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Micropatterned Carbon-on-Quartz Electrode Chips for Photocurrent Generation from Thylakoid Membranes

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ABSTRACT

Harvesting the energy generated by photosynthetic organisms through light-dependent reactions is a significant step towards a sustainable future energy supply. Thylakoid membranes are the site of photosynthesis, and thus particularly suited for developing photo-bioelectrochemical cells. Novel electrode materials and geometries could potentially improve the efficiency of energy harvesting using thylakoid membranes. For commercial applications, electrodes with large surface areas are needed. Photolithographic patterning of a photoresist, followed by pyrolysis, is a flexible and fast approach for the fabrication of carbon electrodes with tailored properties. In this work, electrode chips consisting of patterned carbon supported on quartz were designed and fabricated. The patterned electrode area is 1 cm\(^2\), and the measurement chamber footprint is 0.5 cm\(^2\), one order of magnitude larger than previously-tested electrodes for thylakoid membrane immobilization. The use of a transparent substrate allows back-side illumination, protecting the bioelectrochemical system from the environment and vice versa. Two different mediators, monomeric ([Ru(NH\(_3\))\(_6\)]\(^{3+}\)) and polymeric ([Os(2,2-bipyridine)\(_2\)-poly(N-vinylimidazole)\(_{10}\)Cl]\(^{1+/2+}\)) are used for evaluating photocurrent generation from thylakoid membranes with different electrode geometries. Current densities up to 71 \(\mu\)A cm\(^{-2}\) are measured upon illumination through the transparent electrode chip with solar simulated irradiance (1000 W m\(^{-2}\)).

1. Introduction

Ever since the energy crisis of the 1970s, the energy demand has been increasing at both global and consumer level\(^{1-3}\). In the context of climate change, it is important not only to produce...
sufficient energy for our ever-growing needs, but also to move towards clean, renewable energy sources as a step towards a sustainable future\(^4-6\). In 2010, renewable sources accounted for 16.6% of the energy consumed worldwide, and there is a projected increase to almost 50% by 2040\(^6\).

One of the main renewable energy sources is the Sun. Therefore, there is considerable focus on the development of methods for harvesting solar energy\(^7-10\). Solar energy is the primary energy source for organisms capable of photosynthesis, such as algae, green plants and cyanobacteria. For these organisms, photosynthesis takes place inside thylakoid membranes (TM)s, which for algae and plants are found within chloroplasts\(^11,12\). The natural photosynthetic system is complex and involves numerous processes after photoexcitation. Two coupled protein complexes, photosystems I and II (PS I and PS II) are the key components in the light reactions of photosynthesis\(^13-15\). A variety of photosynthetic systems have been studied for harnessing sunlight in photo-bioelectrochemical cells (PBECs), e.g., isolated PS I and PS II\(^14-16\), TM\(s\)\(^17-19\), cyanobacteria\(^20\), Rhodobacter sp.\(^21,22\), and algae\(^23\). However, in order to commercially explore PBECs, their efficiency needs to be substantially improved\(^24\) by increasing the rate of the extracellular electron transfer and the viability of these systems. There is an interest in developing mediator-less devices, but so far these show very low efficiency, with current densities below 2 \(\mu\text{A cm}^{-2}\) \(^{18,25-27}\). This is why most approaches use different soluble redox species and/or osmium redox polymers as mediators between photosynthetic components and electrodes\(^28,29\). An improvement in the electrochemical interactions in such systems can be achieved at several levels, e.g., i) engineering of photosynthetic systems\(^30\); ii) improvement in the mediated electron transfer between the photosynthetic reactions centers and the electrodes; iii) development of new electrodes, which maximize the active surface area while minimizing...
diffusion distances, thus enhancing light distribution to the photosynthetic components and facilitating electron transfer.

In addition to improving the efficiency of PBECs, another important step towards commercial applications is fabricating the PBECs as closed systems in order to avoid possible undesired interactions between its components and the environment. One option for building a closed system, which still allows light to access the photosynthetic components, is the usage of a transparent bioanode.

Photovoltaic systems make use of transparent electrode materials as an integral part of the device architecture and transparent conducting oxides (TCOs) are the most commonly used materials because of their good conductivity and transparency. Several options are available, from well-known indium-based materials, such as ITO (indium tin oxide), to doped ZnO, TiO$_2$ and SnO$_2$ materials. Recently, fluorinated-tin oxide (FTO), was used as electrode material in a PBEC. However, illumination was done from the top, without taking advantage of the transparency of FTO. An ITO biosupercapacitor was also recently reported. Due to high costs and limited availability, researchers are interested in potential alternatives to TCOs, with graphene, carbon nanotubes and metal nanowire electrodes showing most promise.

Carbon, with its different allotropes, is one of the most widely used electrode materials, especially for fuel cell applications, because of its numerous advantages: abundance, low fabrication costs, wide potential window, relatively inert chemistry, biocompatibility and good electrocatalytic activity. Photolithography can be used to deposit and pattern a polymeric photoresist onto a substrate of choice in a simple, scalable process. Moreover, photolithography potentially enables increasing the surface area by facilitating patterning of three-dimensional structures. By pyrolyzing the polymer precursor in inert atmosphere at temperatures above
900 °C, glass-like carbon is obtained, while the pattern defined in the precursor is preserved\textsuperscript{45}. Quartz is transparent in the UV-Vis domain, hard, durable and thermally resistant up to 1600 °C\textsuperscript{46}. These properties identify it as an excellent substrate for developing transparent chips with patterned carbon electrodes on a large area.

Here we report the application of carbon electrode chips, fabricated through the pyrolysis of photoresist, for harvesting the energy generated by a photosynthetic system. Different carbon electrode patterns were defined on quartz substrates to investigate i) the trade-off between the transparent area available for photosynthesis and the opaque carbon electrode area required for photocurrent collection (open area fraction) and ii) the influence of the distance between the photosynthetic reaction centers (PRCs) and the carbon electrode surface on photocurrent generation. Additionally, the influence of the illumination power density on photocurrent collection was investigated. TMs were used as model light-harnessing systems, since they are easier to extract and purify than PS I and PS II, and at the same time more stable, because all the protein complexes retain their native environment. Photocurrent generation from TMs was tested with one soluble mediator, [Ru(NH\textsubscript{3})\textsubscript{6}]\textsuperscript{3+}, and one redox polymer mediator, [Os(2,2’-bipyridine)\textsubscript{2}-poly(N-vinylimidazole)\textsubscript{10}Cl]\textsuperscript{+2+}.\textsuperscript{29} Experimental data was analyzed through a theoretical diffusion model, which provides insight into the processes taking place in the bioelectrochemical system.

2. Results and discussion

2.1. Measurement system and micropatterned electrode chips

2.1.1. Measurement system and redox reactions

The fabricated electrode chips consist of carbon electrodes on a transparent carrier substrate. The quartz substrate is transparent in the visible range, but it is non-conductive\textsuperscript{46}. The carbon
electrodes are opaque, but good electrical conductors. Since photocurrent generation requires light and photocurrent collection requires conductivity, there is a trade-off between the degree of exposed quartz and carbon areas of the chip. The measurement holder (Supporting information (SI) Figure S1) defines the electrode footprint in the center of the patterned electrode area and it ensures that i) light from the source can reach the sample in a reproducible manner and ii) the path that the electrons need to travel outside the patterned area is the same regardless of the design. The same holder was employed for all the experiments in this study.

A side-view schematic representation of the measurement system, using illumination from the backside of the electrode chips, is shown in Figure 1a. Light passes through the transparent carrier substrate and reaches the TMs in the areas with openings in the carbon layer. The TM layer is immobilized on the surface of the electrode chips with the aid of a dialysis membrane, which is held in place by an O-ring and the measurement holder. The reference electrode and the counter electrode are immersed in the electrolyte solution on the side of the measurement chamber, without being directly exposed to light.

A mediator, in its oxidized form, can be reduced by electrons generated in the water splitting photoreaction activated by the exposure of TMs to light. The reduced mediator can be re-oxidized at the electrode surface, provided that the electrode is polarized at a suitable potential. Here, the soluble mediator, \([\text{Ru(NH}_3\text{)_6}]^{3+}\) is first reduced to \([\text{Ru(NH}_3\text{)_6}]^{2+}\) in contact with TMs exposed to light by the generated photoelectrons. \([\text{Ru(NH}_3\text{)_6}]^{2+}\) can then diffuse freely in the system. When it reaches the electrode surface, which is polarized at 0 V (vs. Ag|AgCl\(_{sat}\)), \([\text{Ru(NH}_3\text{)_6}]^{2+}\) is re-oxidized to \([\text{Ru(NH}_3\text{)_6}]^{3+}\) and the corresponding photocurrent is recorded. Similarly, for the osmium redox polymer as mediator, Os\(_{3}^{3+}\) moieties are reduced to Os\(_{2}^{2+}\) by electrons generated by TMs when exposed to light. Charge is then transported in the redox
polymer by σ bonds and an electron hopping mechanism\textsuperscript{49} to the electrode, which is polarized at +0.22 V (vs. Ag|AgCl\textsubscript{sat}). At the electrode surface, the re-oxidation of Os\textsuperscript{2+} to Os\textsuperscript{3+} takes place, and the corresponding photocurrent is recorded. A schematic representation of the redox reactions and charge transport taking place in the measurement system is shown in Figure 1b.
Figure 1: (a) Schematic representation of the measurement system – cross-sectional view: thylakoid membranes (TMs) immobilized on the surface of the patterned electrode area with the use of a dialysis membrane. The sample is illuminated from the backside and light reaches the TMs through the openings in the carbon layer, which represents the carbon working electrode (CWE). The reference electrode (RE) and counter electrode (CE) are placed on the side to avoid direct exposure to light; (b) Schematic of reactions taking place in the system – top view: light passes through the electrode chips and reaches the TMs, which then perform photolysis of water. Electrons generated in the process reduce a mediator present in the system, which is then transported and subsequently re-oxidized at the electrode surface; (c,d): Microscopy images at 5x magnification of 100 µm wide openings with different open area fractions (0-100%) (c) and of openings of different widths (20-400 µm) for a constant open area fraction of 40% (d). The black areas show the carbon and the gray areas show the quartz substrate exposed by the openings.

The efficiency of the mediating system is expected to be dependent on three parameters: i) the thermodynamic driving force, which is related to the formal potential, \( E^{\circ\ast} \), of the mediator; ii) the likelihood for the mediator to reach the reaction centers in the TMs and pick up the charge; and iii) the diffusion coefficient of the mediator in the system. The \( E^{\circ\ast} \) of the Os\(^{3+}/\)Os\(^{2+}\) redox...
couple in the Os polymer is higher (+0.22 V vs. Ag|AgCl sat) than that of the
[Ru(NH$_3$)$_6$]$^{3+}$/[Ru(NH$_3$)$_6$]$^{2+}$ redox couple (-0.1 V vs. Ag|AgCl sat)$^{50}$ and thus the Os polymer has a
higher thermodynamic driving force to act as an efficient mediator. However, the monomeric
[Ru(NH$_3$)$_6$]$^{3+}$ ions should have easier access to the reaction centers in the TMs. It is also
expected that the diffusivity of the monomeric [Ru(NH$_3$)$_6$]$^{3+}$ ions within the system is higher
than that of the Os$^{3+}$/Os$^{2+}$-redox moieties bound to the end of the side chains of the polymeric
backbone. Furthermore, due to the strongly cationic nature of the Os redox polymer it is
expected that there will be strong electrostatic interactions between the negatively charged TMs
and the polymer. Thus, the Os redox polymer will act as both mediator and immobilization
matrix for the TMs, which the monomeric [Ru(NH$_3$)$_6$]$^{3+}$/[Ru(NH$_3$)$_6$]$^{2+}$ ions will not be able to do.

2.1.2. Micropatterned electrode chips

Each electrode chip design is characterized by two parameters: i) the open area fraction, which
is expressed as the percentage of the area that is not covered by carbon and therefore transparent
to light; and ii) the width of the openings in the carbon layer (in µm). The trade-off for
photocurrent generation between the amount of light reaching the TMs and the electrode surface
area was investigated using electrode chips with square openings with a width of 100 µm and
open area fractions between 0% and 100% (Figure 1c). There are two particular cases: 0% open
area fraction, denoted as “full C”, and 100% open area fraction, denoted as “full opening”. The
influence of the distance between the photosynthetic sites and the electrode surface was
evaluated by using electrode chips with a constant open area fraction of 40% and with square
openings with widths between 20 µm and 400 µm (Figure 1d).

2.2. Photocurrent generation using thylakoid membranes
TM s were immobilized on the patterned electrode chips and tested for photocurrent generation using back-side illumination with an irradiance of 500 W m$^{-2}$ with both the soluble mediator (Figure 2a and 2c) and the osmium redox polymer (Figure 2b and 2d). Arrows in the figures indicate when the lamp was turned on and off. For all the experiments, no photocurrent was generated in the absence of light, i.e., when the lamp was off. When the lamp was turned on, the current increased instantly, after which it slowly declined while the sample was illuminated. This current decline was previously reported to be due to photo-induced damage of PS II$^{29,51}$.

2.2.1. Influence of the open area fraction on photocurrent generation

Figure 2: Current-time trace for photocurrent collection from TMs using different electrode chip patterns and mediators. (a,b): Constant opening width of 100 µm and open area fractions between 0 and 100% (see also Figure 1c); (c, d): Constant open area fraction of 40% and varying opening widths between 20 and 400 µm (see also Figure 1d). Measurement conditions: 1 mM [Ru(NH$_3$)$_6$]$^{3+}$ mediator in PBS at E = 0 V vs. Ag/AgCl$_{sat}$(a, c) or osmium redox polymer mediator at E = +0.22 V vs. Ag/AgCl$_{sat}$(b, d).
Since TMs need light to generate energy, an increase in photocurrent generation can be expected when the area exposed to light increases. This was verified for both mediators by data in Figure 2a, 2b, Figure 3 and Table 1S, showing how an increase in the open area fraction from 20% to 60% for a constant opening width of 100 µm, led to increasing photocurrent densities from 29.3±0.4 µA cm\(^{-2}\) to 39.4±0.8 µA cm\(^{-2}\) for the soluble ruthenium mediator, and from 15.5±0.4 to 31.1±0.6 µA cm\(^{-2}\) for the osmium redox polymer mediator. When using an open area fraction of 0% (full C), no photocurrent was recorded, which is explained by the lack of light activation in the TMs, because the pyrolytic carbon is not transparent to light in the UV-visible range. In the case of the full opening, the entire surface area is exposed to light, but the absence of carbon in the measurement area should theoretically lead to no recording of photocurrent. However, low photocurrent values were recorded with the full opening: 1.8±0.2 µA cm\(^{-2}\) for the ruthenium complex mediator, and 1.6±0.3 µA cm\(^{-2}\) for the osmium redox polymer. Since the opening is surrounded by carbon, the low photocurrent recorded is likely due to reactions taking place at the edges of the opening, in the vicinity of the surrounding electrode surface.

2.2.2. Influence of the distance between the photosynthetic reaction centers and the electrode surface on photocurrent generation

In Figure 2c, 2d, 3 and Table S1, it can be seen that for a constant open area fraction of 40%, varying the width of the openings has a remarkable influence on the generated photocurrent.
When decreasing the width of the openings from 400 \( \mu \text{m} \) to 20 \( \mu \text{m} \), the current density increased by a factor of two, from 23.1\( \pm 0.4 \) \( \mu \text{A cm}^{-2} \) to 53.2\( \pm 0.8 \) \( \mu \text{A cm}^{-2} \), for the soluble ruthenium mediator and by a factor of four, from 11.6\( \pm 1 \) \( \mu \text{A cm}^{-2} \) to 42.8\( \pm 0.6 \) \( \mu \text{A cm}^{-2} \), for the osmium redox polymer.

Regardless of the electrode pattern, the measured photocurrent values are higher when using soluble mediator, as opposed to polymeric mediator. This can be most likely attributed to the fact that the \([\text{Ru(NH}_3)_6]^{3+}\) ions can more easily access the PRCs, as discussed in Section 2.1.1. Additionally, the maximum photocurrent intensity is recorded after 5 s with the soluble mediator, and after 25 s with the osmium redox polymer, indicating that the electron transfer is slower in the latter case.

The experimental results show that the size of the openings has a major effect on the measured photocurrent. This can be attributed to the diffusion-based mechanism through which the charge is transported in the system by the two mediators. In order to understand the influence of the distance between the PRCs and the electrodes on photocurrent generation, a theoretical diffusion model described in the Experimental section and in the SI was employed\(^{52,53}\). The diffusion model assumes that the TMs are uniformly distributed on the surface area of the openings, which implies that electrons are generated with the same probability in all the points on the surface of the opening. In order to reach the electrode surface, electrons are transported in the system by the mediators in a manner approximated by isotropic two-dimensional diffusion. When a mediator in its reduced form reaches the electrode surface, an electron is transferred to the electrode and the mediator is re-oxidized, leading to photocurrent collection. The probability for an electron transported by a mediator to reach the electrode after a certain time interval is denoted as “exit
probability”. The sum of all exit probabilities of the electrons generated on the surface of the opening is considered to be proportional to the current intensity peak.

Figure 4a shows the theoretically calculated and experimentally obtained maximum current densities for electrode chips with an open area fraction of 40% as a function of the width of the openings. The theoretical model is in excellent agreement with the experimental data for both mediators. Figure 4b shows the exit probability as a function of the initial position of the electron for openings of increasing size after a time interval that corresponds to the maximum current intensities recorded for the two mediators using amperometry (5 s for the soluble ruthenium mediator and 25 s for the osmium redox polymer mediator). The color-maps clearly indicate that the probability for electrons generated near the edge of the opening to reach the electrode surface within the time interval is higher. As the distance between a PRC and the electrode increases, the exit probability for a generated electron after the time interval corresponding to the maximum recorded photocurrent decreases (SI Figure S7). For distances above 35 µm, the exit probability is significantly lower than 1, meaning that an electron generated in the center of a large opening will not contribute to photocurrent collection. As it can be seen in Figure 4b, the exit probability is approximately 1 for the entire surface of the 20 µm openings. Based on the experimental results and the model, the distance between individual PRCs and the electrode should be 10 µm or lower for maximum photocurrent collection, where more or less all electrons generated by TMIs are collected by the electrodes in the described setup.
The fit of the experimental current intensities to the theoretical model yields estimates of the effective two-dimensional diffusion coefficients, D. For the ruthenium complex, \( D_{\text{Ru}} = 17\pm3 \, \mu m^2/s \), which is 30 times lower than the reported diffusion coefficient of \([\text{Ru(NH}_3]_6^{3+}\) in 0.1 M phosphate buffer at pH 7.0 (\( D = 548 \, \mu m^2/s \))\(^{54} \). The large discrepancy can be ascribed to the high viscosity of the TM dispersion compared to the buffer solution, as well as to the fact that the 2D model neglects out-of-plane displacement.

The electron transport through the osmium redox polymer occurs by an electron-hopping mechanism and can also be approximated as a diffusive process\(^{49,55} \). The effective in-plane diffusion coefficient was calculated to be \( D_{\text{Os}} = 4\pm1 \, \mu m^2/s \), which is in good agreement with experimental values reported in literature\(^{49} \).

2.2.3. Photocurrent generation in different illumination conditions
Since only the openings in the carbon layer allow light to pass through the electrode chips and reach the TMs, it is expected that not all the thylakoids present on the electrode surface contribute to photocurrent generation when using back-side illumination. To investigate this, chips with 20 µm openings and an open area fraction of 40% were tested using both back-side and top-side illumination with 1 mM [Ru(NH$_3$)$_6$]$_3^{3+}$ as mediator. The 20 µm opening width was chosen since, as discussed in the previous section and shown in Figure 4b, the entire surface area of the openings contributes to photocurrent generation. For the top-side illumination, the light source was positioned to illuminate the top of the chip and all the other parameters were unaltered. Since the open area fraction for the tested chips was 40%, top-side illumination leads to a 2.5 times increase in the surface area exposed to light, as compared to back-side illumination. However, the maximum current measured in top-side illumination mode was 45.2±0.5 µA, which is only 1.7 times higher than in back-side illumination mode. One possible explanation is that, in the case of top-side illumination, the thylakoid moieties, which receive the most light, are positioned at the top of the TM layer, and the soluble mediator carrying the generated electrons can escape into the electrolyte solution instead of diffusing through the TM layer towards the electrode. Moreover, the TM layer attenuates the light, so it is expected that the thylakoids in close proximity to the carbon surface thus receive light with an intensity below 500 W m$^{-2}$. Correspondingly, one of the advantages of the back-side illumination mode is that the light first reaches the lowermost part of the TM layer. This means that the electrons generated in the photosynthetic process reduce the mediator entities, which are already diffusing in the TM layer. Thus, the electrons are more likely to reach the electrode surface and lead to photocurrent generation, instead of diffusing into the electrolyte solution.
As shown in Figure 5, the photocurrent decrease in the top-side illumination mode is higher than in the back-side illumination mode. After the first 200 s of exposure to light, the photocurrent measured in back-side illumination mode is higher than in top-side illumination mode. This indicates that back-side illumination might reduce light-induced damage in PS II.

For real-world applications in the energy sector, it is important to know how the electrode chips behave in different irradiance conditions. The value at which solar cells are tested is of 1000 W m\(^{-2}\), which is also denoted as 1 sun\(^{56}\). This value corresponds to normal illumination (when the sun is at zenith and its rays fall perpendicular to the ground). However, effective daytime irradiance has lower values, due to geometrical factors, terrain and atmospheric attenuation\(^{57}\). Figure 6 shows amperograms (Figure 6a) and the maximum photocurrent density (Figure 6b) generated with the electrode chips with 20 µm openings, an open area fraction of 40% and soluble mediator for different irradiance values, using back-side illumination. As seen in Figure 6a, for the tested interval of 200 – 1000 W m\(^{-2}\) higher irradiance values lead to higher currents, but also to a faster decrease in the photocurrent measured throughout the experiment. The latter is most likely a consequence of light-induced damage in PS II. The current density measured for an irradiance of 1 sun was 71 µA cm\(^{-2}\).
2.3. Comparison to state-of-the-art

In general, the photocurrent densities recorded in this work (see Table S1) for both [Ru(NH$_3$)$_6$]$_{3+}$ and osmium redox polymer using back-side illumination are higher than values previously reported on graphite electrodes using top-side illumination (between 24.9 and 51.7 µA cm$^{-2}$ using different mediators and an irradiance of 6800 W m$^{-2}$)$^{29}$. For a pattern with 20 µm openings and an open area fraction of 40%, the photocurrent density achieved using back-side illumination and soluble mediator (71±0.6 µA cm$^{-2}$ for an irradiance of 1000 W m$^{-2}$) is similar to values previously reported on thylakoid-carbon nanotube composites in top-side illumination (68 µA cm$^{-2}$ for an irradiance of 800 W m$^{-2}$)$^{58}$. Higher photocurrent density values have previously been reported in top-side illumination mode by i) Hasan et al., for gold electrodes and parabenzoquinone as mediator (up to 130 µA cm$^{-2}$); however, the light power of 6800 W m$^{-2}$ was much higher than the one used in this work; ii) Pinhassi et al., for FTO electrodes employed in contact with a suspension of thylakoids (up to 500 µA cm$^{-2}$). In this case, the chlorophyll content of the system was 0.1 mg of chlorophyll, which is one order of magnitude higher than the chlorophyll content employed in this work (11.5 µg of chlorophyll).
The quantum efficiency of the photosynthetic process for the 20 µm / 40 % chips was 0.64 % for the [Ru(NH₃)₆]³⁺ as mediator and 0.52 % for the osmium redox polymer as mediator. This is in good agreement with previously reported quantum efficiency values, which are in the range 0.3 to 3.3 %.

3. Experimental section

3.1. Fabrication of electrode chips

The electrode chips were fabricated using UV photolithography and pyrolysis. 500 µm thick quartz wafers were employed as substrate and SU-8 2005 was used as carbon precursor. The process flow was as follows: overnight dehydration bake of the wafers at 250 °C, spin coating of SU-8 2005: 30 s, acceleration of 2000 rpm min⁻¹ (RCD8 T spinner from Süss MicroTec, Germany), 3 h solvent evaporation at room temperature, proximity exposure, 12 µm gap, through chromium mask and a dose of 100 mJ cm⁻² (MA6 contact aligner from Süss MicroTec, Germany), 1 h bake at 50°C, development: PGMEA, 2x5 min, followed by isopropanol washing and N₂ blow-drying, 16 h hard bake at 90°C, pyrolysis (PEO-601 furnace from ATV Technologie GmbH, Germany). The pyrolysis process was performed as previously reported. The resist thickness was 5.9±0.2 µm before pyrolysis and the obtained carbon layer had a thickness of 790±30 nm, as measured with a DektakXT Stylus profilometer (Bruker, Billerica, MA, USA). A dicing saw (DAD 321 DISCO automatic, Japan) was used to dice the wafers. Each electrode chip has a footprint of 22x22 mm and a patterned area of 10x10 mm with the same positioning on all the different chip designs. In the patterned area, square openings permeating the carbon layer were designed in order to expose the transparent substrate material (Figure 1c and 1d) and thus to allow light to reach the TMs. Nine different chip designs were fabricated and tested. All chips were oxygen-plasma treated for 3 min using a power of 50 W with a 13.56 MHz
RF generator-equipped Atto Plasma System (Diener Electronic, GmbH, Ebhausen, Germany) before performing the electrochemical measurements.

3.2. Photosynthetic process

The thylakoid membranes (TMs) were extracted from Spinacia oleracea as described elsewhere. The chlorophyll content of the extracts was measured in accordance with literature. The TM solution had a chlorophyll content of 3.2 mg mL\(^{-1}\) and it was preserved as deep-frozen aliquots (-80 °C) and thawed before performing the experiments.

A 150W 220 V fiber optic illuminator (Titan Tool Supply, Inc. Buffalo, NY, USA) providing white visible light was used to induce photosynthesis in the TMs. The irradiance was calibrated using a light intensity meter (Techtum Lab AB, Umeå, Sweden) and adjusted to 500 W m\(^{-2}\) unless otherwise specified. The TMs were illuminated through the transparent electrode chips (back-side illumination) for all experiments unless otherwise specified. The correlation between the open area fraction and irradiance measured through the chips is shown in Figure S4.

The quantum efficiency of the system represents the ratio between the number of electrons generated and the number of photons absorbed by the sample, in percentage form. We calculated the apparent quantum efficiency of our system for the 20 µm / 40 % chips, as this is the only electrode pattern in which all the generated electrons are shown to be harvested in the system (Figure 4). The number of electrons was calculated from experimental current data points. The number of photons that the sample was exposed to was calculated from the irradiance value measured through the 20 µm / 40 % chips, considering an average photosynthetic response wavelength of 520 nm. The calculation assumes that all the photons that pass through the electrode chip are absorbed by the sample.

3.3. Electrochemical measurements
MgCl$_2$ was purchased from Scharlau Chemie S.A. NaCl (433209, ≥99%), Hexaaammineruthenium (II) chloride (303690, ≥99.9%) and hexaaammineruthenium (III) chloride (262005, ≥98%) were purchased from Sigma Aldrich. [Os(2,2’-bipyridine)$_2$-poly(N-vinylimidazole)$_{10}$Cl]$^{3+/2+}$ was synthesized as previously reported$^{62}$. Millipore water (18.2 MΩ resistivity) was used for preparing all solutions.

Cyclic voltammetry (CV) and amperometry (A) measurements were performed using a PalmSens potentiostat (EmStat2, Utrecht, The Netherlands) and the PSTrace software in a conventional three-electrode setup. DRIREF-L, a Ag|AgCl$_{sat}$ electrode from WPI (UK) was employed as reference electrode (RE). A Pt wire (500 µm diameter, from Advent Research Materials, UK) was used as counter electrode (CE). The different electrode chips were interfaced to the potentiostat using copper wires and used as working electrodes (WEs) in a setup designed for these experiments and described in the SI. The measurement chamber defines the active electrode footprint area to be 0.5 cm$^2$. The electrolyte is 10 mM phosphate buffer with 10 mM NaCl and 5 mM MgCl$_2$ (PBS). The electrolyte solution was degassed by purging with nitrogen for at least 10 min before experiments. 1 mM [Ru(NH$_3$)$_6$]Cl$_2$/[Ru(NH$_3$)$_6$]Cl$_3$ in PBS was used to characterize the electrode behavior (SI, Figure S2 and S3).

For experiments with TMs and soluble mediator, 3.6 µL of TM extract (optimized value, corresponding to 11.5 µg of chlorophyll) was first dropcast on the patterned area of the electrode chip (11.5 µg cm$^{-2}$). After 5 min, a dialysis membrane (Spectrum Laboratories Inc., USA, MWCO: 6-8 kDa) presoaked in buffer was added and the chip was immediately assembled in the designed holder. A was then performed at 0.0 V vs Ag|AgCl$_{sat}$ in 1 mM [Ru(NH$_3$)$_6$]Cl$_3$ in PBS, a more positive potential than the $E^{\circ}$ of the couple, which is -0.1 V vs. Ag|AgCl$_{sat}$.
For experiments with TMs and osmium-redox-polymer (Os-RP) as mediator, a solution of 10 mg mL\(^{-1}\) \([\text{Os}(2,2’-\text{bipyridine})_2\text{-poly(N-vinylimidazole})_{10}\text{Cl}])^{3+/2+}\) (redox potential +0.22 V vs. Ag\|AgCl\(_{\text{sat}}\)) in milli-Q water was first prepared. 15 µL of Os-RP solution was deposited onto the patterned area and allowed to dry at room temperature for 1 h (optimized values for volume and drying time). After that, TMs and dialysis membrane were added in the same way as for experiments with soluble mediator. A was then performed at +0.22 V vs. Ag\|AgCl\(_{\text{sat}}\) in electrolyte solution.

All data presented is based on three independent experiments and the standard deviation is less than 10 %. Baseline correction was used for all amperometric measurements, as described in the SI and shown in Figure S5. Control experiments without TMs (SI, Figure S6) were used to ensure the currents reported in this paper are due to the photosynthetic process. Maximum current densities are calculated by using the current measured 5 s after the first illumination pulse when using [Ru(NH\(_3\))\(_6\)]\(^{3+}\) as mediator or 25 s after the first illumination pulse when using Os redox polymer as mediator.

### 3.4. Theoretical diffusion model and calculations

Electrons are generated through photosynthetic reactions on the square surfaces representing the openings in the carbon layer. Thus, at the start of the illumination pulse, the electrons are considered to be uniformly distributed on the openings in the carbon layer.

Electrons transported by the mediators were modeled as non-interacting particles freely diffusing in two dimensions. The probability for an electron to escape the irradiated region where it is generated, during an interval of time, was calculated.

An electron is transferred to the electrode as soon as the mediator transports it to the carbon surface, meaning that it diffuses outside the irradiated region. The current intensity is hence
related to the average probability for an electron to reach the boundary during the time interval between the illumination pulse and the measurement of the current intensity peak.

As detailed in the SI (Section S6), the time-dependent exit probability of a particle in a square surface with absorbing boundaries was used\textsuperscript{53,63}, and this parameter was averaged over all starting positions on the square. The average electron exit probability is a function of the width of the opening, the time interval, and the effective diffusion coefficient of the mediator transporting the electron. Experimental current intensities were fitted to the average electron exit probability.

4. Conclusions

Patterning a conductive material on a transparent substrate is advantageous for both light-induced energy conversion and for efficient energy collection. Carbon-on-quartz transparent electrode chips were fabricated and successfully employed for photocurrent generation from TMs using back-side illumination with solar-simulated irradiance. Square openings in the carbon layer defined the regions of the samples that were exposed to light.

Two design parameters of the micropatterned electrodes were shown to influence photocurrent generation. An increase in the open area fraction up to 60% led to an enhanced photocurrent generation. Increasing the open area fraction further was not feasible in the current system, since it would result in a width of the carbon electrodes smaller than 5 µm, which becomes a challenge for patterning with standard UV photolithography. In the future, larger open area fractions could be achieved with different fabrication methods of the electrode patterns. The second parameter that had a high impact on photocurrent generation was the width of the openings in the carbon layer, which influences the distances between the photosynthetic reaction centers and the electrode. Openings larger than 20 µm require charge transfer over a large distance between the
TM PRC and the electrode, leading to a decrease in the recorded photocurrent. This was explained by a diffusion model where electrons transported by the mediators were modeled as non-interacting particles freely propagating in two dimensions. The model showed that in order to maximize photocurrent collection, the distance between the photosynthetic reaction centers and the electrode surface should be below 10 µm, which corresponds to an opening width of 20 µm.

The patterned electrode chips presented in this work can be fabricated in a scalable process and allow photocurrent generation from TMs with competitive results, for relevant irradiance values. In addition, they allow minimizing the necessary reagent consumption (low chlorophyll content required) and protecting the system from the environment and vice-versa by using back-side illumination, which can be employed in the future for building closed PBECs. An additional increase in electrode surface area could eventually be achieved by patterning three-dimensional polymer structures before pyrolysis\textsuperscript{42–44} which in principle could lead to enhanced photocurrent generation.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Additional experimental details and results (PDF)

Jupyter Notebook for calculations (native and HTML format)

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ABBREVIATIONS
TM – thylakoid membrane
PBEC – Photo-bioelectrochemical cell
PS I – Photosystem I
PS II – Photosystem II
TCO – Transparent conductive oxides
FTO – Fluorinated tin oxide

ITO – Indium tin oxide

PRC – Photosynthetic reaction center

SI – Supporting information

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