Self-Powered Electrochemical Memristor Based on a Biofuel Cell – Towards Memristors Integrated with Biocomputing Systems

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Electronic Supplementary Information

Chemicals and supplies. Poly(4-vinyl pyridine) (P4VP, M.W. 160,000 g·mole^{-1}, ρ = 1.101 g·cm^{-3}, Sigma-Aldrich), bromomethyldimethylchlorosilane (Gelest), 1-pyrenebutanoic acid succinimidyl ester (PBSE, AnaSpec Inc.), 3-(*N*-morpholino)propanesulfonic acid (MOPS-buffer, Sigma-Aldrich) and other standard inorganic chemicals and organic solvents (Sigma-Aldrich) were used as supplied without any further purification. PQQ-dependent glucose dehydrogenase (PQQ-GDH; E.C. 1.1.5.2, from microorganism – not specified by the company) was purchased from Toyobo Co., Japan, and used as supplied. Laccase (E.C.1.10.3.2, from *Trametes versicolor*) was obtained from Sigma-Aldrich and used in experiments after the purification procedure described elsewhere.[1] Ultrapure water (18.2 MΩ·cm) from NANOpure Diamond (Barnstead) source was used in all of the experiments.

Electrode modification. Indium-tin oxide (ITO) single-side coated conducting glass (20±5 Ω/sq surface resistivity; Sigma-Aldrich) served as a conducting support for the pH-switchable P4VP-modified electrode. The ITO-electrodes were chemically modified with P4VP-brushes using the "grafting to" method[2,3] according to the following procedure. The ITO-coated glass slides were cut into 25 mm × 10 mm strips. They were cleaned with ethanol in an ultrasound bath for 15 min and dried under a stream of argon. The cleaning step was repeated using methylene chloride as a solvent. The initial cleaning steps were followed by immersing the strips into a cleaning solution (heated to 60 °C in a water bath) composed of NH_4OH, H_2O_2, and H_2O in the ratio of 1:1:1 (v/v/v) for 1 hour. (*Warning: This solution is highly reactive and extreme precautions must be taken upon its use.*). Subsequently, the glass strips were rinsed several times with water and then dried under argon. The freshly cleaned ITO strips were reacted with bromomethyldimethylchlorosilane, 0.1% (v/v), in toluene for 20 minutes at 70 °C. The silanized ITO was rinsed with several aliquots of toluene and dried under argon. Then 60 μL of the P4VP solution in nitromethane, 10 mg·mL^{-1}, were applied to the surface of each ITO glass strip, dried to form a polymer coating, and left to react in a vacuum oven at 140 °C overnight. The final cleaning steps, to remove the unbound polymer, consisted of soaking for 10 minutes in ethanol, followed by additional 10 minutes in a dilute solution of H_2SO_4 (pH 3). Modified electrodes were stored under ethanol.

Buckypaper composed of compressed multi-walled carbon nanotubes (CNTs) (Buckeye Composites; NanoTechLabs, Yadkinville, NC) was used as the electrode material for preparation of biocatalytic enzyme-modified electrodes, Figure SI-1a. Electrodes were washed with isopropyl alcohol with moderate shaking for 15 min at room temperature prior to their modification. The electrodes were incubated with PBSE, 10 mM, in ethanol with moderate shaking for 1 hour at room temperature, subsequently rinsed with ethanol to remove any excess of PBSE and then with MOPS-buffer (50 mM, pH 7.0) to remove

ethanol. The biocatalytic anodes were prepared by the immobilization of PQQ-GDH: the PBSE-functionalized electrodes were incubated for 1 hour in the solution of PQQ-GDH (2.4 $mg \cdot mL^{-1}$) in MOPS-buffer (50 mM, pH 7.0) containing Na_2SO_4 (100 mM) and 1 mM $CaCl_2$ (1 mM), Figure SI-1b. The biocatalytic cathodes were prepared by the immobilization of laccase: the PBSE-functionalized electrodes were incubated for 1 hour in the solution of laccase (1.5 $mg \cdot mL^{-1}$) in potassium phosphate buffer (10 mM, pH 7.0). The immobilization reactions proceeded at room temperature with moderate shaking. Then the enzyme-modified electrodes were stored (4 °C) in the same buffer until use. Characterization of the enzyme-modified electrodes (cyclic voltammetry, enzyme content, etc.) was described in details elsewhere.[4,5]

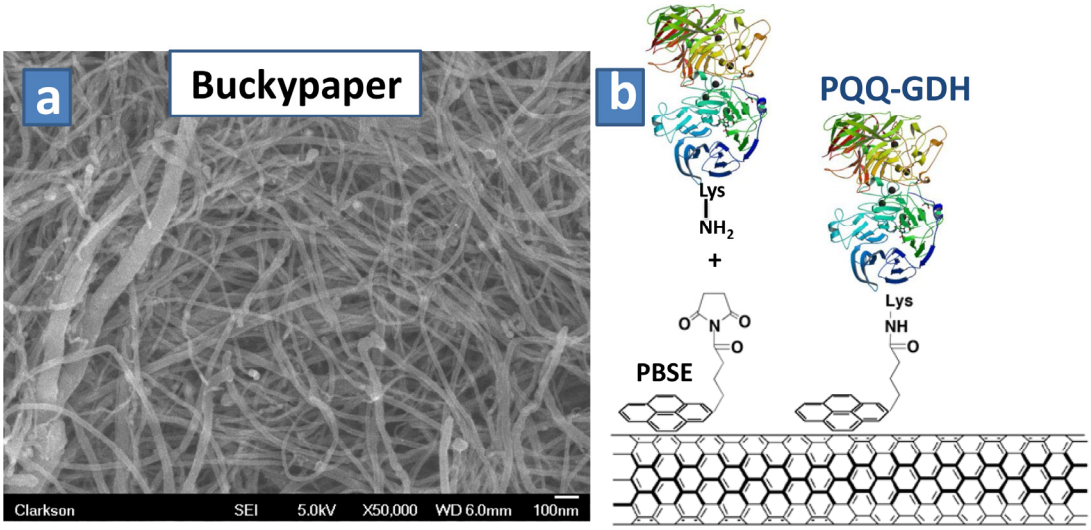

Figure SI-1. (a) SEM image of the buckypaper used as the conductive support for the enzyme-modified electrodes. (b) Immobilization of the PQQ-GDH on CNTs with the help of the heterobifunctional linker 1-pyrenebutanoic acid succinimidyl ester (PBSE), which provides covalent binding with amino groups of protein lysine residues through the formation of amide bonds and also interacts with CNTs via π-π stacking of the polyaromatic pyrenyl moiety. Note that laccase was attached to the buckypaper in the same way.

Electrochemical Measurements. The measurements were carried out with an ECO Chemie Autolab PASTAT 10 electrochemical analyzer using the GPES 4.9 (General Purpose Electrochemical System) software package. All the measurements were performed at an ambient temperature (23±2°C) in a proprietary sandwich cell. The working electrode for the impedance measurements was a P4VP-modified ITO-glass electrode with a geometrical area of ca. 50 mm^2 (note that the typical surface roughness factor for ITO electrodes is ca. 1.6±0.1).[6]

Electrochemical Cell Construction. All experiments were performed in a custom designed electrochemical cell, Figure SI-2. This was composed of two rubber O-rings separated by Nafion, an industry standard proton exchange membrane, held between a conductive ITO-electrode and a glass slide with two buckypaper enzyme-modified electrodes. The ITO-electrode was modified with P4VP by the previously mentioned protocol. The buckypaper electrodes were modified with PQQ-GDH and laccase according to the procedure specified above. A glass slide cut to the same dimensions as the ITO electrode was used to provide mechanical enforcement to the buckypaper enzyme-modified electrodes with a non-conducting gap of ca. 1 mm between the PQQ-GDH anode and laccase-cathode. The electrodes (P4VP-ITO) and both buckypaper enzyme-electrodes were wired using standard alligator clip test leads (wires).

It should be noted that due to the micro-design of this cell (approx. 250 µL working volume) no reference electrode was used. The inner volume of the O-ring facing the P4VP-ITO-electrode was filled with $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1 mixture; 2 mM each) redox probe dissolved in a 5 µM acetate buffer solution containing 0.1 M sodium perchlorate. The inner volume of the O-ring facing the biocatalytic electrodes included 25 mM glucose and O_2 (in equilibrium with air) dissolved in the same 5 µM acetate buffer and 0.1 M sodium perchlorate solution as the opposite chamber.

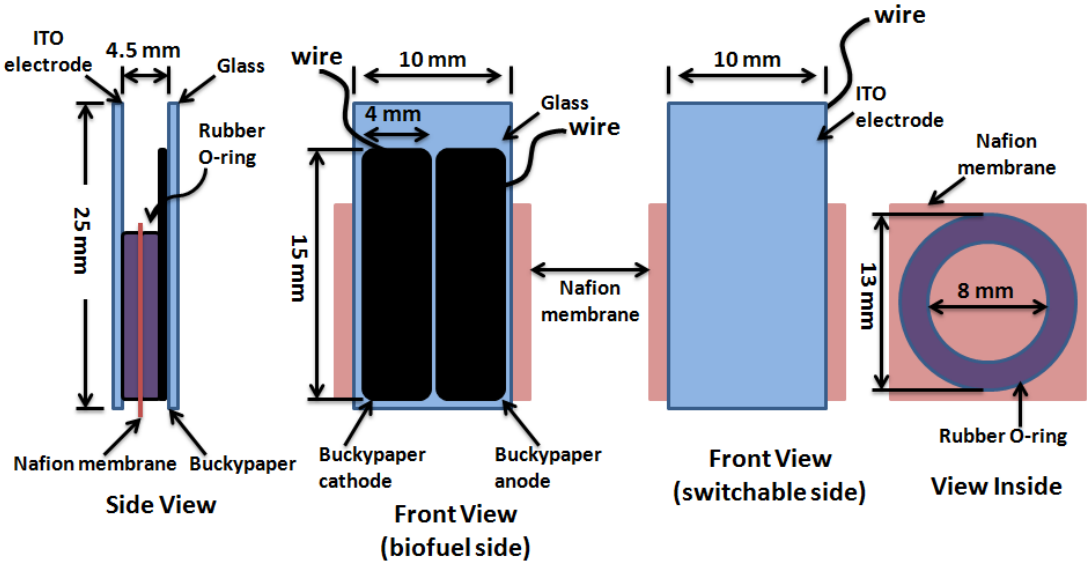

Figure SI-2. Schematics of the electrochemical cell. Note that the ITO-electrode was modified with P4VP-polymer brush and the buckypaper electrodes were modified with PQQ-GDH and laccase (on the biofuel cell anode and cathode, respectively).

The pH changes generated in the system upon operation of the biocatalytic electrodes.

The kinetics measurements of pH change over time due to bioelectrocatalytic activity of the biofuel cell electrodes were measured using standard pH paper strips; therefore, the pH measurements reported have an intrinsic level of error, as much as 0.2 pH unit, as they required the comparison of colors for final analysis. It should be noted that the electrolyte solution included a very low buffer concentration (5 µM acetate buffer) to allow the pH variation upon biocatalytic transformations on the enzyme-modified electrodes. Particularly, the low $pK_a = 3.7$ value[7] of the biocatalytically produced gluconic acid allowed the pH change in the solution.

Hysteresis loop (I-V curve).

One of the features of the classical solid-state memristor devices is the hysteresis loop in the current-voltage function, Figure SI-3, inset.[8,9] This feature was also nicely confirmed for the studied electrochemical device, Figure SI-3.[10] It should be noted that the I-V curve of the memristor device is recorded on the pH-switchable polymer-modified electrode and it is independent on the presence or absence of the biofuel cell in the system. In other words, the I-V curve demonstrating the hysteresis loop reported in the previous study[10] is the same in the present system.

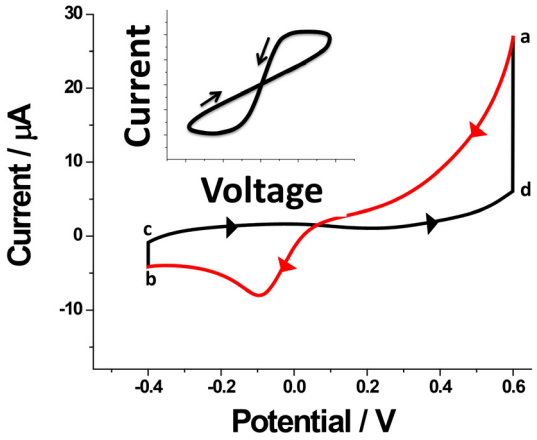

Figure SI-3. Cyclic voltammogram demonstrating the hysteresis loop in the current-voltage function: (a) "open" electrode at initial pH 4.0, (b) the switchable electrode was connected to the biofuel cathode modified with laccase for 30 min to allow switching from the "open" to "closed" state, (c) "closed" state of the electrode at pH ca. 6 produced electrochemically, (d) the switchable electrode was connected to the biofuel anode modified with PQQ-GDH for 30 min to allow switching from the "closed" to "open" state. Potential scan rate between points "a-b" and "c-d" was 50 mV/s. Inset: Schematic hysteresis loop in the current-voltage function characteristic of a memristor device.

Reproducibility of the experimental results and stability of the bioelectronic system.
The pH-switchable electrode was able to switch reversibly between ON and OFF states more than 10 times without any evidence of the polymer film degradation. The biofuel cell operated at the steady-state current/voltage generation over 5 hours. Each set of measurements reported in the paper was repeated at least 3 times with different modified electrodes, thus demonstrating good repeatability. Overall, the system demonstrated good stability and reproducibility on the time scale of the performed experiments. However, the systematic study of the system operation for longer periods of time where outside the scope of the present preliminary study.

References:

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