Electronic Supplementary Information

Chloroplast biosolar cell & self-powered herbicide monitoring

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Isolation of intact chloroplast

Intact chloroplasts were isolated from spinach (*Spinacia oleracea*) purchased from a local grocery store (Salt Lake City, Utah). The entire process was performed under refrigeration (2−4°C). Briefly, 30 g of spinach leaves washed with Milli-Q water were transferred to a blender with 120 mL of 1 M chloroplast isolation buffer (CIB) (pH 7.8) containing 0.1% of (w/v) BSA. The 1 M CIB was prepared as follows (per 1 L of solution): 0.33 M sorbitol (60 g), 0.05 M HEPES (11.92 g), 10 mM NaCl (0.58 g), 2.6 mM EDTA (0.74 g), and 10.6 mM $MgCl_2$ (1 g).

The leaves were processed with 2−4 blender strokes within 5 s. The obtained macerate was gradually passed through three layers of cheesecloth. The filtrate was evenly distributed into four 50 mL tubes and centrifuged at 200g for 3 min (Allegra X-15R benchtop centrifuge, Beckman Coulter) to remove all unwanted whole-cell and cell-wall debris. Supernatants from each tube were transferred to fresh, chilled 50 mL tubes and centrifuged again at 1000g for 7 min. The chloroplasts appeared as a green pellet on the bottom of the tubes. After discarding the supernatants, the pellet was gently dislodged and resuspended with 1−2 mL of CIB buffer containing 0.1% of BSA. Separation of intact chloroplast from broken ones was performed by centrifuging at 1700g for 6 min on a Percoll (40%) layer. A 10 mL portion of 40% Percoll solution was used for each 6 mL of chloroplast suspension. After the centrifugation, intact chloroplasts were collected from the bottom of the tube and resuspended in 0.5 mL of CIB without BSA.

Prior to preparing glycerol stocks of the isolated chloroplasts, the concentration of chlorophyll inside chloroplasts was estimated according to the procedure reported by Arnon.[1] Briefly, 10 µL of the freshly isolated chloroplasts was suspended in 1 mL of 80% acetone and centrifuged at 3000g for 2 min. The supernatant was collected, and its absorbance was measured at 652 nm. The chlorophyll concentration in the isolated chloroplasts was set at 1 mg mL^{-1} and stored at −80°C. All steps were performed under refrigeration (2−4°C).

Biophotoanode and control photoanode preparation

The EGDGE-modified chloroplast photoanode was prepared by utilizing a carbon paper electrode (1 cm^2 geometric surface area) as support. The surface was modified by drop-casting 103.77 µL of a chloroplast solution prepared as follows: 30 µL of the isolated chloroplast glycerol stock (1 mg mL^{-1} chlorophyll concentration) + 70 µl Milli-Q water + 3.77 µL EGDGE solution (10 mg mL^{-1}). A picture of the chloroplast photoanode is shown in Fig. S1. The modified electrodes were allowed to dry at room temperature for approximately 30-40 min before their employment for the electrochemical characterization. The regular chloroplast photoanode (no EGDGE) was prepared following the same procedure of the EGDGE-modified biophotoanode, but utilizing a chloroplast solution composed of 30 µL of the isolated chloroplast glycerol stock (1 mg mL^{-1} chlorophyll concentration) + 73.7 µl Milli-Q water. Finally, abiotic control photoanodes were prepared utilizing bare carbon paper electrodes or carbon paper electrodes modified with 100 mL of Milli-Q water + 3.77 µL EGDGE solution (10 mg mL^{-1}).

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Chloroplast biosolar cell set up

The setup of the chloroplast biosolar cell is shown in Fig. S1. Specifically, the chloroplast photoanode was placed in a L-shaped transparent electrochemical cell with the side with deposited chloroplasts facing toward the glass to illuminate the electrode with a fiber optic light (Dolan-Jenner, Fiber-Lite Model 190) providing a light intensity of 76 mW cm^{-2}. The Pt-free air-breathing cathode was placed on the opposite side/opening of the electrochemical cell. The cathode was prepared as previously reported.[2, 3] Briefly, 3.5 g of activated carbon (NORIT SX Ultra, Sigma) were mixed with 0.5 g carbon black (Alfa Aesar). Following, 4 mL of a 30% dispersion of poly(tetrafluoroethylene) in water (Fuel Cell Earth) were added, together with 2-3 ml of Milli-Q water added while mixing the mixture on a vortex. The obtained paste was pressed onto a stainless steel mesh disk (McMaster-Carr, Robbinsville, BJ, USA) at 140˚C with 5000 psi. The electrochemical cell was filled with 40 ml of sterile (125˚C for 15 min) 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$. The electrical circuit between the photoanode and the air-breathing cathode was completed by connecting them with an 8000 Ω resistor.

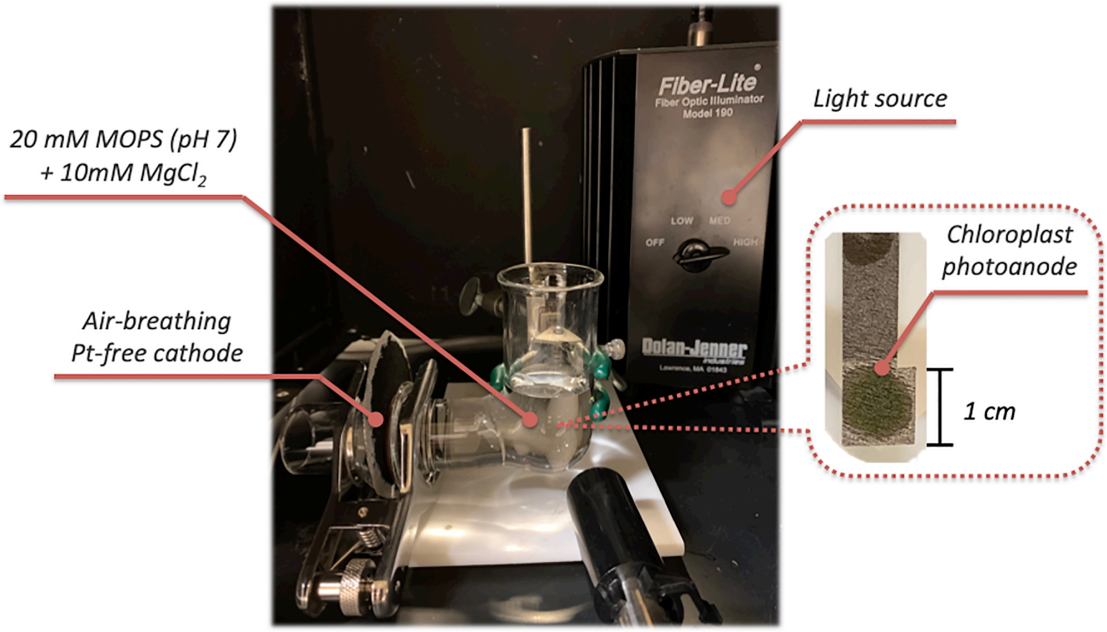

Fig. S1 Components of the chloroplast biosolar cell. The callout shows the chloroplast photoanode with immobilized chloroplasts (green area).

Electrochemical setup

For the initial characterization of the biophotoanodes, a transparent three-electrode cell was set up utilizing a saturated calomel electrode as the reference electrode (SCE, CHI 150, CH Instruments, Inc.) and a Pt mesh as a counter electrode. The electrochemical cell was filled with 20 ml of sterile (125˚C for 15 min) 20 mM MOPS buffer (pH 7) + 10 mM $MgCl_2$. The (bio)photoanode was placed facing the glass in order to illuminate the electrode with a fiber optic light (Dolan-Jenner, Fiber-Lite Model 190) providing a light intensity of 76 mW cm^{-2}. Cyclic voltammetry and amperometric i-t experiments were performed with CH Instruments potentiostats (CH Instruments, Inc.). For the characterization of the biosolar cells, quasi-stationary polarization curves at 0.1 mV s^{-1} with the cathode as the working electrode and the photoanode as the counter and reference electrode were performed to determine the power output, both under light and dark conditions. The power density was calculated based on the formula $P = E \times I$, where P is the power density, E is the potential, and I is the current density. The current density is calculated based on the anode geometric area of 1 cm^2. At least three independent replicate experiments were performed for all the studies, and errors indicate one standard deviation.

Diuron inhibition of chloroplast photoanodes

Diuron additions to the 20 ml of 20 mM MOPS (pH 7) + 10 mM $MgCl_2$ electrolyte were performed while stirring to investigate the biotic origin of the photocurrent obtained from EGDGE-modified chloroplast photoanodes. As shown in Fig. S2, after illuminating the chloroplast photoanode, the expected increase in current response was obtained, reaching 0.7±0.1 μA cm^{-2}. The following additions of diuron up to a final concentration of 5.0 μM resulted in a 93±2% inhibition of the photocurrent (continuous red line). Chloroplast photoanodes with no diuron addition showed only a 30±10% inhibition of photocurrent after 1800 seconds (continuous black line), while abiotic carbon paper electrodes modified with EGDGE showed no current or light-related response (black dashed line). Accordingly, the results confirm the photocatalytic activity of the immobilized chloroplasts performing the light-driven water-splitting reaction. Furthermore, as shown in Fig. S2 B, a 0.1 – 1.0 μM concentration of diuron resulted in a linear inhibition ratio %. Motivated by this result, we explored the possibility of utilizing the EGDGE-modified chloroplast photoanode for the development of a biosolar cell functioning as a self-powered biosensor, as discussed in the manuscript.

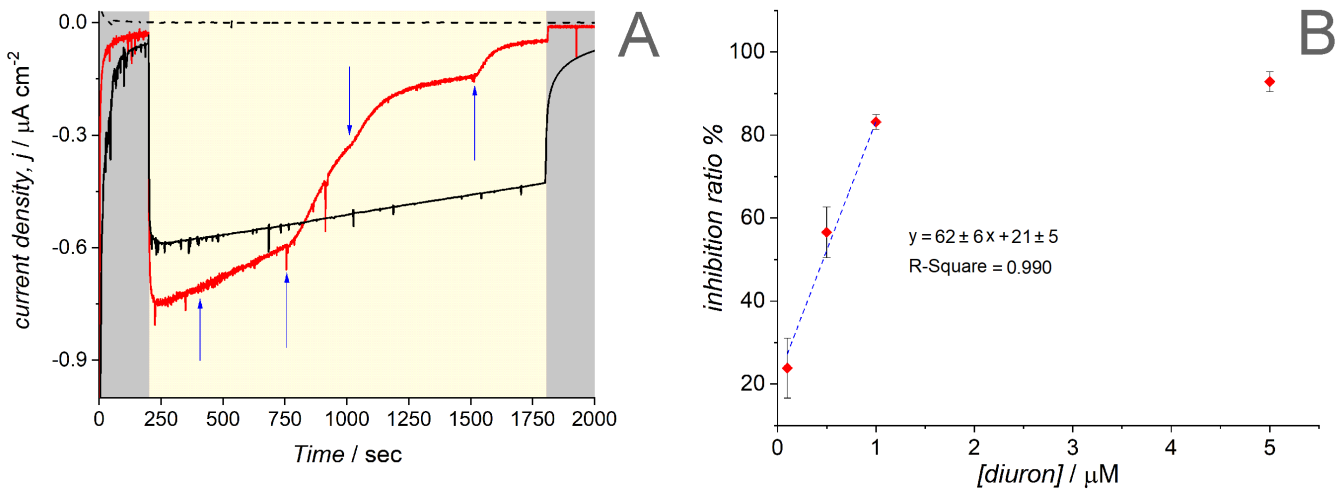

Fig. S2 Diuron inhibition on biophotocurrent production from the EGDGE-modified chloroplast photoanode. A) Representative amperometric i-t traces at 0.1 V vs. SCE for EGDGE-modified chloroplast photoanodes without (black continuous line) or with increasing additions of diuron (red continuous line) in 20 mM MOPS (pH 7) + 10 mM $MgCl_2$. Control experiment with abiotic carbon paper electrode modified with EGDGE (black dashed line). Reference electrode: SCE. Counter electrode: Pt. Yellow background indicates light conditions. Light intensity: 76 mW cm^{-2}. B) inhibition ratio % vs. diuron concentration.

Reversibility of diuron inhibition

The reversibility of the inhibiting effects of diuron was investigated in the three-electrode setup previously introduced. Specifically, amperometric i-t tests were performed exposing EGDGE-modified chloroplast photoanodes to 1.0 μM diuron and following placing the electrodes in 20 ml of fresh 20 mM MOPS (pH 7) + 10 mM $MgCl_2$ for 1 hour. After this time, the amperometric i-t tests were repeated, and the biophotocurrents were compared to the response obtained before the addition of diuron. Fig. S3 shows representative amperometric i-t traces for a EGDGE-modified chloroplast photoanode exposed to 1.0 μM diuron at 500 sec (red line), and for the same photoanode tested after being left for one hour in a diuron-free solution (green line). It can be noted that after one hour a clear recovery of the biophotocurrent response was obtained, however, the inhibition was not 100 % reversible. Specifically, it was possible to recover 70±8% of the biophotocurrent prior diuron addition. It should be noted that leaving the photoanodes in diuron-free solutions for longer periods might allow a further recovery of the photocurrent. These results are in agreement with recent studies showing that the kinetics of diuron release from photosynthetic biological entities is slower than its binding rates.[4]

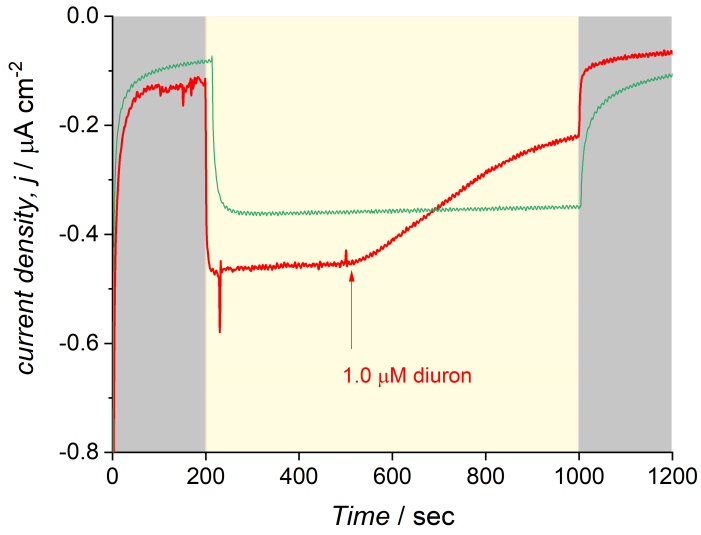

Fig. S3 Representative amperometric i-t curves for a EGDGE-modified chloroplast photoanode with 1.0 µM diuron addition at 500 sec (red line) and for the same photoanode after being in a diuron-free solution for 1 hour (green line). Yellow background indicates light conditions.

Power density of the Pt-free biosolar cell

The power densities obtained for the different biosolar cells are shown in Fig. S4. The use of EGDGE-modified chloroplast photoanodes (green) allowed a remarkable increase in power density, reaching 430±90 µW m^{-2} under illumination, compared to 180±60 µW m^{-2} for the biosolar cells with regular chloroplast photoanodes (red). A clear light-related response was obtained, as in the dark power densities decreased to 33±6 and 30±10 µW m^{-2} for biosolar cells with EGDGE-modified and regular chloroplast photoanodes, respectively (dashed green and red lines). Control cells with abiotic photoanodes resulted in no significant power production for both light and dark conditions (< 10 µW m^{-2}, black), further confirming the biotic origin of the photocurrent.

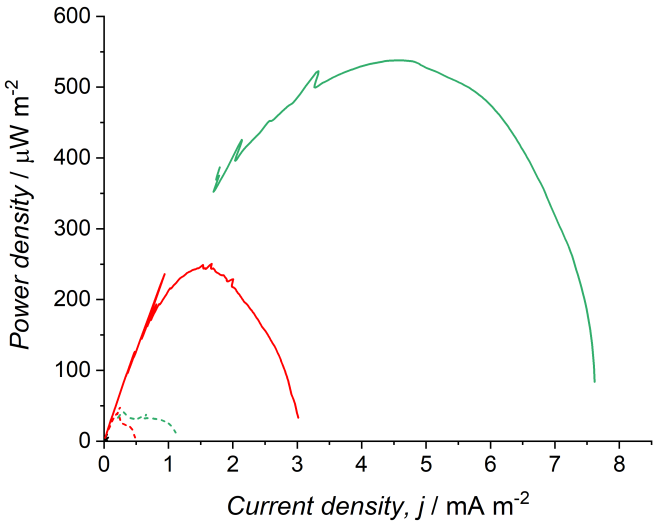

Fig. S4 Power density under illumination (continuous lines) and dark (dashed lines) for EGDGE-modified (green) and regular (red) chloroplast biosolar cells. Control cells with abiotic photoanodes (black).

Notes and references

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