Living battery – biofuel cells operating *in vivo* in clams

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Supplementary information

Chemicals: 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] 1-pyrenebutanoic acid succinimidyl ester (PBSE), 3-(*N*-morpholino)propanesulfonic acid (MOPS-buffer), 2,6-dichlorophenolindophenol (DCIP) and other standard chemicals were purchased from Sigma-Aldrich and used as supplied without any further purification. Water used in all of the experiments was ultra pure (18.2 MΩ·cm) from a NANOpure Diamond (Barnstead) source.

Clams: The live specimens of hard clam (*Mercenaria mercenaria*) used in this study were purchased in a supermarket "Price Chopper" (NY). The specimens measured in average 6 cm in shell diameter that is a usual size for adults of this species. The clams were held in 1-gallon plastic aquariums in highly aerated, dechlorinated water of mild salinity at a temperature of 4-5 ºC.

Electrode preparation: Buckypaper composed of compressed multiwalled carbon nanotubes (Buckeye Composites; NanoTechLabs, Yadkinville, NC) was used as the electrode material (geometric area 0.25 cm^2). Indium-tin oxide (ITO, 20±5 Ω/sq surface resistivity; Sigma-Aldrich)

was used as the conducting support for the mechanical reinforcement of the buckypaper which was attached to ITO using with silver conductive epoxy glue (MG Chemicals, Surrey, Canada). Electrodes were incubated with PBSE (10 mM in DMSO) with moderate shaking for 1 h at room temperature and subsequently rinsed in DMSO (5 min) to remove excess PBSE and then washed in potassium phosphate buffer (10 mM, pH 7.0; 5 min). PQQ-GDH and laccase were used for the modification of the anode and cathode, respectively. The PBSE-functionalized electrodes were immediately incubated with enzyme solutions, 1 $mg \cdot mL^{-1}$ for PQQ-GDH and 1.5 $mg \cdot mL^{-1}$ for laccase, in potassium phosphate buffer (10 mM, pH 7.0) for 1 h at room temperature with moderate shaking. In the case of PQQ-GDH, the solution also included 1 mM $CaCl_2$. The modified electrodes were stored (4 ºC) in potassium phosphate buffer (0.1 M, pH 7.0) until implanted in clams for *in vivo* use or transferred to an electrochemical cell for *in vitro* measurements.

Electrochemical characterization of the modified electrodes: Cyclic voltammetry measurements were carried out with an ECO Chemie Autolab PASTAT 10 electrochemical analyzer, using the GPES 4.9 (General Purpose Electrochemical System) software package. The working electrode was made of buckypaper attached to ITO support (0.25 cm^2 geometrical area) and modified with PQQ-GDH or laccase. A Metrohm Ag|AgCl|KCl, 3M, electrode served as a reference electrode and a Metrohm Pt wire was used as a counter electrode. Cyclic voltammograms were recorded at a scan rate of 1 $mV \cdot s^{-1}$ in an electrolyte solution composed of 0.1 M phosphate buffer, pH 7.0, for the laccase-electrode and 50 mM MOPS-buffer, pH 7.0, with added 1 mM $CaCl_2$ for the PQQ-GDH-electrode. Cyclic voltammograms of the PQQ-GDH-electrode were obtained in the absence and presence of glucose (20 mM), while cyclic voltammograms of the laccase-electrode were obtained in the absence and presence of oxygen (in equilibrium with air). Anaerobic conditions were achieved by purging the solution with argon.

It should be noted that the enzyme-modified electrodes demonstrated cyclic voltammograms slightly different from those reported in our previous paper.[2] The reasons for the difference are the following: (*i*) The buckypaper composed of compressed multiwalled carbon nanotubes was mechanically reinforced by attaching to an ITO electrode, thus the electrodes have different

composition. (*ii*) More importantly, the commercial buckypaper has significant variation from sample-to-sample in its conductivity and hydrophilicity / hydrophobicity, therefore resulting in different bioelectrocatalytic properties of the enzyme-modified electrodes prepared from another sample of the paper.

Biofuel cell measurements: The implantable electrodes were inserted into the clams through two holes cut with a small circular saw in a dorso-posterior part of their shells and placed into the hemolymph between body wall and heart, dorsal to the visceral mass with the distance between the electrodes of ca. 1.5 cm (varied in different specimens by ±2 mm), Figure SI1. The geometrical area of the electrodes immersed in the hemolymph was 0.25±0.02 cm^2. The voltage and current generated by the biofuel cell were measured by a multimeter (Meterman 37XR) on a variable resistance used as an external load. The individual biofuel cells were integrated into batteries which included 3 clam-biofuel cells in parallel or serial connections, Figure SI2. All measurements were carried out at ambient temperature (23±2ºC).

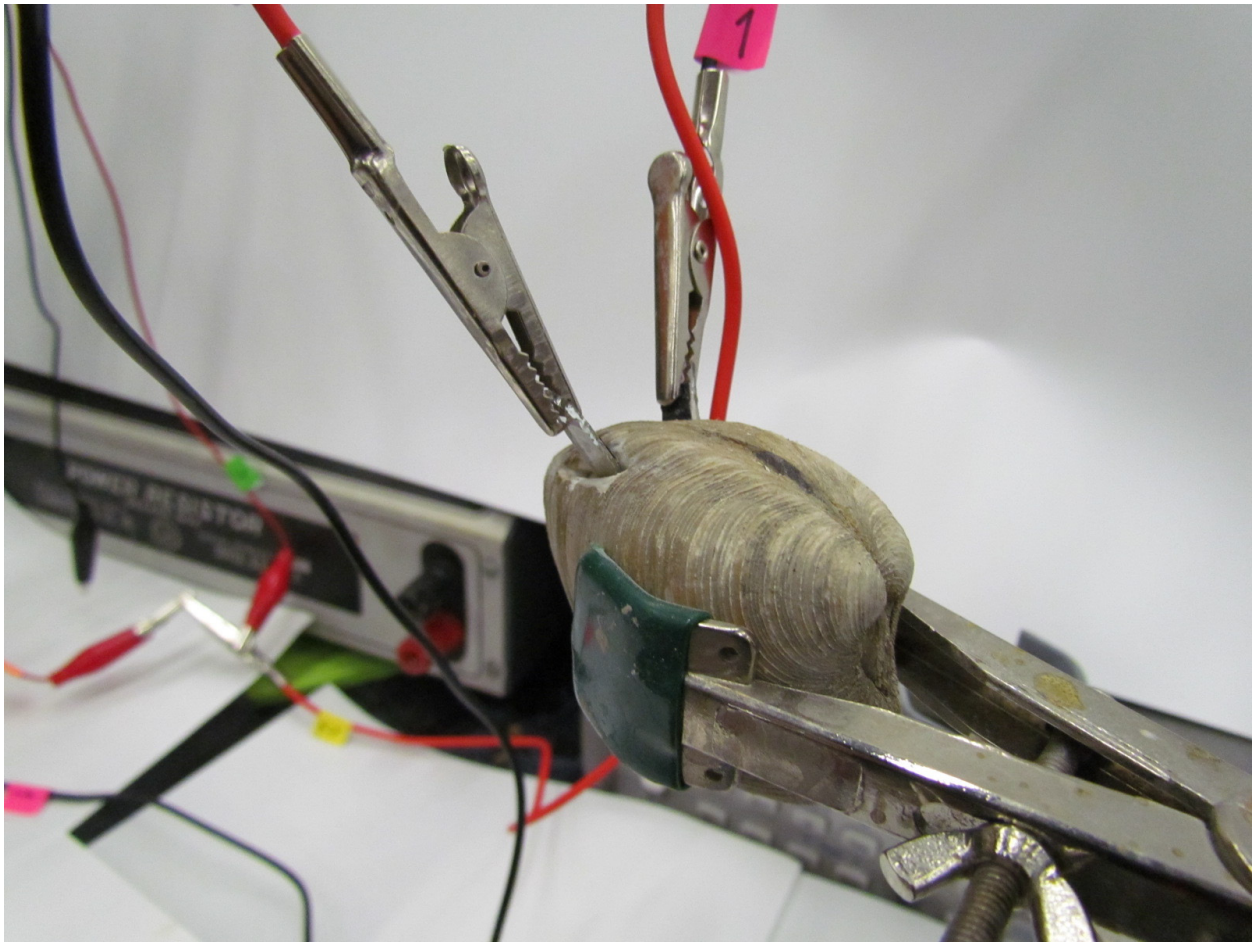

Figure SI1: *The electrical wiring of the biocatalytic electrodes implanted in a clam.*

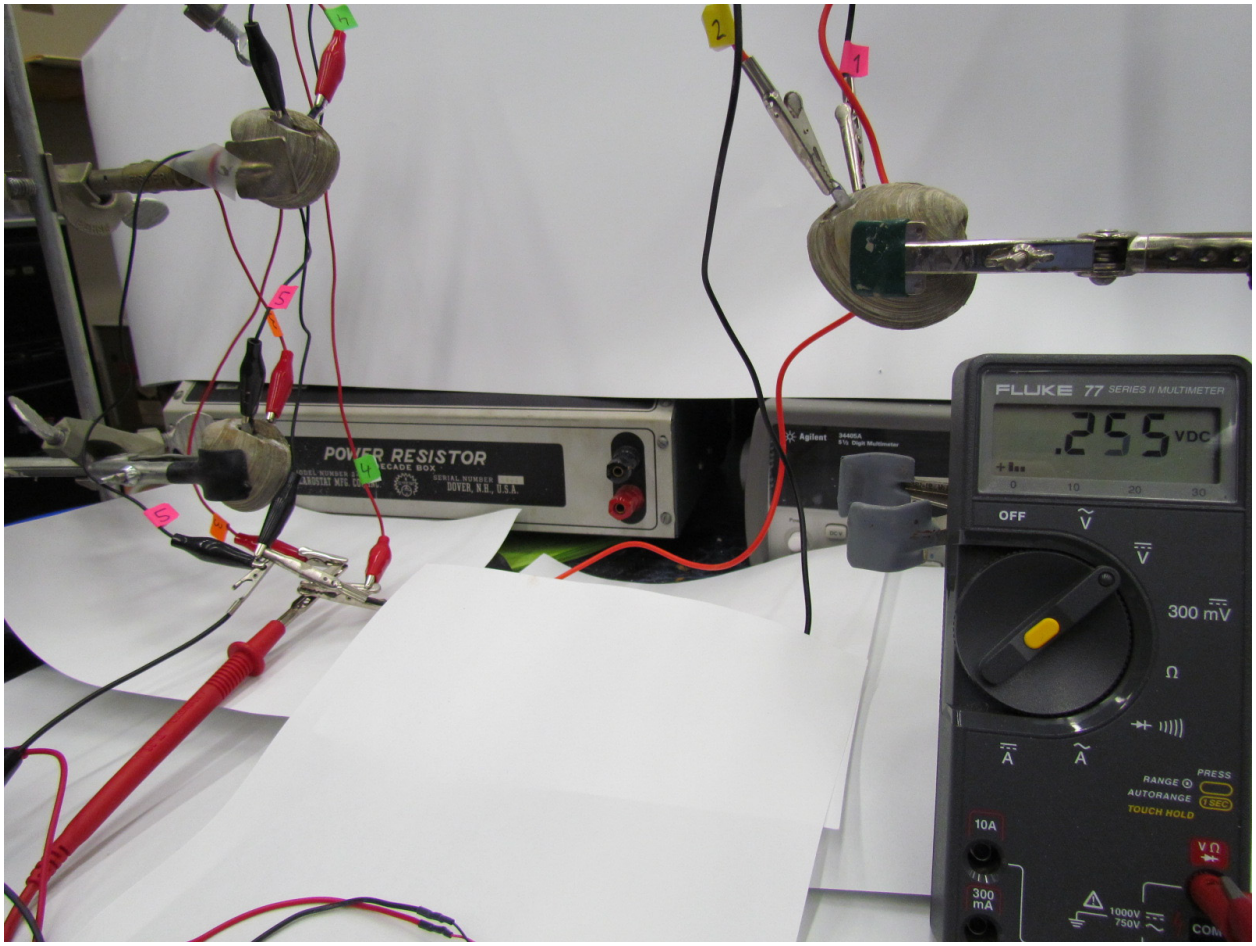

Figure SI2: *The setup for electrical measurements on the battery composed of 3 clam-biofuel cells.*

Activity assays of the enzyme-modified electrodes: The activity of PQQ-GDH immobilized on the anode was determined according to the protocol published elsewhere[3] by measuring decrease in absorbance of 2,6-dichlorophenolindophenol (DCIP) upon its biocatalytic reduction with glucose. The PQQ-GDH-modified electrode was immersed in a MOPS-buffer solution (10 mM, pH 7.0) containing 10 mM glucose, 120 μM PMS, 200 μM DCIP, 1 mM $CaCl_2$ and the absorbance change (λ = 600 nm; ε_{DCIP} = 21 $mM^{-1} \cdot cm^{-1}$) in time was measured. The PQQ-GDH activity was measured as 250 mU per electrode. The activity of laccase immobilized on the cathode was determined according to the standard protocol[4] by measuring increase in the absorbance upon oxidation of $K_4Fe(CN)_6$ by oxygen biocatalyzed by laccase. Although the optimum pH for laccase is ca. 5.5-6.0,[5] the assay was performed at pH 7.0 mimicking operational

conditions of the biofuel cell. The laccase-modified electrode was immersed in phosphate buffer (10 mM, pH 7.0) containing 1 mM $K_4Fe(CN)_6$ and oxygen (in equilibrium with air) and the change in the absorbance (λ = 420 nm; ε = 1 $mM^{-1} \cdot cm^{-1}$) in time was measured. The laccase activity was measured as 460 mU per electrode.

References:

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