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ARTICLE TYPE

Supplementary information

Experimental Section

Materials

Synthesis of the redox polymers was achieved by adapting literature procedures[1] using $(NH_4)_2OsCl_6$ (Aldrich) as starting material to prepare the *cis*-Os(4,4'-dimethoxy-2,2'-bipyridine)$_2Cl_2$ and *cis*-Os(4,4'-dichloro-2,2'-bipyridine)$_2Cl_2$ complexes, which were then complexed, via ligand substitution reaction in ethanol/water solvent, to a previously pre-synthesised polyvinylimidazole (PVI) polymer.[2] Glucose oxidase (GOx) from (*A. Niger*) was obtained from Sigma – Aldrich and *Th*Lacc was donated by VTT Technology, Finland (K. Kruus). Unless otherwise stated all other chemicals were obtained from Sigma-Aldrich. All buffers were prepared from solutions of the selected base then adjusted to the desired pH using solutions of the acid.

Apparatus

Graphite disc electrodes (3 mm diameter), formed by shrouding graphite rods (Goodfellow) in glass tubes using heat-shrinkable tubing and establishing an electrical connection to copper rods (Farnell) at the rear with silver epoxy resin (Farnell), were used as working electrodes. Cyclic voltammetry was carried out with a CHI 650 potentiostat, using a graphite electrode, Ag/AgCl (3 M KCl) and platinum wire as working, reference and counter electrodes, respectively (IJ Cambria). EFCs were assembled by insertion of anode and cathode into a compartment-less electrochemical cell containing 5 mL of electrolyte solution. The anode and cathode were externally connected through a resistance box (IET Labs) over a resistance range of 5 MΩ to1 kΩ, and the voltage between the electrodes measured with a multimeter (Keithley) for each load.

Film assembly was monitored at each step with a quartz crystal microbalance (QCM, USI Japan) with 9 MHz QCM resonators (AT-cut, International Crystal Mfg). The gold resonators were first treated with 4 mM 3-mercaptopropionic acid in ethanol overnight to form negative monolayer surface to mimic graphite surface. Films were assembled onto negatively charged gold resonator surface and were dried in a stream of nitrogen before measuring the frequency change (ΔF).[3]

Enzyme activity

Glucose oxidase was obtained from Sigma – Aldrich (10 mg/ml stock solution was prepared in phosphate buffer pH 7.4) and *Trametes hirsuta* (*Th*Lacc) was provided by VTT (Finland) as (3.6 mg/mL) stock solution in citrate buffer pH 5. Enzyme activity was calculated using spectrophotometric assays (Agilent 8453). Glucose oxidase activity was monitored at 460 nm, where the increase in absorbance is a result of the oxidation of dianisidine through a peroxidase coupled system, using an extinction coefficient of 11300 M^{-1} cm^{-1}, in phosphate buffer pH 6.[4] The enzymatic activity of the laccase was measured by monitoring the oxidation of 5 mM ABTS in 50 mM acetate buffer pH 4.5 at 420 nm over a period of 10 minutes using extinction coefficient of 36 000 M^{-1} cm^{-1}.[5]

Methods

Electrodes were prepared, based on LBL self assembly of redox polymer and enzyme.[6] For anode and cathode 10 µL redox polymers (8–10 mg/ml solution/suspension in water) were adsorbed over graphite electrode for 20 minutes, then rinsed with milli Q water. Further enzymes 10 µL GOx of 10 mg/ml (1500 U/ml), or *Th*Lacc (390 U/ml) was adsorbed for 20 minutes, then washed with Milli Q water, further repeating these steps to form (polymer /enzyme)$_n$ films. Prepared films were dried for 12 h before testing electrochemically or EFC assembly. Unless otherwise stated, current and power densities were measured at 37 °C in 0.1M potassium phosphate buffered solutions containing 0.15 M NaCl, 0.1 M glucose and saturated O_2.

Supplementary figures

Figure S1. QCM frequency shifts for alternate redox polymer – enzyme a) polymer I/GOx and b) polymer II/*Th*Lacc adsorbed on gold resonators first coated with a monolayer of 3-mercaptopropionic acid rendering a negatively charged surface that mimics the self assembly at graphite electrode.

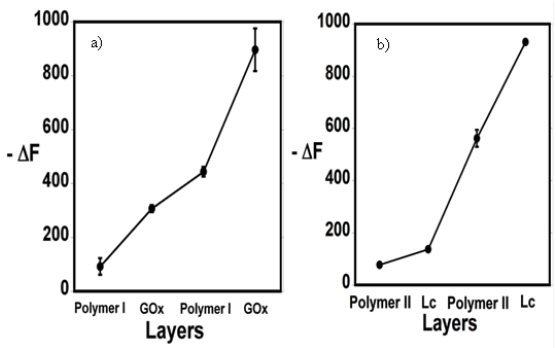
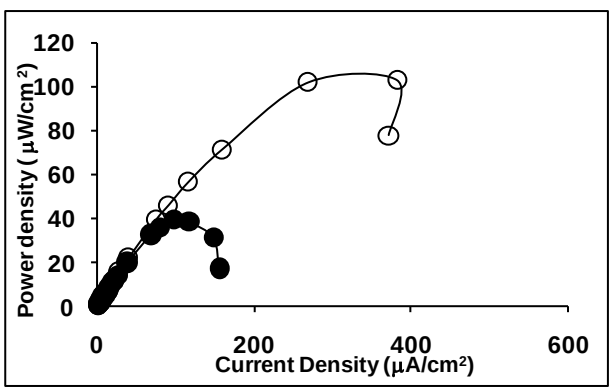


Figure S2. Power versus current density curve of an EFC composed (polymer I /GOx)$_2$ and (polymer II/ *Th*Lacc)$_2$ films in oxygen-saturated 0.1 M potassium phosphate buffer containing 0.15 M NaCl at 37 ºC, in the presence of 0.1 M glucose at pH 7.4 (closed circles) and 5.5 (open circles).



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