

Supplementary Material

High Photo-Electrochemical Activity of Thylakoid-Carbon Nanotube Composites for Photosynthetic Energy Conversion

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Chlorophyll Concentration Measurements:

The chlorophyll concentration (C_{ch}) was calculated by using the data from UV-Vis spectrum into the equation (E1)

$$C_{ch} \left(\frac{mg}{mL} \right) = \frac{8.02 \times A_{663} + 20.2 \times A_{645}}{10} \rightarrow (E1)$$

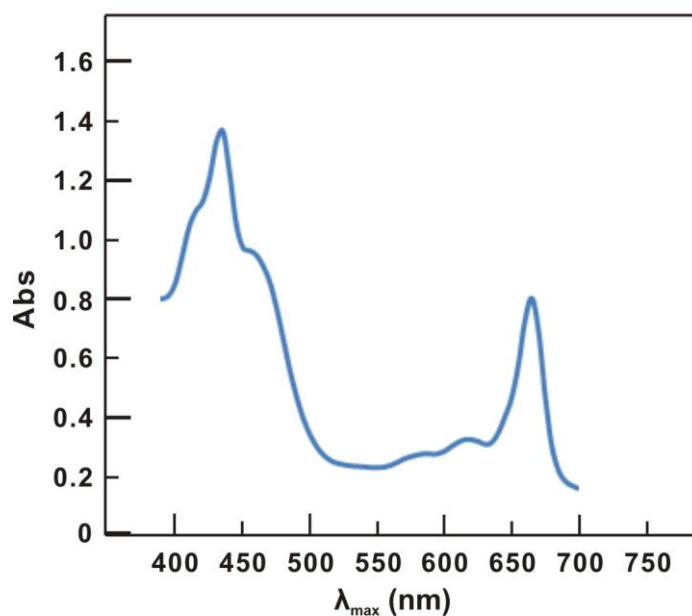


Figure S1. UV-Vis spectrum of suspended thylakoid membranes to calculate chlorophyll concentration.

Optimization of Composite Composition

The plots of current versus time at fixed anode potentials were used as a guiding tool for optimizing the thylakoid-MWNT composite electrode (Figure S2). The parameters optimized were thylakoid immobilization time (Fig. S2a) and thylakoid concentration in the immobilization mixture (Fig. S2b), mediator concentration (Fig. S2c), and the anode polarization potential (Fig. S2d). The results showed the photocurrent was directly proportional to thylakoid concentration, however only a maximum of $0.44 \mu\text{g cm}^{-2}$ of thylakoids could be immobilized due to the limited geometric size of our electrodes. Similarly, we noticed that incubation durations (for immobilization of thylakoids on MWNT matrix) beyond 1 h did not result in a significant increase in photocurrents. The photocurrent was also proportional to the mediator concentration, but the percent decrease of photocurrent per duty cycle varied. The optimized mediator concentration obtained was 1.5 mM, where the photocurrent was $0.9 \mu\text{A}$ for the first cycle while still maintaining stability through multiple cycles. The anode potential was also optimized at 0.2 V to observe noticeable photo-activity.

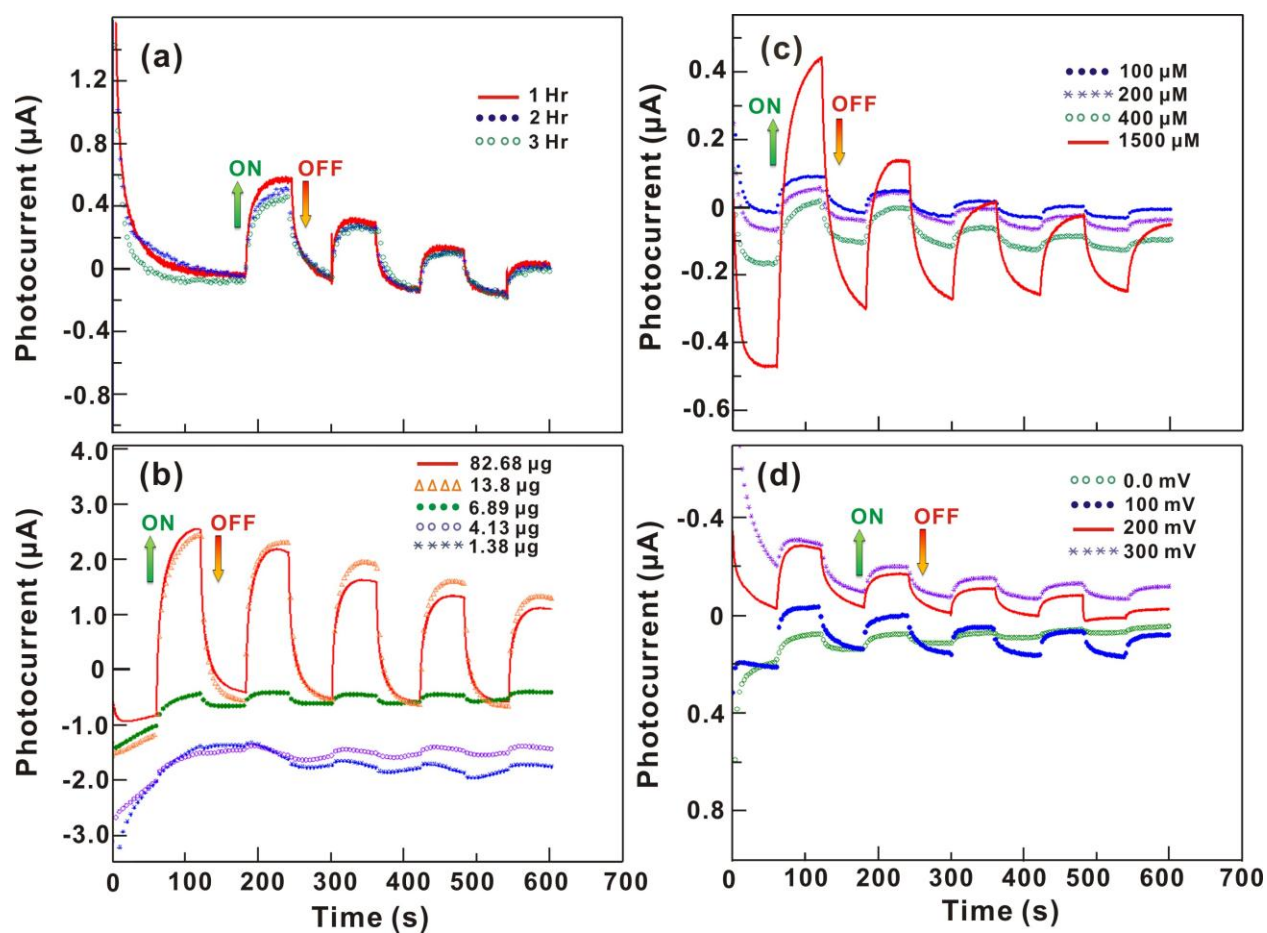


Figure S2. Photocurrent analysis of thylakoid-MWNT composites for the optimization of (a) thylakoid immobilization time, (b) chlorophyll loading, (c) mediator concentration and (d) applied potential. ↑ represents light condition and ↓ represents dark condition. An applied potential of 0.2 V, 1 hr thylakoid immobilization time, and 1.5 mM mediator concentration were the most favorable conditions for this technique.

Inhibition of Plastocyanin by KCN:

The thylakoid-MWNT composites prepared using KCN in the immobilization mixture exhibited a significant reduction the plastocyanin activity by up to 73 %. Although KCN only inhibits plastocyanin, the redox activity of *cyt b₆f* (peak at 0 V) was also reduced by 23 %. This could be due to the lack of an electron acceptor for *cyt b₆f* (when plastocyanin was inhibited), which may result in an excited *cyt b₆f* (electron rich) that reacts with oxygen to form peroxides that degrade the *cyt b₆f* activity over time as suggested by Fuerst et.al.[†]

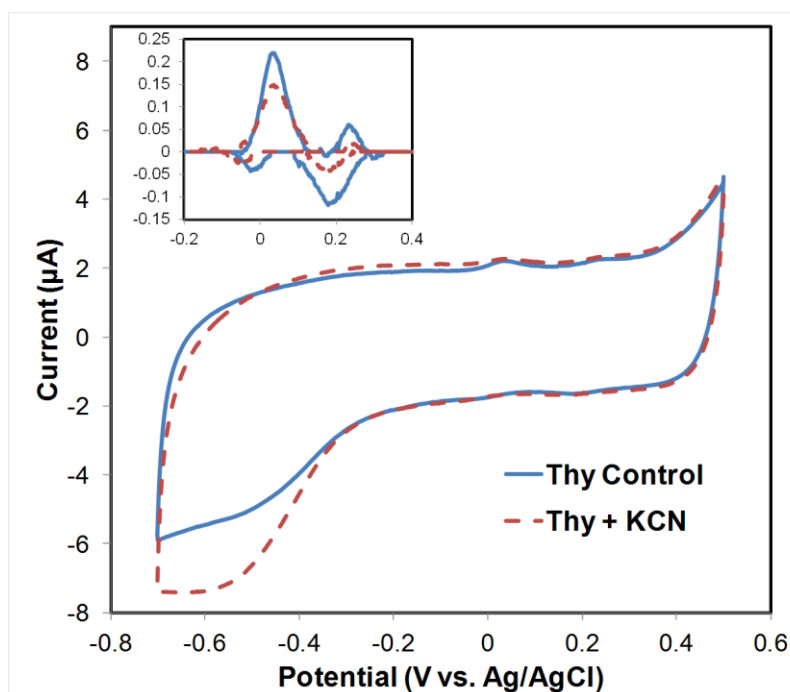


Figure S3. Cyclic voltammograms of thylakoid-MWNT composites in the presence and absence of 10 mM KCN as plastocyanic inhibitor.

[†] E.P. Fuerst and M.A. Norman, Interaction of herbicides with photosynthetic electron transport. *Weed Science* **39**, 458-464 (1991).

Photoactivity of Mediators:

Constant potential measurements on unmodified MWNT electrodes in the presence of mediators at 0.2 V under light on-off conditions showed that the ferricyanide $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple exhibited less photo-response than the benzoquinone complexes used by others in the literature. Therefore the observed photocurrent activity in our thylakoid-MWNT composite electrodes can be directly attributed to thylakoids and not the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ mediator.

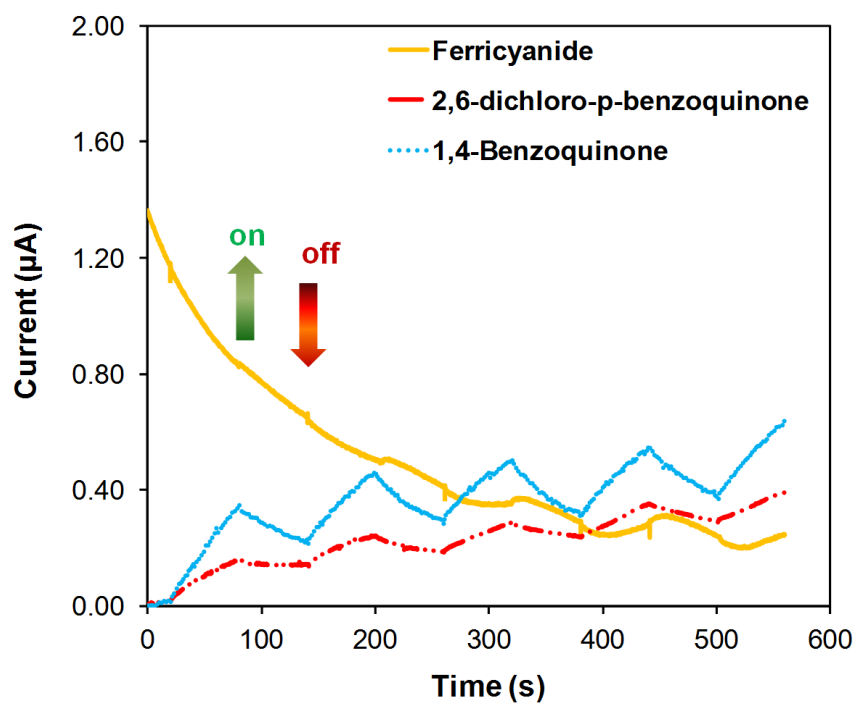


Figure S4. Photocurrent responses of 1.5 mM 2,6-dichloro-p-benzoquinone and 50 mM 1,4-benzoquinone mediators on MWNT electrodes in the absence of thylakoids. The photocurrent response of ferricyanide mediator is also shown for comparison.

Absorption Spectra of Mediators and Inhibitors:

The peak at 673 nm indicates absorbance via chlorophyll-*a*. The absorption spectrum for the thylakoid-free MWNT electrode did not contain the chlorophyll peak (Fig. S5a), indicating that the mediator does not compete chlorophyll for absorbing light in the 660-675 nm range. Also the thylakoid-MWNT composite electrode in the presence of mediator showed the 664 nm chlorophyll-*a* peak, indicating that the light absorbance of chlorophyll was not affected by the presence of mediator. Similar absorption spectra were also obtained for the composite electrodes in the presence of herbicide, DCMU and the inhibitors, KCN and Paraquat/diquat.

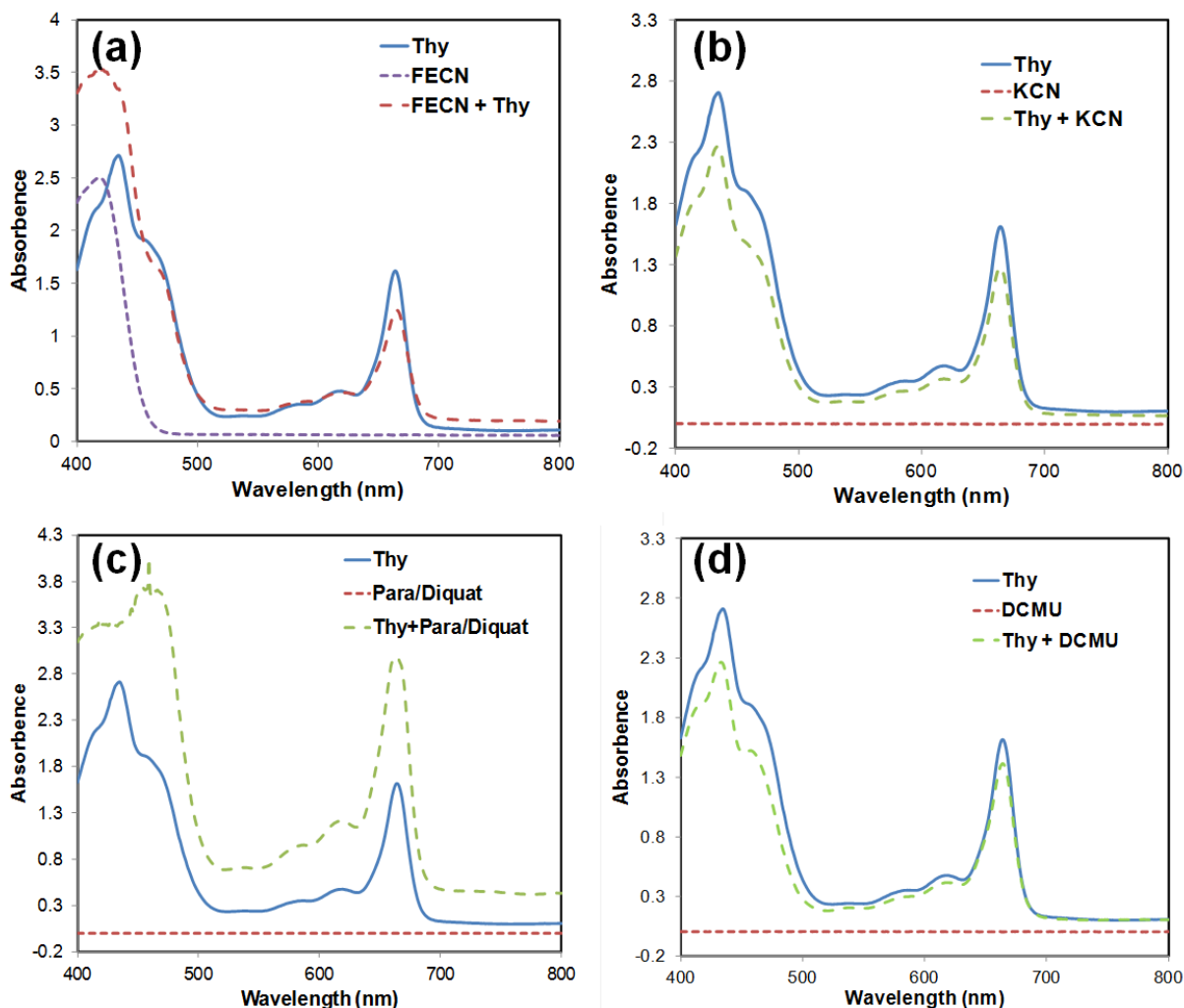


Figure S5. Absorption spectrum of thylakoid membranes with exposure to (a) ferricyanide mediator, (b) KCN, (c) paraquat/diquat and (d) DCMU herbicide.

Effect of Light Intensity on Photocurrent:

The photo-electrochemical response of the thylakoid-MWNT composite modified electrodes varied with light intensity as shown in Figure S6. Initially the system was in dark. A high intensity light (80 mW cm^{-2}) was illuminated after 60 sec, which resulted in the increase in measured photocurrent at 0.2 V to a stable value of $2.6 \mu\text{A}$. At time 540 s, the light intensity was decreased to "medium" setting in the lamp that resulted in the decrease in photocurrent to $1.9 \mu\text{A}$. When light intensity was further decreased to "low" setting, the photocurrent of the composite further decreased to $0.9 \mu\text{A}$. The results demonstrate very good dependency of thylakoid photo-response on the intensity of the incident light. In these experiments the light passes through the glass cell and buffer solution to reach the electrode, not all the intensity of light was fallen on the electrode surface from the light source.

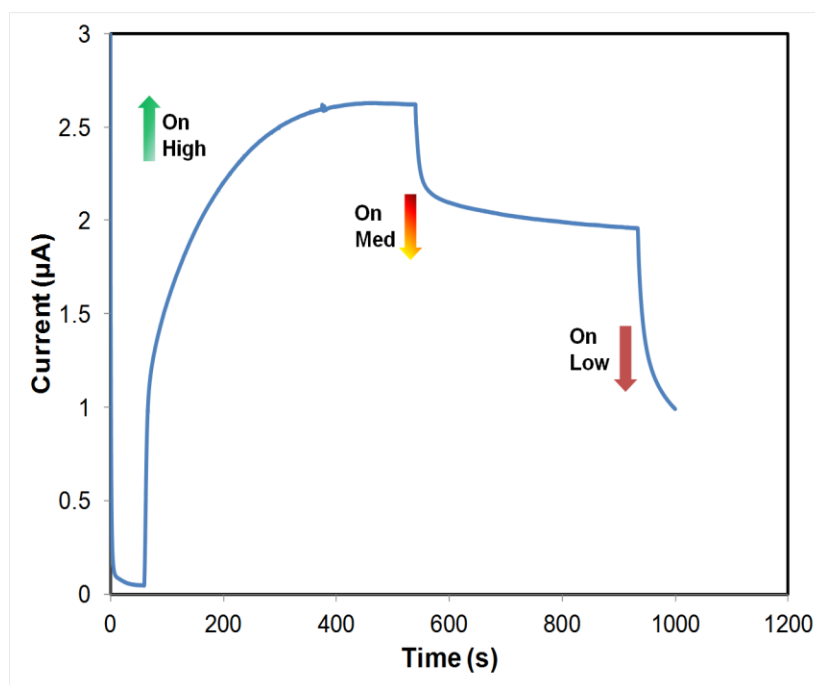


Figure S6. Effect of light intensity on thylakoid-MWNT composites. High light intensity was turned on at 60 s, medium at 540 s and low light at 920 s.

Advantages of Thylakoid Immobilization:

To understand if there are significant advantages associated with immobilizing thylakoids for carrying out photo-electrochemical reactions, rather than suspending them in the solution, separate experiments were performed. Figure S7a reveals that the immobilized thylakoids exhibited a fairly stable and reproducible photo-electrochemical activity over several duty cycles, whereas the thylakoids suspended in solution showed a gradual loss in the photocurrent activity with less reproducibility. Moreover, despite a high concentration of chlorophyll in solution (up to 400 times more), the photocurrents of suspended thylakoids were significantly lower than that of immobilized thylakoids. This was expected, as the electron flux of mediators for immobilized thylakoids would be higher than for suspended thylakoids due to the proximity of thylakoid membrane proteins to the electrode, which reduces the diffusion distance for mediators. It can also be noticed that although high concentration of suspended thylakoids increases the photocurrents, the trend is reversed at exceedingly high thylakoid concentrations due to the issues of high turbidity and low light penetration in the electrolyte, a case that was carefully avoided in our experiments. The cyclic voltammograms (Figure S7b) showed redox activities for immobilized thylakoids arising from the direct interaction of surface bound proteins with the electrode. For the case of thylakoids suspended in the solution there was no such redox activity. Therefore the immobilization method is vital for enhanced electron transfer and high photocurrents.

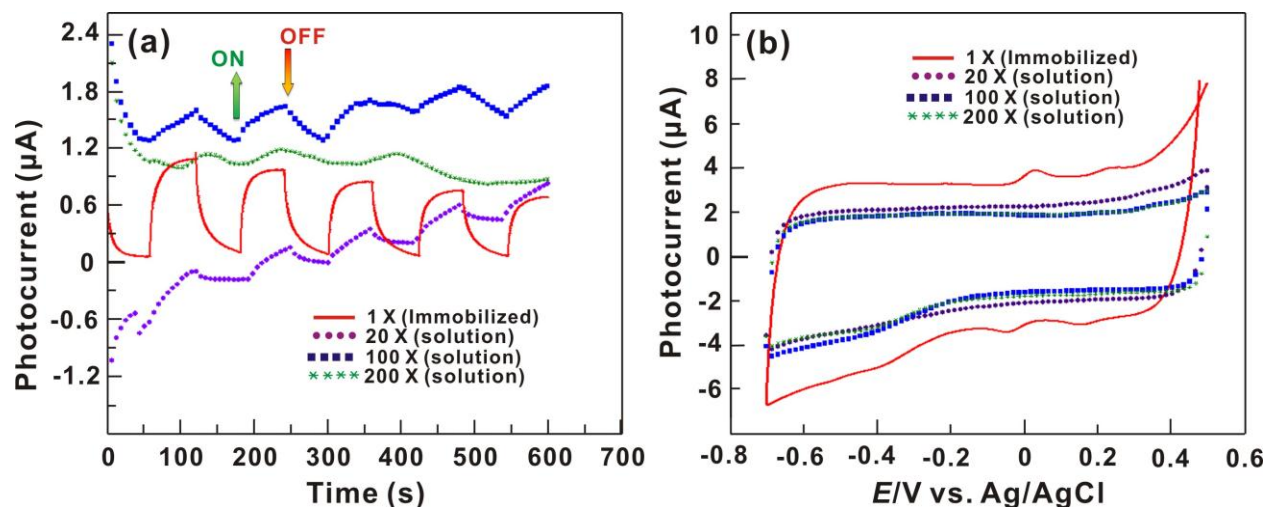


Figure S7. Comparison of immobilized thylakoids (1X) with various quantities (20X, 100X, 200X) of suspended thylakoids in solution: (a) photocurrent response (\uparrow represents light condition and \downarrow represents dark condition); and (b) cyclic voltammograms under light. Note: 1X corresponds to 0.014 mg_{chl} .

Steady State Analysis:

When light was illuminated a large increase in anodic current was observed due to ferrocyanide oxidation at the electrode surface. This would require a continuous ferricyanide reduction by thylakoid membrane proteins in the presence of light. Over time the current generation stabilized to a constant value at approximately $0.675 \mu\text{A}$ (Figure S8a). This indicates that the observed decrease in photocurrent overtime during different light on-off cycles was due to transience in the mediator diffusion, which reaches steady state. The observed phenomenon could partly be due to the establishment of steady diffusion gradients in the system. Initially there was a high concentration of mediator present at the electrode-solution interface. Upon illumination, the redox couple undergoes transition from ferricyanide to ferrocyanide and which results in a decreased concentration of the ferricyanide at the interface. This slows down electron transfer to the electrode until it reaches equilibrium upon which a steady photocurrent was observed. In the absence of light (Figure S8b), the photocurrent was stabilized to $\sim 1 \text{ nA}$. As we can see from the figures, over time the currents from both experiments reached the same steady value. This suggests that the decrease in the photocurrent over time was partly due to the transience in mediator diffusion. However a loss of photo-electrochemical activity or composite dissolution from the electrode surface over time can neither be verified nor confirmed based on our experimental results.

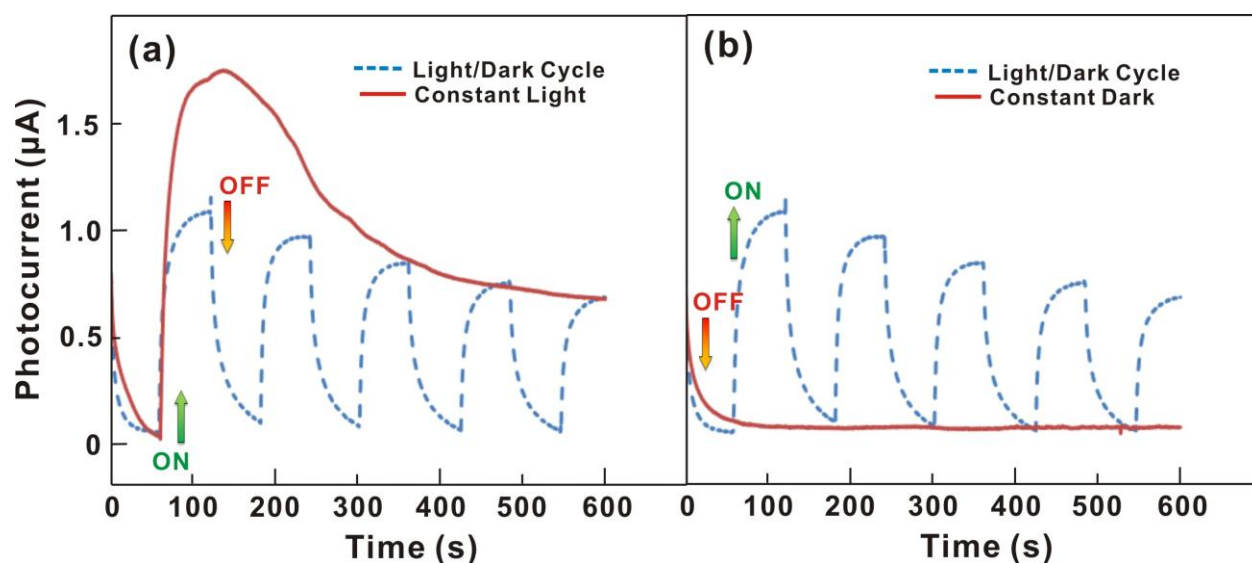


Figure S8. Photocurrent analysis of thylakoid-MWNT modified gold electrode under: (a) constant light and (b) constant dark conditions.

Herbicide Inhibition of Photosystem II:

Upon exposing the thylakoid to DCMU herbicide that inhibits PSII activity, no photo-electrochemical activity was noticed in the light on-off tests. However the cyclic voltammograms under light showed no loss in the redox activities of both *cyt-b₆f* and plastocyanin, indicating the direct electrochemical activity of the thylakoid membrane redox proteins were not affected by the PSII inhibition.

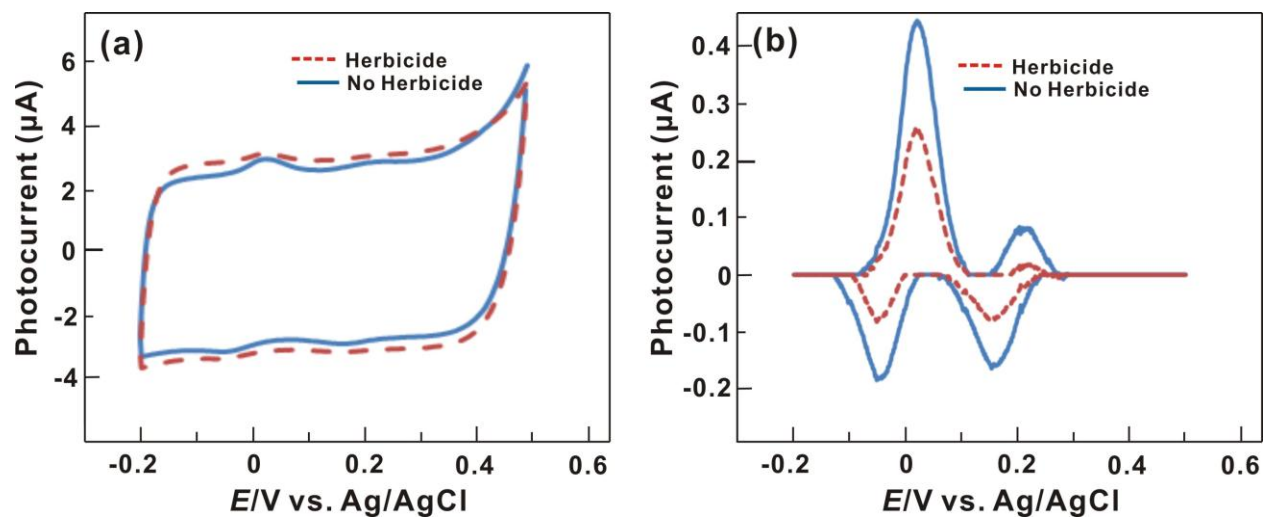


Figure S9. (a) Cyclic voltammograms of thylakoid-MWNT composites with and without exposure to DCMU herbicide. (b) background subtracted cyclic voltammograms showing the retention of redox peaks of *cyt-b₆f* (0 V) and plastocyanin (0.2 V).

Non-Involvement of PSI in Photocurrent Generation

The non-involvement of PSI in photocurrent generation was studied by inhibiting PSI activity by paraquat/diquat solution mixture (of the bipyridillum family). The solution mixture was added to thylakoids prior to immobilization. Paraquat ($E_0 = -0.45$ V) acts as a competitor to ferredoxin (FD, $E_0 = -0.51$ V) for accepting the electrons from the F_a/F_b site of PSI ($E_0 = -0.56$ V) in the photosynthetic pathway. As shown in Figure S10, the presence of the PSI inhibitor did not significantly reduce the photo-electrochemical response of the thylakoid-MWNT composites. Therefore a major fraction of the electron flux generated at the OEC site must have been diverted towards the electrode through $[\text{Fe}(\text{CN})_6]^{3-/4-}$ mediator as depicted in Figure 1 (in main article), rather than to the PSI complex via the natural pathway.

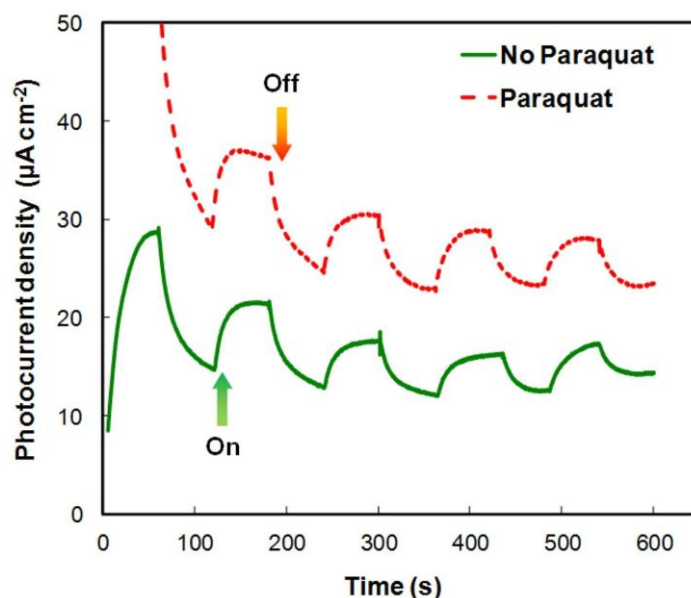


Figure S10. Comparison of the photo-current responses of unexposed and paraquat exposed thylakoid-MWNT composites. ↑ and ↓ represents light on and light off conditions respectively.