



## Improved Performance of a Thylakoid Bio-Solar Cell by Incorporation of Carbon Quantum Dots

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Carbon quantum dots (CQDs) were incorporated into thylakoid bioanodes capable of direct photobioelectrocatalysis in order to increase the photocurrent generation. More thylakoids are in contact with the increased surface area which allows for greater direct electron transfer (DET). Additionally, the fluorescent quantum dots redshift the light which allows for the thylakoid/CQD electrodes to use more of the solar spectrum, increasing the photocurrent. The current density was more than twice as large when CQDs were included in a thylakoid bio-solar cell.

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The development of solar energy conversion devices is a large area of research in a world where the demand for renewable energy sources is quite high.<sup>1</sup> Many of these devices use expensive precious metal catalysts and semiconductors. An alternative to these conventional solar cells would be the use of the biological catalysts responsible for solar energy conversion in nature. This type of system would be safely disposable as well as inexpensive to fabricate, because the catalysts would be extracted from plant matter. Electrodes capable of photocurrent generation using chloroplasts,<sup>2,3</sup> thylakoids,<sup>4–7</sup> individual photosystems,<sup>8</sup> and reaction centers of photosynthetic complexes from cyanobacteria and higher plants<sup>9–12</sup> have been developed.

The Minteer research group has reported previously a bio-solar cell consisting of a thylakoid bioanode and Pt air-breathing cathode capable of generating a short circuit photocurrent density of  $2.14 \pm 0.11 \mu\text{A cm}^{-2}$  with an open circuit voltage (OCV) of 0.46 V.<sup>5</sup> When the cathode was replaced with a laccase oxygen reduction biocathode, the cell had a short circuit photocurrent density of  $14.0 \pm 1.8 \mu\text{A cm}^{-2}$  and OCV of 0.72 V.<sup>6</sup> The major advantage to our system is that the thylakoids are able to perform direct electron transfer (DET) which leads to a larger OCV than reported for mediated thylakoid bio-solar cells.<sup>4</sup> However, the current output of our system is small compared to conventional solar cells, so improving the performance of a DET thylakoid bio-solar cell is crucial for its potential use in future energy generation.

Thylakoid membranes, found in cyanobacteria and chloroplasts of plants, contain all of the necessary components for the light-dependent reactions which convert solar energy into chemical energy.<sup>13</sup> Photosynthesis begins with the oxidation of water to O<sub>2</sub> by photosystem II (PSII). The electrons from PSII are used in the reduction of plastoquinone to plastoquinol which then binds to the cytochrome b<sub>6</sub>f complex. Plastoquinone is regenerated and the resulting protons are released into the lumen while the electrons are transferred to plastocyanin. The reduced Pc is oxidized and its electron are transferred to photosystem I (PSI) and then to ferredoxin. From ferredoxin, the electron flow can proceed by two different pathways.<sup>14</sup> Ferredoxin-NADP<sup>+</sup> reductase oxidizes the reduced ferredoxin and reduces NADP<sup>+</sup> to NADPH.<sup>15</sup> Alternatively, the electrons from ferredoxin can be transferred to plastoquinone for a cyclic pathway. A proton gradient is generated during photosynthesis and is used by ATP synthase to produce adenosine triphosphate (ATP).<sup>13</sup>

Nanostructured carbon materials have been used to improve the performance of a number of bioanodes and biocathodes. The most commonly used carbon material used for this type of application, carbon nanotubes (CNTs), have been implemented for laccase<sup>16</sup> and bilirubin oxidase<sup>17</sup> oxygen reduction biocathodes as well as glucose

bioanodes<sup>18–21</sup> to give increased surface area for DET. Calkins et al. covalently tethered thylakoids to CNTs for their anodes for a bio-solar cell in order to increase the number of thylakoids in contact with the carbon surface.<sup>4</sup> Carbon quantum dots (CQDs) are a fairly new carbon material with a variety of unique optical and electrical properties, including photoluminescence, photoinduced electron transfer, and electrochemiluminescence.<sup>22–25</sup> However, unlike more traditional quantum dots such as CdS or CdSe, carbon quantum dots are chemically inert, biocompatible, and show low toxicity.<sup>26</sup> In this paper, we use carbon quantum dots to improve the performance of a thylakoid bio-solar cell. In addition to the increased surface area the CQDs provide, they are also fluorescent<sup>27</sup> which gives the potential to use more of the solar spectrum than currently used by our bioanodes for solar energy conversion.

### Experimental

Carbon quantum dots were fabricated following a previously reported procedure.<sup>27</sup> Graphite rods were used as anode and cathode in a 99.5:0.5 ethanol/water solution containing 0.3 g NaOH. A current density of 20 mA/cm<sup>2</sup> was applied for 24 hours. MgSO<sub>4</sub> was added to remove the water and the CQD were purified by silica gel column chromatography with ethanol as the solvent.

Thylakoid membranes were isolated from organic spinach leaves following a previously reported procedure.<sup>5</sup> Briefly, intact chloroplasts were obtained and lysed to extract the thylakoids. The thylakoids were resuspended in 330 mM sorbitol, 50 mM HEPES, and 2 mM MgCl<sub>2</sub>, pH 7.8, and stored at –20°C until further use. Chlorophyll concentration was determined spectroscopically to be 1.1 mg/mL. Thylakoid anodes were constructed using 1 cm<sup>2</sup> Toray carbon paper. Catalase from *Aspergillus niger* (Sigma, 9.47 kU mg<sup>–1</sup>, 28.73 mg mL<sup>–1</sup>, 3 μL) was added to 100 μL of the thylakoid suspension. For CQD electrodes, 20 μL of the CQD solution was added to 100 μL of thylakoid suspension. An aliquot of 50 μL is applied to each electrode and allowed to dry. A thin layer of silica is vapor deposited onto the electrodes using tetramethyl orthosilicate following a previously reported procedure.<sup>28</sup> Electrodes were stored at 4°C overnight.

Fluorescence data was acquired with a Hitachi F-7000 fluorescence spectrophotometer. For TEM imaging, 3.5 μL of CQDs in ethanol were placed on a carbon-coated copper transmission electron microscope grid and allowed to dry. The specimen was then imaged in a JEOL JEM1400-Plus TEM (Tokyo) operated at 120 kV with a Gatan Orius SC1000 camera (Pleasanton, California). Electrochemical measurements were performed using a CH Instruments CHI660E electrochemical workstation. Electrodes were illuminated with a 250 W Halogen lamp at 5200 lumens. Laccase biocathodes were used for the bio-solar cell testing. These oxygen reduction cathodes were prepared following a previously reported procedure.<sup>6</sup> All electrochemical experiments were repeated in triplicate and the results show the average for the three trials.

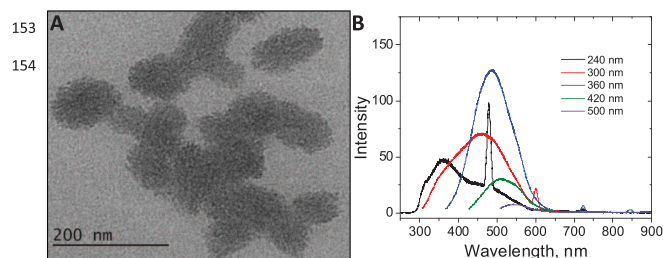
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**Table I. (A) Light and dark currents ( $J_L$  and  $J_D$ ) and current differences ( $\Delta J$ ) for electrodes held at 350 mV vs. Ag/AgCl. (B) Short circuit current densities and OCVs for bio-solar cells in 0.1 M pH 5.5 citrate buffer with laccase cathodes and different anodes: CQDs with no thylakoids, thylakoids only, and thylakoids with CQDs.**

Amperometric testing	$J_D$ ( $\text{nA cm}^{-2}$ )	$J_L$ ( $\text{nA cm}^{-2}$ )	$\Delta J$ ( $\text{nA cm}^{-2}$ )		
CQDs	$17 \pm 6$	$21 \pm 7$	$4 \pm 1$		
Thylakoids	$65 \pm 20$	$430 \pm 70$	$360 \pm 50$		
Thylakoids + CQDs	$620 \pm 80$	$1060 \pm 30$	$450 \pm 60$		
Bio-solar cell testing	$J_{SCD}$ ( $\mu\text{A cm}^{-2}$ )	$J_{SCL}$ ( $\mu\text{A cm}^{-2}$ )	$\Delta J_{SC}$ ( $\mu\text{A cm}^{-2}$ )	OCV <sub>D</sub> (V)	OCV <sub>L</sub> (V)
CQDs	$0.6 \pm 0.1$	$0.9 \pm 0.2$	$0.28 \pm 0.06$	$0.67 \pm 0.02$	$0.67 \pm 0.03$
Thylakoids	$3.7 \pm 0.8$	$6 \pm 1$	$2.0 \pm 0.9$	$0.67 \pm 0.01$	$0.65 \pm 0.01$
Thylakoids + CQDs	$9 \pm 2$	$12 \pm 1$	$3.1 \pm 0.9$	$0.65 \pm 0.02$	$0.62 \pm 0.02$

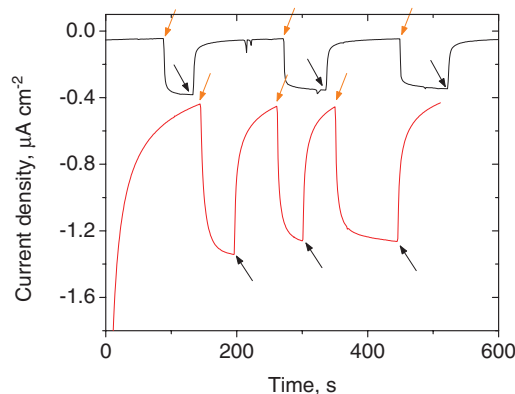


**Figure 1.** (A) TEM image of CQD and (B) Fluorescence emission spectra of CQDs in ethanol at several excitation wavelengths.

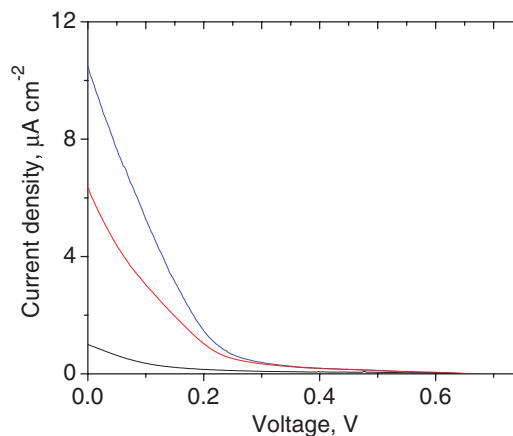
### Results and Discussion

CQDs were imaged with TEM to determine the shape and size of the particles. As seen in Figure 1A, the quantum dots are ovoid in shape with a long axis of  $126 \pm 8$  nm and a short axis of  $77 \pm 7$  nm. The TEM shows that the CQDs aggregate into larger particles. Fluorescence spectra for the CQDs at several excitation wavelengths are shown in Figure 1B and are consistent with previously reported results by Li et al.<sup>27</sup> During photosynthesis, the thylakoid photosystems absorb at 680 nm and 700 nm and also require green-yellow light.<sup>14</sup> The CQDs used in this study absorb at wavelengths that are not necessary for photosynthesis and emit light which is redshifted to 500–600 nm (green-yellow). This makes it possible for the bioanodes to make greater use of the solar spectrum than thylakoid electrodes alone which would not use the light at lower wavelengths. With the addition of CQDs, that light is now being used.

Amperometric experiments at 350 mV vs. Ag/AgCl were performed with thylakoid and thylakoid/CQD electrodes. Representative J-t curves are shown in Figure 2. The current is allowed to stabilize in



**Figure 2.** Current density measured at 350 mV vs. Ag/AgCl for thylakoid (black line) and thylakoid/CQD (red line) electrodes in 0.1 M pH 5.5 citrate buffer. Orange arrows indicate when the electrode was illuminated and black arrows indicate when the light was turn off.



**Figure 3.** Representative J-V curves for bio-solar cells with different anodes: CQDs with no thylakoids (black line), thylakoids only (red line), and thylakoids with CQDs (blue line). The solar cells were tested in 0.1 M pH 5.5 citrate buffer at a scan rate of 5 mV/s while illuminated.

the dark and then the electrode is illuminated until the current stabilizes again. This process is repeated several times. As can be seen in Figure 2, the electrodes containing CQDs take longer to reach a stable current. This is likely due to the increased carbon surface area. The current density in the dark ( $J_D$ ) is used as the background and subtracted from the light current density ( $J_L$ ) to calculate the difference ( $\Delta J$ ). As seen in Table I, when CQDs are included in the bioanode the light and dark current densities are significantly larger (more than 2 times and 10 times larger, respectively). The current difference,  $\Delta J$ , shows only a small change ( $\sim 25\%$  increase). This suggests that most of the performance improvement is due to the increased surface area allowing for more DET from the thylakoids. It is possible that the fluorescent emission of the CQDs is responsible for a small part of the current density increase, but the inherent variability in the system makes it difficult to say this conclusively. Control experiments performed with electrodes containing no thylakoids showed only a small current response.

Representative J-V curves for modified electrodes were obtained in the presence of light (see Figure 3). Thylakoid-modified electrodes generated a short circuit current density of  $6 \pm 1 \mu\text{A/cm}^2$ . This current density decreased rapidly to approximately 0.25 V where it remained close to zero until the open circuit voltage (OCV) of  $0.65 \pm 0.01$  V (see Table I). The addition of CQDs to thylakoids during immobilization increased the current density over the voltage range of 0 to 0.25 V. The short circuit current density with thylakoids and CQDs was  $12 \pm 1 \mu\text{A/cm}^2$ , which is two times larger than electrodes with only thylakoids. Similarly to the amperometric results, when CQDs are included the  $\Delta J_{SC}$  shows a small change. This indicates that a small part of the enhancement is due to the ability of the CQDs to utilize more of the light spectrum. Control electrodes modified with only CQDs showed small currents close to 0 V. The incorporation of CQDs did not affect the OCV for the bio-solar cell.

### Conclusions

Carbon quantum dots were incorporated into thylakoid bioanodes to improve the performance of bio-solar cells. Photocurrent generated during amperometric experiments at 350 mV vs. Ag/AgCl were more than twice as large for thylakoid/CQD electrodes compared to thylakoids only. Bio-solar cell tests showed a similar increase. The results suggest that the CQDs improve performance in two ways. Besides the increased surface area which is true of any carbon material added to a modified electrode, the fluorescence capabilities of the CQDs allow for the system to use more of the light spectrum by converting lower wavelengths that are not used in photosynthesis to higher wavelengths that are. This enhancement would be increased by using larger CQDs that shift the light to even higher wavelengths, because the photosystems absorb deep red light.

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