Bioelectrocatalytic generation of directly readable code: harnessing cathodic current for long-term information relay

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Electronic Supplementary Information (ESI)

Enzyme preparation. Laccase (E.C. 1.10.3.2) from \textit{Trametes versicolor} was purchased from Sigma-Aldrich and used in experiments after the following minor preparation. A suspension of the stock enzyme powder was made (5 mL, 20 mg mL\textsuperscript{-1}) in potassium phosphate buffer (20 mM, pH 7.3) and then dialyzed using a Slide-A-Lyzer dialysis cassette (10 KDa molecular weight cut off; Thermo Fisher Scientific, Inc.) against a series of sequential buffer exchanges (10 mM potassium phosphate, pH 7.0 for 12 h at 4°C) containing: 1) 1 mM CuSO\textsubscript{4}, 2) 1 mM EDTA and 3) buffer only. Following dialysis, the protein concentration of the preparation was determined using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.) according to the manufacturer’s instructions. Protein activity in respect to catalytic oxidation was determined for syringaldizine, using the supplier’s standard method (Sigma-Aldrich). The laccase preparation was normalized to a defined protein concentration (5 mg mL\textsuperscript{-1}) with catalytic activity of \sim 2.1 units mg\textsuperscript{-1} [where one unit will oxidize 1 µM of syringaldizine (ε = 65 mM\textsuperscript{-1} cm\textsuperscript{1}) per min]. Bovine serum albumin (BSA) was used without further treatment.

Electrode preparation. CNT-BP prepared from 100% multi-walled carbon nanotubes (Buckeye Composites; NanoTechLabs, Yadkinville, NC) was used as the electrode material (geometric area 0.1 cm\textsuperscript{2}). Electrodes were incubated with PBSE (10 mM in DMSO) with moderate shaking for 1 h at room temperature, rinsed in DMSO to remove excess PBSE and then in potassium phosphate buffer (10 mM, pH 7.0). The electrode was then immediately incubated with prepared laccase (or BSA, 1 mg mL\textsuperscript{-1}), for 1 h at room temperature with moderate shaking, before washing extensively with potassium phosphate buffer (10 mM, pH 7.0). Electrodes were used immediately or stored in potassium phosphate electrolyte (0.1 M, pH 5.8) at 4°C until transferred to an electrochemical cell. The concentration of electroactive enzyme associated with the CNT-BP surface was determined by integrating the anodic peak according the equation, \Gamma = \frac{Q}{nFA} where \Gamma is the number of moles cm\textsuperscript{-2}, Q is the charge obtained by integrating the anodic peak, n is the number of electrons involved in the reaction (assumed to be 4 for laccase), F is Faraday’s constant and A is the electrochemically available surface area (EASA). EASA was calculated using the capacitance of the electrode obtained in a non-Faradaic region during CV.\textsuperscript{1,2} The specific capacitance of the CNT was taken to be 20 µF cm\textsuperscript{-2}.\textsuperscript{2,3}

Electrochemical measurements. Enzyme-functionalized CNT-BP electrodes were placed into a Teflon cap fabricated to fit a glassy carbon disk electrode and used as the working electrode in a stacked-cell configuration with a platinum gauze (100 mesh; Alfa Aesar) counter electrode and a Ag/AgCl (3M KCl) reference electrode.\textsuperscript{4} The electrochemical cell working volume (4 mL) was supplied with nitrogen- or oxygen-saturated electrolyte (potassium phosphate buffer, 0.1 M, pH 5.8) via two peristaltic pumps at a fixed flow rate (5 mL min\textsuperscript{-1}). Electrochemical measurements were performed with a potentiostat (Versastart 3, Princeton Applied Research) and the associated software. For potentiostatic measurements, a constant potential of 0.4, 0.5 or 0.3 V was applied at the working electrode and current was monitored with respect to time. All electrochemical measurements are described with standard deviation and reported vs. Ag/AgCl throughout.
Biocode generation. To facilitate the timed flow of the programmed two input sequence, a programmable logic controller (Direct Soft 5, Automation Direct, Cumming, GA) was housed in an electrical enclosure with provisions for power and switches to control two external pumps. The PLC directly controls two peristaltic pumps via a binary hexadecimal input, programmed with Relay Ladder Logic (RLL). Code 39 was manually decoded to a series of bits (binary 1 and 0) and 32 bit segments loaded into the PLC memory via 8 hexadecimal numbers in the RLL program. When the most significant bit (MSB) is a binary 1, the pump is activated to feed oxygen-saturated electrolyte: alternatively a binary 0 activates a second feed stream of nitrogen-saturated electrolyte. Following each pump duration the binary PLC memory is shifted 1 bit to the left, thereby discarding the previous MSB and registering a new input until all bits in PLC memory have been read.

References: