

Supporting Information

Electrocatalysis by Subcellular Liver Fractions Bound to Carbon Nanostructures for Stereoselective Green Drug Metabolite Synthesis

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Experimental Section

Chemicals and Materials. Edge plane pyrolytic graphite disk electrodes (geometric area 0.2 cm²) were prepared from pyrolytic graphite blocks (1 in. x 1 in. x 0.5 in., Momentive Performance Ltd., Strongsville, OH) in our machine shop. Multiwalled carbon nanotubes (MWNT, purity 99.1 %) were a gift from SouthWest Nano-Technologies Inc. (SWeNT, Norman, OK). Testosterone, L- α -phosphatidylcholine (denoted as PL), and standard 6 β -hydroxytestosterone were purchased from Sigma-Aldrich. Human liver microsomes (HLM, total protein: 20 mg mL⁻¹, total amount of CYP enzymes: 0.58 nmol mg⁻¹ protein, NADPH-cytochrome c reductase activity: 171 nmol mg⁻¹ protein min⁻¹, cytochrome b₅: 0.439 nmol mg⁻¹ protein, cytochrome P450-NADPH reductase (CPR) concentration: 0.057 nmol mg⁻¹ protein) was purchased from XenoTetch (Lenexa, KS, USA). Recombinant bactosomes containing only human CPR (expressed in *Escherichia coli*) was purchased from Cypex Ltd. (Dundee, Scotland, UK) [CPR concentration: 9.7 nmol mL⁻¹, total protein concentration: 12.8 mg mL⁻¹]. All other chemicals were high-purity analytical grade. Electrochemical measurements were carried out in phosphate buffer containing 0.15 M NaCl, pH 7.0 at 25 °C.

Instrumentation. A 3- electrode electrochemical cell consisting of an Ag/AgCl reference electrode (1M KCl, CH Instruments Inc.), a Pt wire counter electrode, and HLM adsorbed MWNT-coated PGE working electrodes were used. Electrochemical experiments were performed by using CH Instruments (Model: CHI 1040, Texas, USA). Cyclic voltammograms (CVs) were performed under argon purged anaerobic buffer to study direct electron transfer properties. For catalytic oxygen reduction studies, CVs were conducted by rotating disc voltammetry at a rotation rate of 300 rpm (EcoChemie Autolab rotator equipped with motor controller unit, Metrohm Inc., USA).¹ The surface morphology of PGE, PGE/MWNT, and HLM adsorbed PGE/MWNT electrodes were imaged by scanning electron microscopy (Model: JEOL JXM 6400).

Construction of MWNT modified HLM bioelectrode. Prior to use, PGE electrodes were polished on SiC paper (P320 grit), sonicated for 30 s, rinsed in deionized water and dried under nitrogen (N₂). About 10 μ L of MWNT (1 mg mL⁻¹ dispersion in DMF, obtained by ultrasonication at room temperature for 4 h) was dry-coated on polished PGE electrodes by leaving overnight at room temperature. The MWNT coated PGE electrodes (PGE/MWNT) were then washed with deionized water and dried under N₂. 20 μ L of HLM was adsorbed on PGE/MWNT electrodes for 30 min at 4 °C (denoted as PGE/MWNT/HLM). The electrodes were rinsed in deionized water and stored overnight at 4 °C before conducting electrochemical and electrocatalytic measurements.

Electrocatalytic hydroxylation of testosterone. The electrocatalytic conversion of testosterone to 6 β -hydroxytestosterone was identified by liquid chromatography. In brief, four HLM adsorbed PGE/MWNT electrodes were placed in 10 mL beaker containing 250 μ M testosterone 2 mL phosphate buffer, pH 7.0. The electrolysis was carried out at an applied potential of -0.6 V vs Ag/AgCl for 1 h under saturated oxygen conditions using a multipotentiostat (CH Instruments, CHI 1040). The reaction mixture obtained from electrolysis was analyzed using high performance liquid chromatography (HPLC, premier C18 column, length 10 cm, Shimadzu). The mobile phase (30% acetonitrile, 70% water) was delivered at a flow rate 0.3 mL min⁻¹. Testosterone and 6 β -hydroxytestosterone were identified based on chromatograms of standards (Sigma) run under similar conditions.

Hydrogen peroxide (H₂O₂) assay. Hydrogen peroxide strips (EMD Millipore MQuant peroxide test strips, Billerica, MA, USA) were used to identify the H₂O₂ formation after the bulk electrolysis of testosterone hydroxylation by PGE/MWNT/HLM electrodes. The H₂O₂ formation arises from the electrocatalytic oxygen reduction by microsomal heme proteins. The electrolysis was carried out as described above. H₂O₂ strip was placed in 1 mL of 50 % diluted reaction mixture in buffer and the resulting color of the strip was compared with the H₂O₂ concentration scale provided by the manufacturer to identify the peroxide concentration formed in the electrolysis.

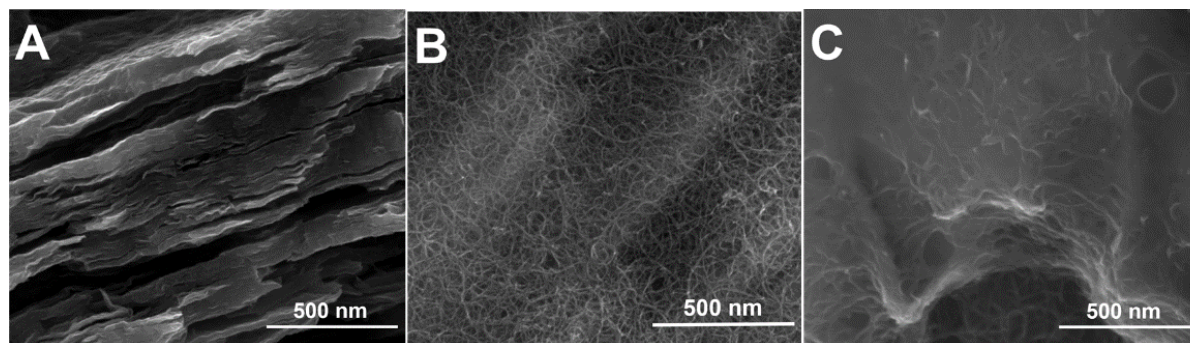


Figure S1. Representative SEM images of **A.** polished bare PGE electrode, **B.** coated MWNT on the PGE electrode and **C.** HLM adsorbed onto the MWNT-modified PGE electrode.

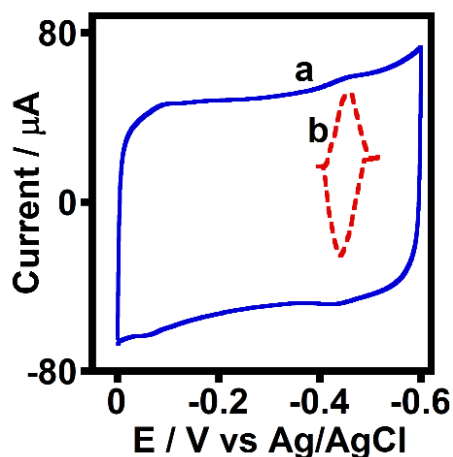


Figure S2. Cyclic voltammograms of (a) unsubtracted and (b) background subtracted PGE/MWNT/CPR electrode in argon atmosphere, phosphate buffer pH 7.0 at 25 °C, scan rate 0.3 V s⁻¹.

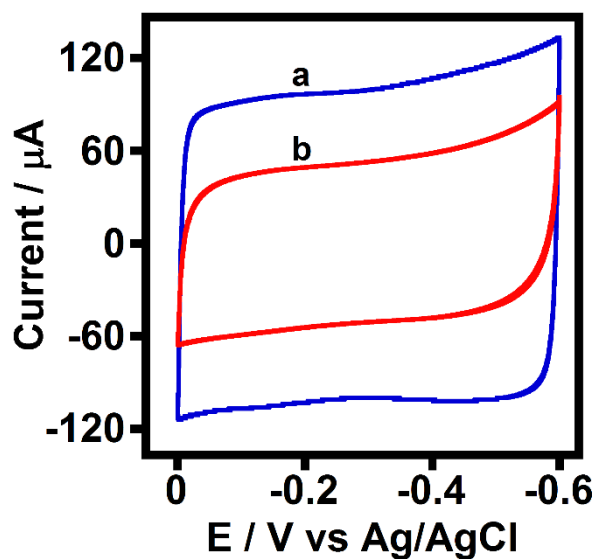


Figure S3. Cyclic voltammograms of (a) PGE/MWNT and (b) PGE/PL electrodes in the absence of HLM in argon atmosphere, phosphate buffer pH 7.0 at 25 °C; scan rate 0.3 V s⁻¹.

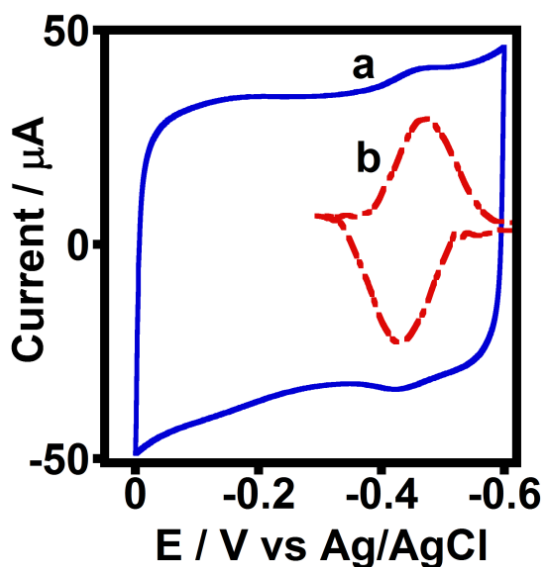


Figure S4. Cyclic voltammograms of (a) unsubtracted and (b) background subtracted PGE/HLM electrode in argon atmosphere, phosphate buffer pH 7.0 at 25 °C, scan rate 0.3 V s⁻¹.

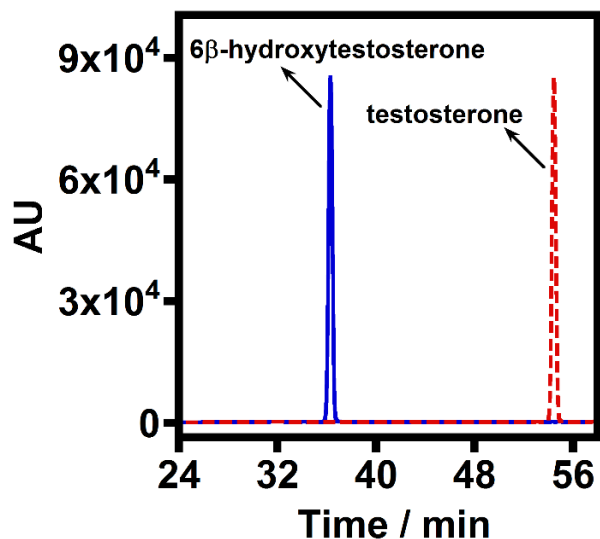


Figure S5. High performance liquid chromatograms of 250 μM testosterone and 6 β -hydroxytestosterone standards in phosphate buffer pH 7.0 at 25 °C.

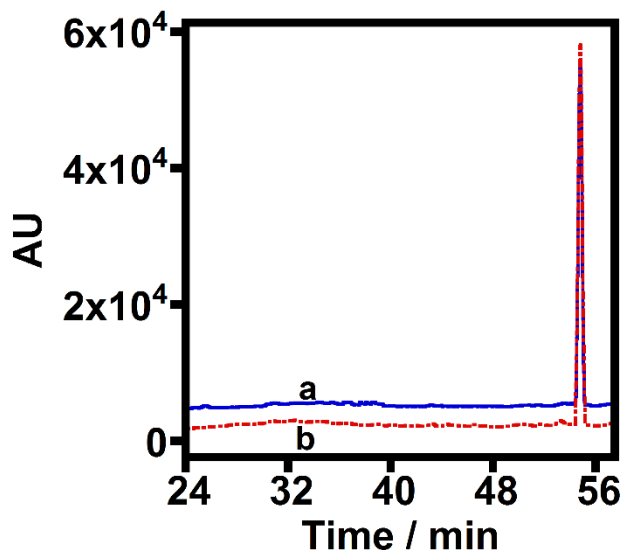


Figure S6. High performance liquid chromatograms of reaction mixtures after 1 h bulk electrolysis of (a) PGE/MWNT and (b) PGE/PL electrodes (no HLM present) in $250 \mu\text{M}$ testosterone, phosphate buffer pH 7.0 at -0.6 V vs Ag/AgCl under saturated oxygen condition at $25 \text{ }^\circ\text{C}$.

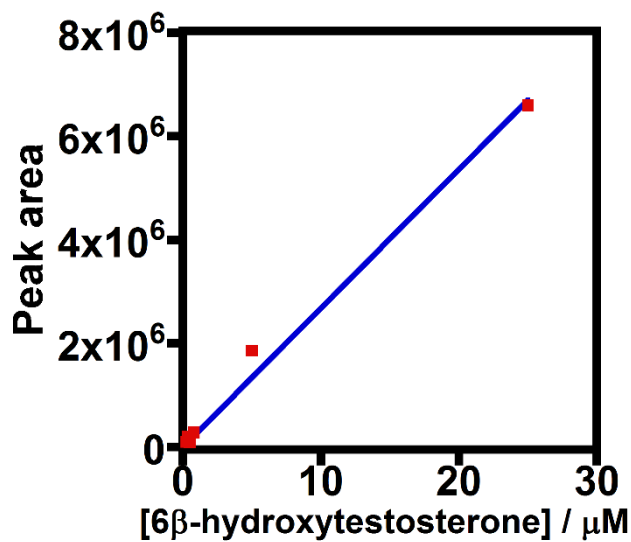


Figure S7. Calibration plot showing the peak area versus concentration of standard 6β -hydroxytestosterone.

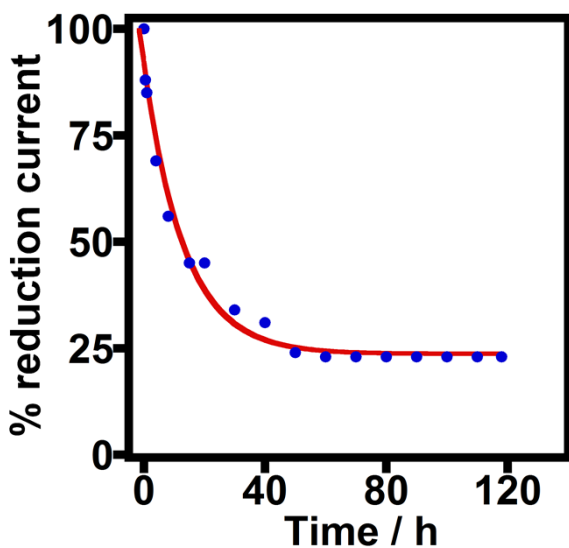


Figure S8. Amperometric *i-t* curve assessing the catalytic oxygen reduction stability of PGE/MWNT/HLM electrodes at an applied potential of -0.6 V vs Ag/AgCl in phosphate buffer, pH 7.0, saturated oxygen, $25 \text{ }^\circ\text{C}$.

1 (a) S. Krishnan, A. Abeykoon, J. B. Schenkman and J. F. Rusling, *J. Am. Chem. Soc.* 2009, **131**, 16215-16224; (b) S. Krishnan and F. A. Armstrong, *Chem. Sci.* 2012, **3**, 1015-1023.