off the annealed ITO nanoparticles from the FTO coated glass into methanol (500 μL). The suspension was sonicated for 15 minutes in iced water, then centrifuged at 12 000 g for 2 minutes. The supernatant was analysed by UV-Vis spectroscopy and the Chl *a* concentration was determined using the extinction co-efficient of Chl *a* at 665.5 nm in methanol (70020 $[mol\ chl\ a]^{-1}$ dm^3 cm^{-1}).[11] Photocurrents were background corrected by subtracting the dark current from the chronoamperometry profile.

Unless otherwise stated, data were analysed based on 3 biological replicates. In each biological replicate, the mean of 3 technical replicates was taken to give an average value for that biological replicate. The mean and standard error across three biological replicates was calculated.

Stepped chronoamperometry experiments were performed in 200 μM PYO in BG11 electrolyte between − 0.1 V and 0.8 V vs SHE with a potential step of 0.1 V and dark/light cycles of 2 minutes off, 2 minutes on, 2 minutes off at each potential. Around 6 dark/light cycles were performed first in BG11 alone until the photocurrent profile reached steady state. Then the PYO was added and the electrolyte bubbled with N_2 gas for 20 minutes to purge the system of O_2 before the stepped chronoamperometry experiment began. A stream of N_2 was maintained in the airspace during the experiment. Data analysis was based on 3 biological replicates.

In the longevity studies, to allow the photocurrent to equilibrate prior to beginning the experiment, 3 light/dark cycles of 2 minutes off, 2 minutes on were run before the dark/light cycles were changed to 3 minutes off, 1 hour on. 15 dark/light cycles of 3 min off, 1 hour on were performed. A current/time graph was plotted and the baseline dark current subtracted from the trace. The total charge delivered by the *Synechocystis*-loaded electrodes during each light cycle was found by integrating the background corrected photocurrent. The number of electrons transferred during each light cycle was found by dividing the total charge delivered by the charge carried by 1 electron. Assuming 100 % efficiency, the moles of O_2 produced was calculated by dividing the no. of electrons transferred by 4 ($2H_2O \rightarrow 4H^+ + 4\ e^- + O_2$) and then diving by Avogadro's constant. This was then divided by the moles of Chl *a* on the electrode and multiplied by 358 to find the turnover number (TON) in moles of O_2 produced per mole of PSII (assuming the ratio of PSI:PSII in the cells is 1:1, the ratio of Chl *a*:PSII in the cell was calculated to be 358:1[12,13]). The turn-over frequency (TOF) was found by dividing the TON by 3600. TONs from consecutive light cycles were summed to find the cumulative TON. The mean and standard error of the cumulative TONs and the TOFs was calculated from 3 biological replicates.

All cyclic voltammetry measurements were recorded in BG11 electrolyte at pH 8.5, unless otherwise stated, at 25°C with a glassy carbon or ITO working electrode. Unless stated otherwise, the electrolyte was bubbled with N_2 gas for 20 min prior to the experiment to purge the system of O_2, and a stream of N_2 was maintained in the headspace during the experiment.

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