Supplementary Information

Hybrid Electrophototroph Enables High-Efficiency Carbon Dioxide Valorization to Fuel Molecules

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This PDF file includes:

Supplementary Text

Efficiency approximation

The inoculum was pre-incubated in the heterotrophic medium as discussed in the previous section. In order to quantify the conversion of electrons taken up by the PSII deficient cyanobacteria for CO_2 reduction, selective acetic acid generation from external electricity was defined as exocellular electrons uptake efficiency (*EEUE*$_{Acetate}$). The $NaH^{13}CO_3$ experiment data were used for this purpose. The *EEUE*$_{Acetate}$ value under electrophototrophic condition can be calculated based on the following equation:

$$EEUE_{Acetate} = \frac{8 \times \Delta n_{^{13}C-labeled\ Acetate} \times F}{\int Idt}$$

where $\Delta n_{^{13}C-labeled\ Acetate}$ is the change of ^{13}C-labeled acetate amount difference between two sampling points, *F* is the Faradaic constant, and $\int Idt$ is the amount of charge that has been passed through the working electrode during the sampling period (see Fig. S10 for accumulated electrons during electrophototrophic period). With the ^{13}C-labeled acetate fractions (Fig. 3c) and the corresponding acetate acid concentrations of ~0.163 mM (initial) and ~0.817 mM (5th day), the calculated average *EEUE*$_{Acetate}$ through electrophototrophic metabolism is 64.56 %.

Considering the illumination involved in this electrophototrophic process, the energy conversion efficiency approximation is conducted in similar fashion to approaches reported in previous literature[1]. Here we define the import energy to acetate efficiency as follows:

$$\eta = \frac{\Delta_r G^0 from\ CO_2\ to\ Acetate}{Electricity\ Energy + Illumination\ Energy}$$

The Gibbs free energy of reaction ($\Delta_r G^o$) for CO_2 reduction to acetate can be calculated corresponding the following chemical reaction:

$2CO_2$ (g) + $2H_2O$ (l) → CH_3COOH (l) + $2O_2$ (g)

$$\Delta_r G^0 = \sum \Delta_f G^0 products - \sum \Delta_f G^0 reactants = 1015.32\ kJmol^{-1}$$

The thermodynamic potential of this reaction is ΔE^o = 1.09 *V*.[2] So the electricity energy passed over the course of our five-day experiment was equal to $\int Idt \times \Delta E^o$ = 0.358 × 1.09 = 0.390 J where 0.358 C is the total number of Coulombs passed over the experimental duration (Fig. S10). The power density of the illumination is 55 μmol $s^{-1}m^{-2}$ (white LED). After passing through the FTO and reaching the carbon felt cathode and the culture, the power density was measured as ~16 μmol $s^{-1}m^{-2}$ (3.328 W m^{-2}).

The illumination energy over the cyanobacteria would be calculated by this power density (3.328 W m^{-2}) with accumulated illumination time (7025.31 s) and FTO glass window area (1.15 cm^2). Therefore, the import energy to acetate efficiency η = ~ 9.85 % while the photo energy conversion efficiency is 11.28 %. Table S2 summarized the efficiency results based on three bio-replicates.

Based on reported methods[1], since the reaction proceeds at 30 °C, the Gibbs free energy compared to the above standard condition should also incorporate the relevant entropy changes, which are assumed to only arise from entropy changes of gaseous CO_2 and O_2 in our reaction.

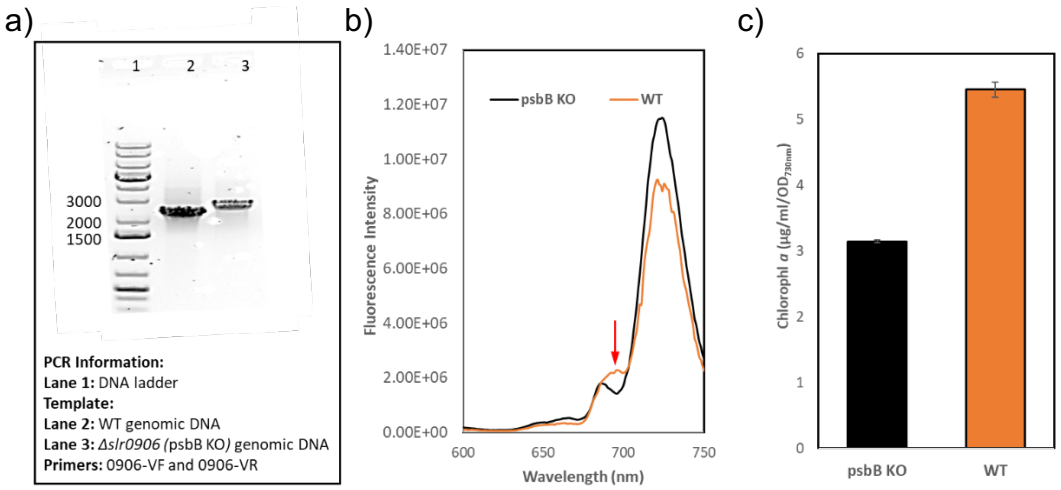
$$\Delta S^0_{rxn} = -(\frac{\partial G}{\partial T})_{P,C} = \Delta S^0 products - \Delta S^0 reactants = 2\Delta S^0(O_2) - 2\Delta S^0(CO_2)$$
$$= -17.2\ J\ mol^{-1}K^{-1}$$

So, the Gibbs free energy change (ΔG) with temperature change from room temperature to incubation temperature is 0.0085 % increase, resulting the neglectable increase of η.

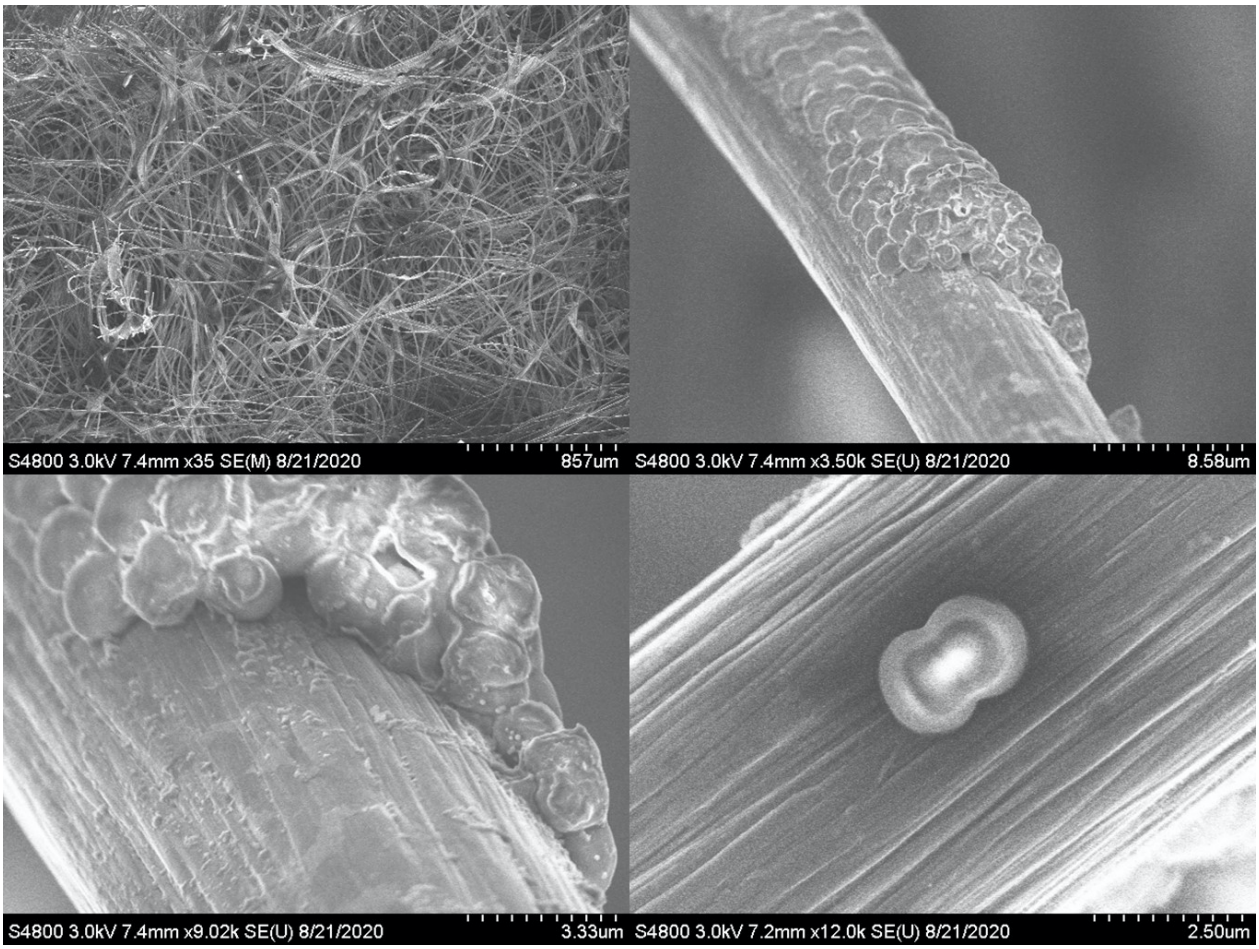
Intermittent electrons supply for electrochemical incubation

To realize exogenous electron-driven CO_2 fixation and conversion, we incubated PSII-deficient cyanobacterial cultures and applied electrical potential with amperometric characterization. Light and electrical bias were investigated. In practice, we found that the increase of accumulated charges supplied to bacterial cells would become slower over time when continuously providing electricity in electrophototrophic process (Fig. S4). The acetate production did not sustain during this process. Ceasing the electricity supply while maintaining the illumination for a certain period could effectively resume the electrophototrophic process. This observation indicates a mismatch occurring between fast injection of exogenous electrons and slow charge transfer in PETC. We speculate that persistent supply of exogenous electrons might over-reduce the redox components in PETC, the re-oxidation of which largely relies on electron consumption in downstream metabolic activities. However, if reoxidation proceeds more slowly than the rate of external electron input, this imbalance may leave the PETC redox carriers in a more reduced state, retarding continuous influx of exogenous electrons over time. Illumination of the biotic system while stopping electricity supply would allow electrons to be consumed during metabolism, therefore regenerating oxidized PETC components that could readily accept additional exogenous electrons. It suggests that a better coupling of exogenous electron injection kinetics with the biological photosynthetic metabolism will be crucial for the electrophototrophic process.

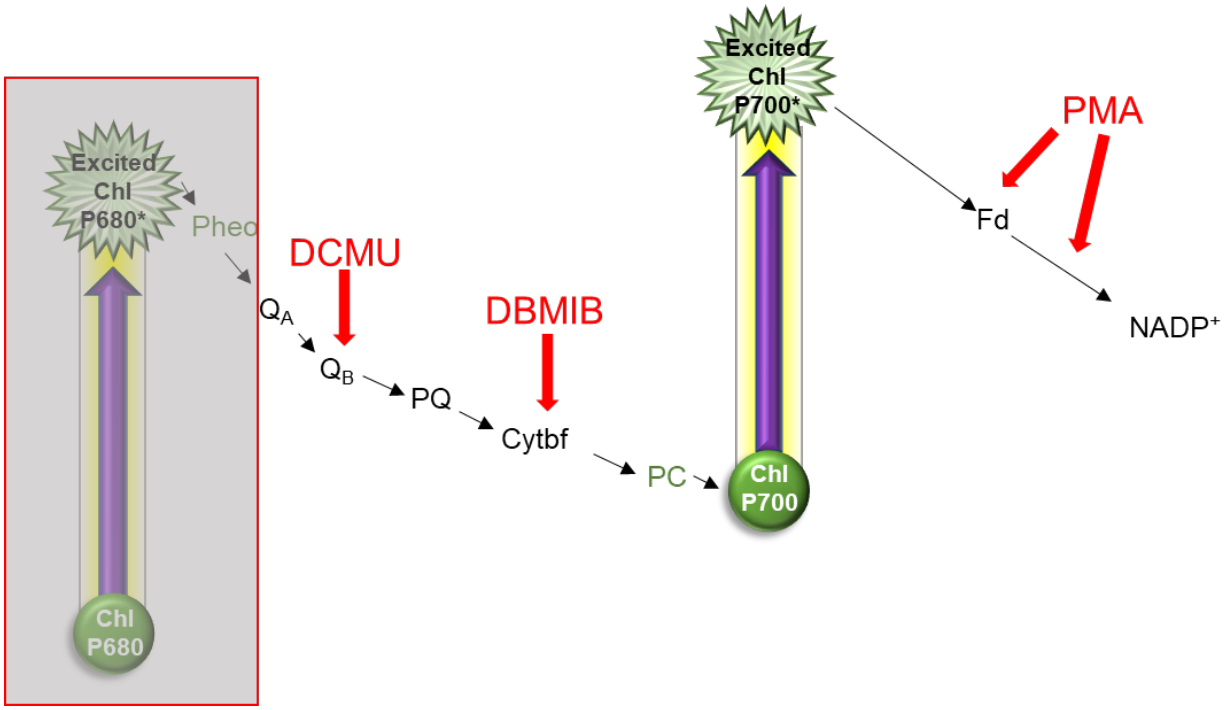
Based on this hypothesis, we developed “intermittent electrons supply” as a strategy. As shown in Fig. S4, much higher charge transfer rate and acetate productivity can be achieved when 30 minutes interval was introduced between 30 second electricity supply than the continuous supply condition. We thus adopted the “30 s + 30 min” electricity supply in the electrochemical chamber for long-term electrophototrophic incubation. The illumination was programmatically controlled along with the electricity supply.



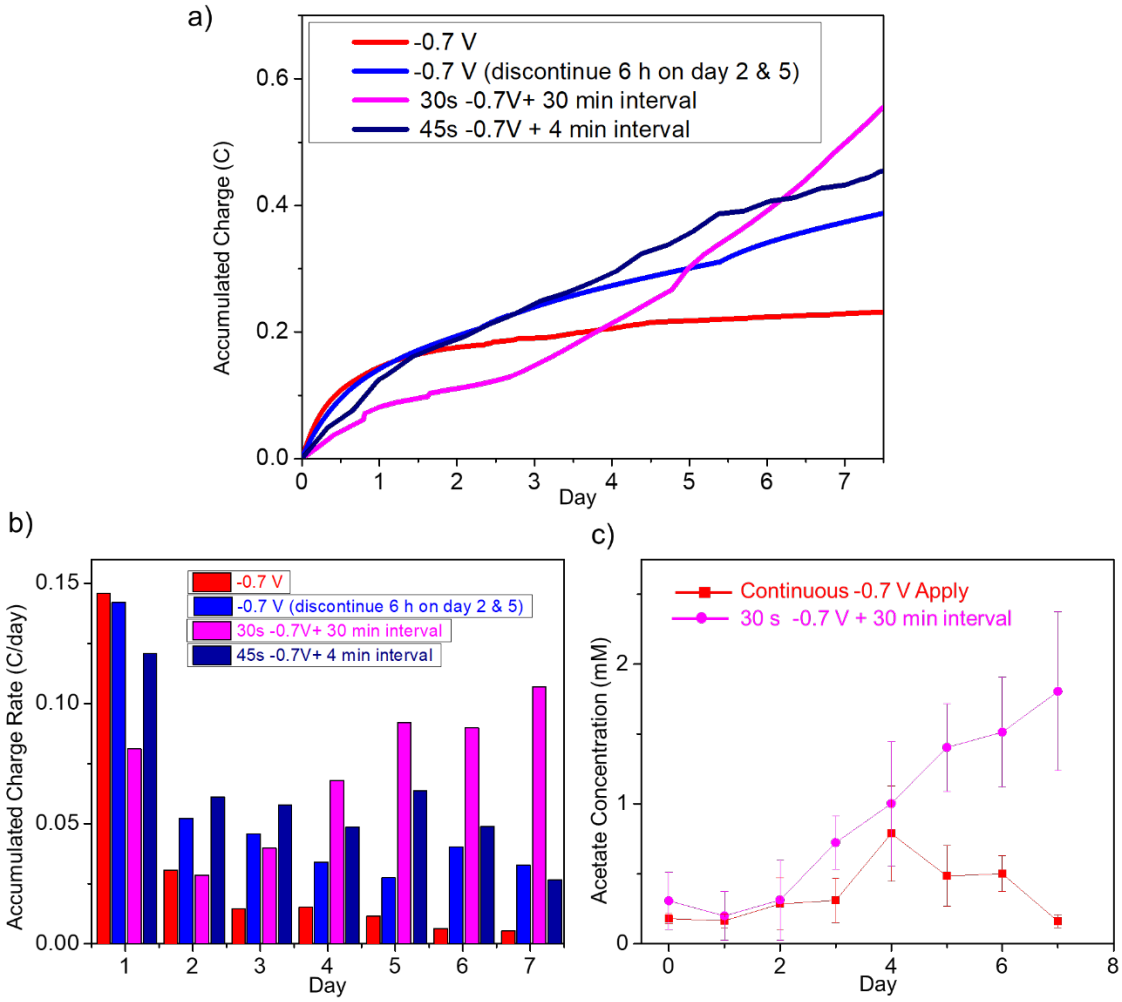
Supplementary Fig. 1. Genotype and phenotype of PSII knockout mutant. a) PCR analysis verified that psbB gene was interrupted by a larger size streptomycin gene cassette replacing partial coding region; b) 77K fluorescence demonstrated disappearance of a shoulder peak at 695 nm (indicated by a red arrow) due to the psbB deficiency; c) Chlorophyll *a* concentrations in the mutant and wild type. Error bars represent biological triplicates.



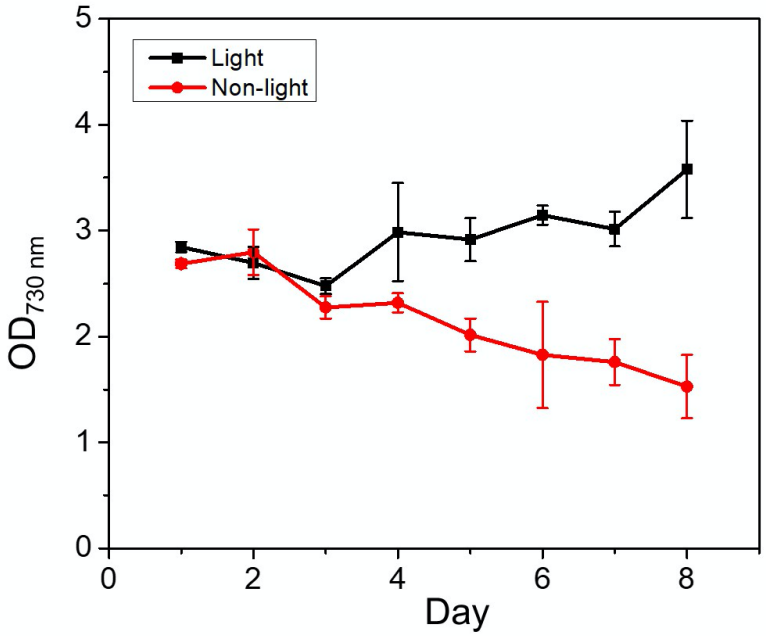
Supplementary Fig. 2. Scanning electron microscopy (SEM) of PSII-deficient *Synechocystis* cells on carbon felt electrode materials in different magnifications.



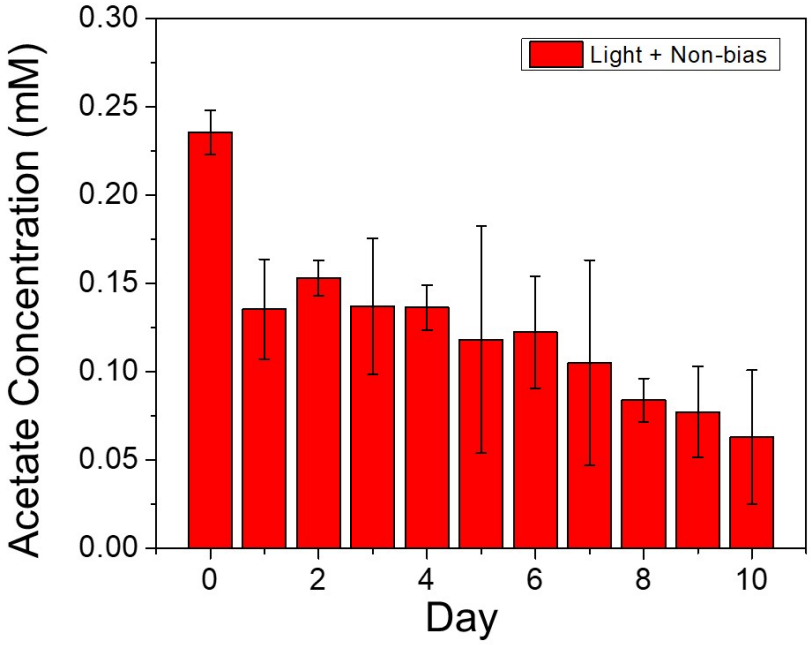
Supplementary Fig. 3. Site-specific inhibitors act on the "Z" scheme of natural photosynthesis. Gray bar indicates the deficient PSII. Three inhibitors: DCMU, DBMIB, and PMA inactivate the Q_B, Cytochrome b6f complex, and components downstream of PSI in the PETC, respectively.



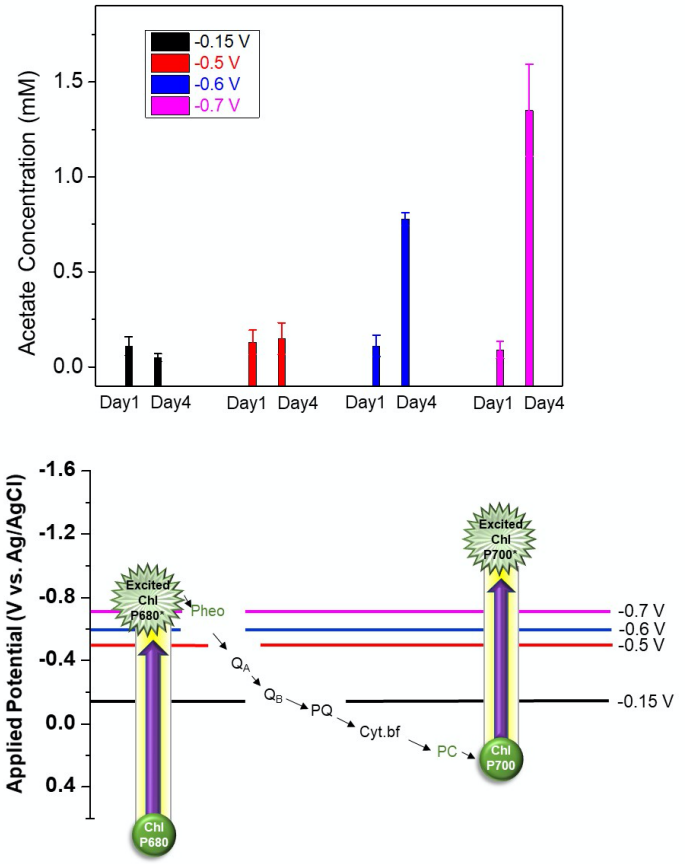
Supplementary Fig. 4. Supplying exogenous electrons to the electrophototrophic hybrid with different strategies. a) Time courses of accumulated charge transfer to ΔPSII with 4 programmed electricity supplies under illumination (white LED, 55 μmol m^{-2} s^{-1}). **b)** Daily accumulated charge rates. The rate for continuous electricity supply (red bar) gradually decreased over time, while intermittent electricity supply (e.g. 30s -0.7V EC with 30 min non-EC interval, pink bar) can maintain stable charge transfer to the bacterium over time. **c)** Time courses of acetate production with continuous EC and intermittent EC applied.



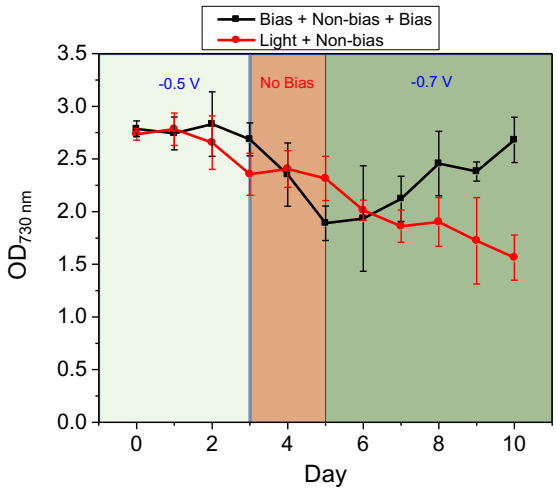
Supplementary Fig. 5. OD_{730} of ΔPSII under external electrical bias (-0.7 V vs. Ag/AgCl) with and without illumination. Error bars represent standard deviations from biological triplicates.



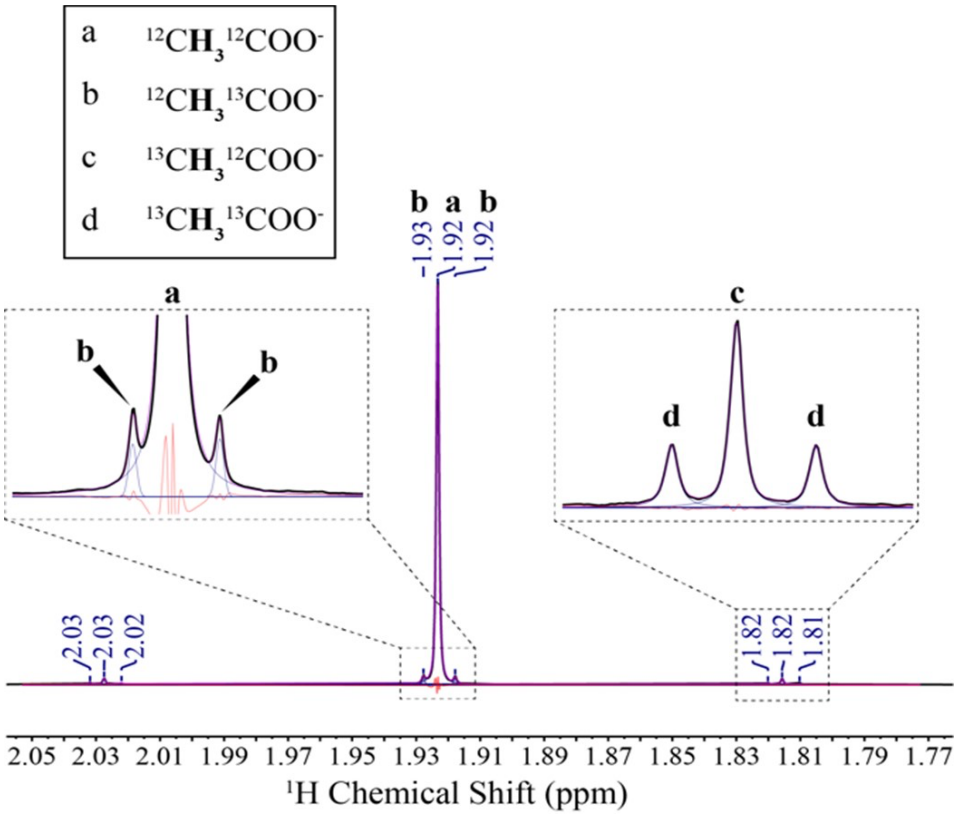
Supplementary Fig. 6. Acetate concentrations in the light-illuminated culture of PSII deficient *Synechocystis* without external electrical bias. Error bars represent standard deviations from biological triplicates.



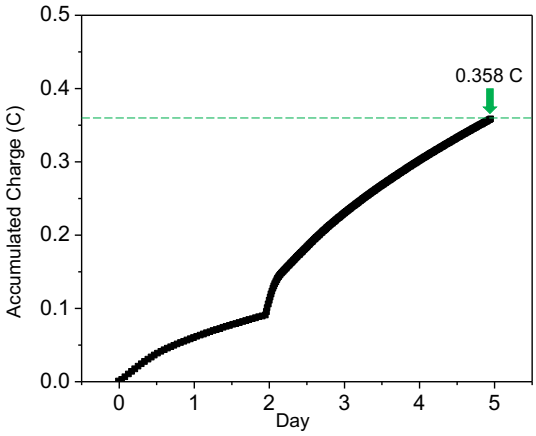
Supplementary Fig. 7. Acetate production in PSII deficient *Synechocystis* (Top) as a function of applied potentials (-0.15 to -0.7V) based on their levels in the "Z" scheme of natural photosynthesis (Bottom). Error bars represent standard deviations from biological triplicates.



Supplementary Fig. 8. OD_{730} of ΔPSII when external electrical bias was manipulated. The black curve shows the -0.5 V (vs. Ag/AgCl) bias applied for the first 3 days, no bias for next 2 days and then -0.7 V (vs. Ag/AgCl) bias applied for final 5 days under the constant illumination(white LED, 55 μmol m^{-2} s^{-1} on FTO glass). Red curve indicates the OD_{730} without external electrical bias under constant illumination. Error bars represent standard deviations from biological triplicates.



Supplementary Fig. 9. ^{1}H-NMR spectra indicating the acetate was labeled in both methyl and carboxyl carbons from ^{13}C-bicarbonate. Samples are supernatant collected from the electrophototrophic system under illumination (white LED, 55 μmol m^{-2} s^{-1} on FTO glass) and intermittent external electrical bias supply (-0.7 V vs. Ag/AgCl) for 5 days.



Supplementary Fig. 10. Accumulated electrons transferred to hybrid electrophototrophic system within 5 days. Green arrow and dash line indicate the total charge consumed which is used in the energy efficiency calculation.

Supplementary Table 1. MDV of proteinogenic amino acids in PSII deficient *Synechocystis* under biased (-0.7 V vs. Ag/AgCl) and unbiased conditions.

MDV		m0	m+1	m+2	m+3	m+4	m+5
Ala_57(PYR1-3)	Biased	0.91261	0.07920	0.00692	0.00127	0	0
	Unbiased	0.96247	0.03618	0.00001	0.00134	0	0
Ala_85(PYR2-3)	Biased	0.96666	0.02754	0.00580	0	0	0
	Unbiased	0.98055	0.01944	0.00001	0	0	0
Ser_57(PGA1-3)	Biased	0.92125	0.06662	0.00946	0.00267	0	0
	Unbiased	0.95670	0.04327	0.00001	0.00001	0	0
Ser_85(PGA2-3)	Biased	0.94565	0.03507	0.01928	0	0	0
	Unbiased	0.97935	0.02064	0.00001	0	0	0
Gly_57(SER1-2)	Biased	0.29693	0.66770	0.03537	0	0	0
	Unbiased	0.97242	0.02758	0.00001	0	0	0
Gly_85(C1)	Biased	0.63306	0.36694	0	0	0	0
	Unbiased	0.99025	0.00975	0	0	0	0
Glu_57(2OG1-5)	Biased	0.79504	0.19492	0.00742	0.00148	0.00070	0.00045
	Unbiased	0.92860	0.07125	0.00001	0.00001	0.00001	0.00011
Asp_57(OAA1-4)	Biased	0.77960	0.16755	0.04555	0.00001	0.00729	0
	Unbiased	0.92537	0.07305	0.00001	0.00045	0.00112	0
Phe_302(PEP1-2)	Biased	0.92676	0.06637	0.00687	0	0	0
	Unbiased	0.96572	0.03427	0.00001	0	0	0
Thr_57(OAA1-4)	Biased	0.74364	0.22885	0.01414	0.000004	0.01336	0
	Unbiased	0.92519	0.06412	0.00597	0.00471	0.00001	0

Supplementary Table 2. Summary of calculated energy conversion efficiencies in the electrophototrophic system.

Exocellular electrons up-taken efficiency ($EEUE_{Acetate}$)	Import energy to acetate efficiency (η)	Import photo-energy to acetate efficiency
61.84 ± 5.05 %	9.32 ± 0.73 %	10.64 ± 0.83 %

Supplementary Material References

1 Liu, C., Colon, B. C., Ziesack, M., Silver, P. A. & Nocera, D. G. Water splitting-biosynthetic system with CO(2) reduction efficiencies exceeding photosynthesis. *Science* **352**, 1210-1213 (2016).

2 Liu, C. *et al.* Nanowire-bacteria hybrids for unassisted solar carbon dioxide fixation to value-added chemicals. *Nano Lett* **15**, 3634-3639 (2015).