## **Supplementary Information**

# Orientation of photosystem I on graphene photocathode through cytochrome $c_{553}$ leads to an improvement in photocurrent generation

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#### Preparation of single layer graphene by chemical vapor deposition (CVD) method

The conventional CVD procedure was used to grow the large area single layer graphene (SLG) on Cu foil, with methane as a carbon source. Pure Cu substrate with a size of  $1.5 \times 1.5 \text{ cm}^2$  and thickness of 0.025 mm was used as a catalyst. For preparation of SLG, the Cu foil was cleaned for 10 min. consecutively with acetone, isopropanol, and deionized water, then airdried before placing in the CVD system (**Fig. S1**). Dried Cu foil was placed in a tube furnace with a quartz boat and the system was vacuumed to  $10^{-6}$  Torr. The furnace was heated up to a temperature of 1000 °C by providing H<sub>2</sub> and Ar gases. When the temperature of the oven reached the desired value, the Cu foil was subjected to a heat treatment under H<sub>2</sub>/Ar gas flow. This annealing process was performed to ensure the formation and growth of Cu seeds. Subsequently, CH<sub>4</sub> was used as a carbon source for the growth of the SLG sheets on the Cu

foil for 30 min. Reaction pressure was maintained at the ambient pressure throughout the entire process. Finally, the furnace was closed and allowed to reach temperature of 150 °C and to cut off  $H_2$  and Ar gas flow. When the Cu foil was cooled to room temperature under vacuum,  $N_2$  gas was supplied to bring the system to the room pressure followed by removal of the Cu foil from the CVD system. The obtained graphene layers were analyzed by Raman scattering spectroscopy (**Fig. S2**), which allows to determine the number of graphene layers, and probe the density of defects in the structure and the presence of undesirable impurities.<sup>1–3</sup>



**Figure S1.** Schematic representation of the graphene coating process by CVD on Cu substrate at high vacuum and temperature using  $CH_4$  as a carbon source. The furnace was maintained at 1000 °C under the ambient pressure throughout the production process.

#### Transfer of large-area SLG onto FTO substrate

The graphene layer grown on the Cu foil, was then transferred onto the fluorine-doped tin oxide (FTO) substrate as described below. Firstly, a 5% polymethyl methacrylate (PMMA) solution was overlaid homogeneously onto the graphene layer by spin coating at 4000 rpm for 40 s to provide a support for the transparent graphene layer. Next, 6 ml of deionized water were mixed with 2 ml of a 69 % solution of nitric acid and incubated with the graphene-coated Cu substrate for 3 min. Subsequently, 200 g of iron (III) nitrate dissolved in 400 ml deionized water was deposited onto the graphene-coated Cu substrate for 1 day to etch Cu substrate. The PMMA-graphene layer was washed with deionized water, placed in 2% HCl (2 ml HCl + 15 ml ultrapure water) for 10 min. to remove metal contaminants, then washed again in deionized water. The graphene-PMMA layer was transferred onto the FTO-coated glass surface. The PMMA/graphene/FTO substrate was left in 99.9 % pure acetone at 40 °C for 30 min. to remove the PMMA layer. The resultant SLG on the FTO surface was analyzed by Raman scattering spectroscopy, Scanning Electron Microscopy, and Atomic Force Microscopy, as described in Materials and Methods.



**Figure S2.** Average Raman spectrum of a high-quality defect-free SLG on FTO substrate. The D, G and 2D bands represent the main Raman features of a high-quality graphene layer.



**Figure S3.** Top-view SEM images of FTO substrate covered with SLG. Panels (a) and (b) show FTO surface coated with SLG visualized at two different magnifications, as indicated. Micrographs (c) and (d) show intact SLG coverage of the FTO surface.

Further structural characterization of SLG/FTO samples was performed by SEM and polarized light microscopy (see **Fig. S3-S5**). Due to high transmittance of SLG, the FTO surface was clearly visible (see **Fig. S3c,d**). Figure S3 a,b show the difference between the SLG-coated FTO electrode and bare FTO. The polarized light microscopy analysis shows that the SLG completely covers the whole surface of the FTO substrate (see **Fig. S4b**).



**Figure S4.** The polarized light microscope images of the SLG/FTO. (a) shows areas fully covered with SLG and bare FTO, (b) FTO covered with SLG.



**Figure S5.** Cross-sectional SEM image of the Ni-NTA-pyrene/SLG/FTO electrode. Ni-NTApyrene/SLG is an ultra-thin layer consist of Ni atom and NTA-pyrene/SLG stack with the thickness of approximately 1.4 nm.



**Figure S6.** AFM topographic images of SLG on FTO surface. Panels (a) and (b) show topology of FTO covered with SLG and bare FTO area at two different magnifications.

To get a better insight into any putative defects of the obtained SLG structure, we performed the AFM analysis of the SLG/FTO electrode. To this end, we identified the area of FTO surface that was coated with SLG and which displayed relatively high roughness (see **Fig. S6a,b**). **Figure S7** shows the roughness of SLG/FTO and FTO surfaces. While the average surface roughness (Ra, red line) of SLG/FTO was almost 12.6 nm, the surface roughness (Ra, green line) of FTO was measured at 23.9 nm, indicating that the graphene layer reduces the roughness of the FTO surface.



**Figure S7.** AFM visualization of SLG on FTO surface. Shown is a topographic image of SLG on FTO surface (a), as well as the AFM height profiles (b) of SLG/FTO surface (red line) and bare FTO surface (green line).

#### Integration of pyrene-NTA with graphene surface

In order to facilitate attachment of photosystem I to the graphene surface we applied noncovalent functional structures with molecules containing common  $\pi$ -systems, such as pyrene and its derivatives. Integration of the pyrene-NTA molecule on the graphene surface was confirmed by XPS measurements based on assaying the C1s contribution originating from the pyrene and graphene structures. The XPS peaks of NTA-pyrene/SLG/FTO and SLG/FTO are shown in Figure S8. The XPS spectra reveal the presence of oxygen, carbon and nitrogen in the NTA-pyrene/SLG/FTO substrate (Fig. S8a). The signals corresponding to Sn3d5/2, O1s, C1s and N1s were determined at 486.7 eV, 530.6 eV, 284.1 eV and 399.1 eV. The level of the C1s contribution corresponding to the sp<sup>2</sup> carbon, which is the main component of graphene and the pyrene structures, is indicated in the XPS spectrum (Fig. S8b). The C1s component was determined at 283.4 eV and 284.1 eV for SLG/FTO and NTA-pyrene/SLG/FTO, respectively. This shift in the bonding energy of the XPS C1s spectrum indicates the presence of the functional structures of pyrene-NTA and graphene. The N1s component was determined at 399.1 eV in the NTA-pyrene/SLG/FTO sample (Fig.S8c), indicating the presence of the pyrene-NTA moiety on the SLG/FTO surface. The very low level of oxides as well as the relatively small amount of sp<sup>3</sup> carbon indicate the high quality and low defect level of the NTApyrene/SLG/FTO substrate.4,5





**Figure S8.** XPS analysis of pyrene-functionalized SLG. Panel (a) shows the broadscan XPS spectra of NTA-pyrene/SLG/FTO and SLG/FTO surfaces. Panels (b) and (c) display C1s and N1s XPS spectra of pyrene-NTA layer (red line), respectively, in comparison to the SLG/FTO samples (black line).

### Setup for electrochemical characterization



**Figure S9.** Picture of the photoelectrochemical cell used in this study. Panels show pictures of the electrochemical cell including the electrodes (WE – FTO/SLG/pyrene-NTA-Ni/protein; REF – Ag/AgCl; CE – Pt wire) used for the chronoamperometric measurements under the illumination with white light of 100 mW·cm<sup>-2</sup>

#### Fluorescence spectroscopy of biophotoelectrodes



**Figure S10**. Fluorescence image of the PSI@SLG photoelectrode together with fluorescence spectra acquired for selected locations in the image. The spectra are compared with the reference spectrum of PSI (green). The data was obtained for the excitation wavelength of 485 nm.



**Figure S11.** Fluorescence decay curves measured for (a) PSI+SLG, (b) PSI@SLG, and (c) the reference of PSI only sample, together with fitted curves. Residuals are also shown. The data was obtained for the excitation wavelength of 485 nm.



#### Electrochemical characterization of the biophotoelectrode

**Figure S12.** Photochronoamperometric analysis of PSI@SLG electrode depending on the illumination direction. (a): Chronoamperometric measurement at -200 mV (*vs* Ag/AgCl) for the sample recorded directly through the electrode and through the electrolyte. (b): photocurrent densities generated depending on the illumination direction.

In order to evaluate the influence of direct *versus* indirect exposure of PSI@SLG sample to the light illumination on the photoelectrochemical activity, the bioelectrode was illuminated through the electrode and through the electrolyte (see **Fig. S12**). The photochronoamperometric analysis indicated the production of higher photocurrents values for sample directly exposed to the white light compared to residual photocurrents recorded while illuminating the electrode through the electrolyte, what stays in agreement with the literature.<sup>6</sup>

To test the effect of direct *versus* indirect exposure of biophotoelectrodes to white light illumination on the electrochemical performance, the samples were illuminated either through

the electrolyte or directly through the electrode during the photochronoamperometric measurements (see **Fig. S12**). For the FTO-based samples, the residual photocurrents were recorded while illuminating the samples through the electrolyte in contrast to the much higher photocurrents when light was applied directly through the sample, in agreement with the previous study.<sup>6</sup>



**Figure S13.** Long-term stability for FTO/SLG/pyrene-NTA-Ni/cyt/PSI electrode. Photocurrent density generated from PSI@SLG electrodes used in this study was measured after 6-12 months of their initial preparation (time 0).



**Figure S14.** Cyclic voltammetry analysis of the PSI-based SLG electrodes. Cyclic voltammograms were recorded at 100 mV/s in Ar-saturated 5 mM phosphate buffer (pH 7.0), in the dark.

In order to additionally verify the presence of PSI within the SLG electrodes, we analysed the samples by cyclic voltammetry. Figure S14 shows cyclic voltammograms recorded for oriented PSI@SLG and randomly deposited PSI+SLG (randomly deposited PSI) bioelectrodes. Although we were unable to distinguish the specific peaks associated with PSI, most likely due to their overlap with NTA and cyt c553 redox peaks, the PSI@SLG assembly exhibits higher values of capacitive currents observed in the potential range between -0.05 V and +0.2 V. The latter phenomenon is likely due to the presence of a higher redox active surface of PSI@SLG electrode compared to PSI+SLG sample due to the presence of ordered PSI layer. We have confirmed that PSI deposited on the electrode surface is indeed active by measuring the cyclic voltammograms of electrodes composed of PSI physisorbed onto FTO using the same experimental conditions for the electrode assembly as for the PSI-SLG samples. Figure S15 shows a pair of well-defined peaks at -0.2 V and -0.5 V which can be attributed to highly active PSI layer deposited on the FTO electrode.



**Figure S15.** Cyclic voltammograms for FTO-based electrode with physisorbed PSI complex, CVs were recorded at 100 mV/s in Ar-saturated electrolyte (5 mM phosphate buffer, pH 7) in the potential range of between -0.8 V and +0.2 V, in the dark.



**Figure S16.** Photochronoamperometry of PSI-based SLG electrodes. Graph shows the dependence of generated currents *versus* time for three analyzed structures: NiNTA/SLG (blue), PSI+SLG (red), and PSI@SLG (black). After 2-min. current stabilization at -300 mV potential under dark conditions, photochronoamperometric signals were recorded with a 30-s. illumination cycle, as described in Materials and Methods.

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