Supporting information for:

Freestanding HRP–GOx redox buckypaper as biocathodes for biofuel cell applications

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S1- Materials and methods

All reagents, glucose oxidase (GOx, 168 Umg^{-1}), HRP (193 U mg^{-1}), laccase (120 Umg^{-1}) from *Trametes versicolor*, Succinyl-concanavalin A, glucose, pyrenemethylammonium hydrochloride and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) potassium salt are purchased from Aldrich and stored at 4°C. All reagents and chemicals products purchased from Aldrich were of reagent grade quality and were used as received unless it is mentioned. Commercial grade thin Multi-Walled Carbon Nanotubes (MWNT) (9.5 nm diameter, purity >95%), obtained from Nanocyl were used as received without any purification step. Succinyl-concanavalin A was purchased from Sigma. Bis-pyrene ABTS [1] and pyrrole concanavalin A [2] were synthesized as described.

^{1}H-NMR spectra were recorded on a Bruker AVANCE 400 operating at 400.0 MHz. ESI mass spectra were recorded with a Bruker APEX-Qe ESI FT-ICR mass spectrometer. All electrochemical experiments were performed in an electrochemical cell with a conventional three-electrode configuration by using an Autolab pgstat100 potentiostat. The BP films were studied as working electrode with a geometric surface of 1 cm^2. A platinum wire was used as counter-electrode and a saturated calomel electrode (SCE) served as reference. Electrochemical measurements were performed using 0.1 mol L^{-1} phosphate buffer saline (PBS) as electrolyte in aqueous media at room temperature. The solutions were deoxygenated

by purging with argon prior to each experiment. For the performance measurements of the biocathode the solutions were re-oxygenated with a constant oxygen flow during the whole experiment or in air.

FE-SEM images were recorded using ULTRA 55 FESEM based on the GEMINI FESEM based on the GEMINI FESEM column with beam booster (Nanotechnology Systems Division, Carl Zeiss NTS GmbH, Germany) and tungsten gun with an accelerating voltage of 3 kV and WD of 5.4 mm. The texture properties were analyzed using advanced data processing software (ADP version 5.1, Thermo Electron Corporation).

The electric resistance of the buckypaper was measured by a standard programmable DC voltage/current four-point probe method using a Jandel, Universal probe connected to a current generator (Jandel, RM3).

Confocal scanning microscopy is used for measuring the thickness using a Keyence 3D Laser Scanning microscope.

S2- Preparation of biocathodes:

Bis-Pyr-ABTS-BPs were prepared as described in reference [1]. These PBs were incubated at room temperature in HRP or laccase solution (5 mg/mL in BP, pH 7.4) for 1 or 4h, respectively. Finally, unbound HRP or laccase was eliminated by washing with PB (0.2 M, pH 7.4).

The as prepared BPs were functionalized by pyrrole-concanavalin A as followed: 200 µL of 0.1 mol. L^{-1} phosphate buffer (pH 7.4) containing pyrrole-concanavalin A (1 mg/ml) was drop-casted on the PB. The solvent was allowed to dry at room temperature. After the electropolymerization step in 0.2 mol.L^{-1} phosphate buffer (pH 7.0) free of monomer, each electrode was rinsed with phosphate buffer and then immersed for 2 h in phosphate buffer containing $CaCl_2$ (0.5 mmol.L^{-1}) and $MnCl_2$ (0.5 mol.L^{-1}).

For the specific anchoring of GOx onto the poly(protein) film, the poly(pyrrole-concanavalin A) BPs were incubated in GOx solution (5mg/mL in phosphate buffer, pH 7.0) for 4 h. All steps were carried out at room temperature. When not in use, the electrode was stored at 4 °C in a refrigerator.

S3- Electrochemical measurements

All electrochemical experiments were carried out in a conventional three-electrode cell. Solutions of glucose were allowed to mutarotate during one day and were kept refrigerated. Electropolymerization was performed by controlled potential electrolysis ($E_{applied}$ =+0.65 V versus SCE, Q = 10 mC) in PBS. The resulting modified electrodes were then rinsed with phosphate buffer.

S4- Synthesis:

N-(3-Aminopropyl) pyrrole [3] and pyrrole concanavalin A [2] were synthesized as described in the corresponding reference.

References

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(3) Gall, T. L.; Passos, M. S.; Ibrahim, S. K.; Morlat-Therias, S.; Sudbrake, C.; Fairhurst, S. A.; Queiros, M. A.; Pickett, C. J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1657.