

## Supplementary Material For Carbon Nanoweb: A Promising New Bioelectrode Material.

Shannon J. Little, Stephen F. Ralph, Nicolas Mano, Jun Che and Gordon G. Wallace

### Details of reagents used

Bilirubin oxidase (BOD; EC1.3.3.5) from *Trachyderma tsunodae* was purchased from AMANO and purified as previously reported.<sup>1</sup> Glucose oxidase (GOD) from *Aspergillus niger* (EC 1.1.3.4, 208 U/mg) was purchased from Fluka and further purified.<sup>2</sup>

HiPCO single-walled carbon nanotubes (SWNTs) (4% Fe catalyst, batch no. P0341) were obtained from CNT Nanotechnology (Rice University, Houston, USA). Reticulated vitreous carbon (RVC) was purchased from Duocel, ERG Materials and Aerospace Corporation, while glassy carbon plates were obtained from Hochttemperatur-Werkstoffe GmbH. Methods for preparing the polymers PAA-PVI-[Os(4,4'-dichloro-2,2'-bipyridine)<sub>2</sub>Cl]<sup>2+/+</sup> and (PVP-[Os(N,N'-dialkylated-2,2'-bipyridine)])<sup>3+/2+</sup> were prepared by previously reported methods.<sup>3,4</sup> Poly (ethylene glycol)diglycidyl ether (PEGDGE) was obtained from Polysciences Inc. (Warrington, PA, USA) as a 8% (w/w) solution.

### Instrumentation

Raman spectra were obtained using a Jobin Yvon Horiba HR800 Raman spectrometer and LabSpec software. Spectra were measured using a 632.8 nm laser or a 1800 lines.mm<sup>-1</sup> grating at room temperature, with the peaks obtained being analysed using a Gaussian fitting procedure.

Scanning electron microscopic (SEM) images were obtained using a JEOL JSM 7500FA cold-field-gun field emission microscope. Samples were mounted on a stage using adhesive carbon tape and a drop of conductive silver paint.

Electrochemical experiments were conducted using a Princeton Applied Research model 363 potentiostat/galvanostat and model 401 E-corder connected to a Dell GX 270 PC running AD Instruments software (EChem version 2.0.7 and Chart version 5.0.2). A three electrode cell was employed using a platinum mesh (2 cm x 2 cm) auxiliary electrode and Ag/AgCl reference electrode with a 3 M NaCl salt bridge. The working electrode was a glassy carbon plate or disk, piece of RVC, or one of these materials that had been modified with NanoWeb and, in some instances, various enzymes and other polymers.

### **Preparation of modified electrodes**

Polished glassy carbon disk electrodes were modified with SWNTs or chemically converted graphene via a drop casting method. 1 mg.mL<sup>-1</sup> dispersions of SWNTs in dimethylformamide were prepared via ultrasonic sonication, while chemically converted graphene was provided by Dr Sanjeev Gambhir of the University of Wollongong as a 0.5 mg.mL<sup>-1</sup> dispersion in dimethylformamide. The modified glassy carbon electrodes were prepared by drop casting 3 μL (3 x 1 μL aliquots) of a dispersion of the required material onto the electrode and then evaporating the solvent slowly either by application of heat or vacuum. All electrodes were washed with distilled water prior to use.

### **Preparation of NanoWeb materials**

The high surface area NanoWeb was grown using chemical vapour deposition (CVD) onto either glassy carbon (GC) or reticulated vitreous carbon (RVC; 45 pores per inch). The substrate was first coated with a thin layer of catalyst solution consisting of 10% (w/w) iron(III) *para*-toluenesulfonate (Baytron) in ethanol. In the case of glassy carbon sheets, the catalyst solution was applied by spin coating using a speed of 1500 rpm and a commercial spin-coater (Laurell Tech). The solvent was immediately evaporated using a hot plate (60°C) in order to avoid crystallisation of the iron(III) salt, and the coated substrate then stored in a 100 oven. RVC substrates were briefly immersed in the 10% (w/w) catalyst solution before being removed, shaken to remove excess solution, and then allowed to dry on laboratory tissue paper until all

the excess oxidant had drained from the electrode. The solvent was then removed using a 100 °C oven, in which the electrode was subsequently stored.

CVD growth of the NanoWeb material was achieved using a Thermal CVD system (Atomate) that provides computer control over synthesis conditions. Initially the system was flushed with argon ( $200 \text{ mL}\cdot\text{min}^{-1}$ ) for 30 minutes, after which the furnace temperature was increased to 600°C whilst a mixture of argon ( $150 \text{ mL}\cdot\text{min}^{-1}$ ) and hydrogen ( $20 \text{ mL}\cdot\text{min}^{-1}$ ) were passed through the furnace. The furnace temperature was then maintained at 600°C for 10 minutes, resulting in reduction of the iron(III) catalyst to iron nanoparticles. Growth of the NanoWeb was then initiated by ramping the temperature up to 800°C at which point acetylene gas ( $10 \text{ mL}\cdot\text{min}^{-1}$ ) was passed through the furnace whilst maintaining a constant flow of argon ( $200 \text{ mL}\cdot\text{min}^{-1}$ ) and hydrogen ( $3 \text{ mL}\cdot\text{min}^{-1}$ ). Synthesis of the NanoWeb was complete after 30 min., at which point the furnace, acetylene and hydrogen were turned off, and the system flushed continuously with argon ( $150 \text{ mL}\cdot\text{min}^{-1}$ ) until the temperature was less than 100 °C.

#### **Preparation of GOx Modified NanoWeb/RVC Electrodes**

NanoWeb/RVC electrodes prepared as described above were covered with 42  $\mu\text{L}$  of a solution consisting of 5 mg of purified GOx dissolved in 75  $\mu\text{L}$  of 0.1 M PBS and 25  $\mu\text{L}$  of 5% (w/w) Nafion solution. After the mixture was allowed to soak into the NanoWeb/RVC the electrode was placed in a fridge until the solvent had evaporated (~ 2h.). Immediately prior to use the GOx modified NanoWeb/RVC electrodes were washed with 0.1 M PBS to remove any loosely attached enzyme.

#### **Preparation of BOD Modified NanoWeb Electrodes**

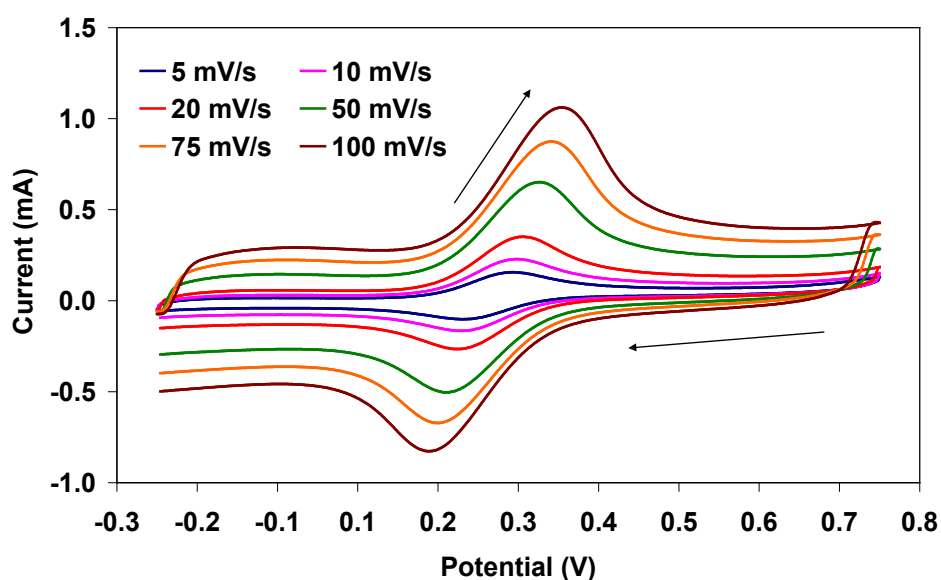
All NanoWeb electrodes were modified using a drop cast solution that was prepared by diluting 1  $\mu\text{L}$  of a  $100 \text{ mg}\cdot\text{mL}^{-1}$  stock solution of purified BOD in phosphate buffer with 20  $\mu\text{L}$  of 20 mM phosphate buffer. The resulting solution was then cast onto the NanoWeb electrodes. After allowing the enzyme to adsorb for 10 min., the electrode was immersed in 500  $\mu\text{L}$  of fresh buffer solution briefly to remove excess enzyme, and then allowed to dry at 4 °C for 18 h.

### Supplementary Material References

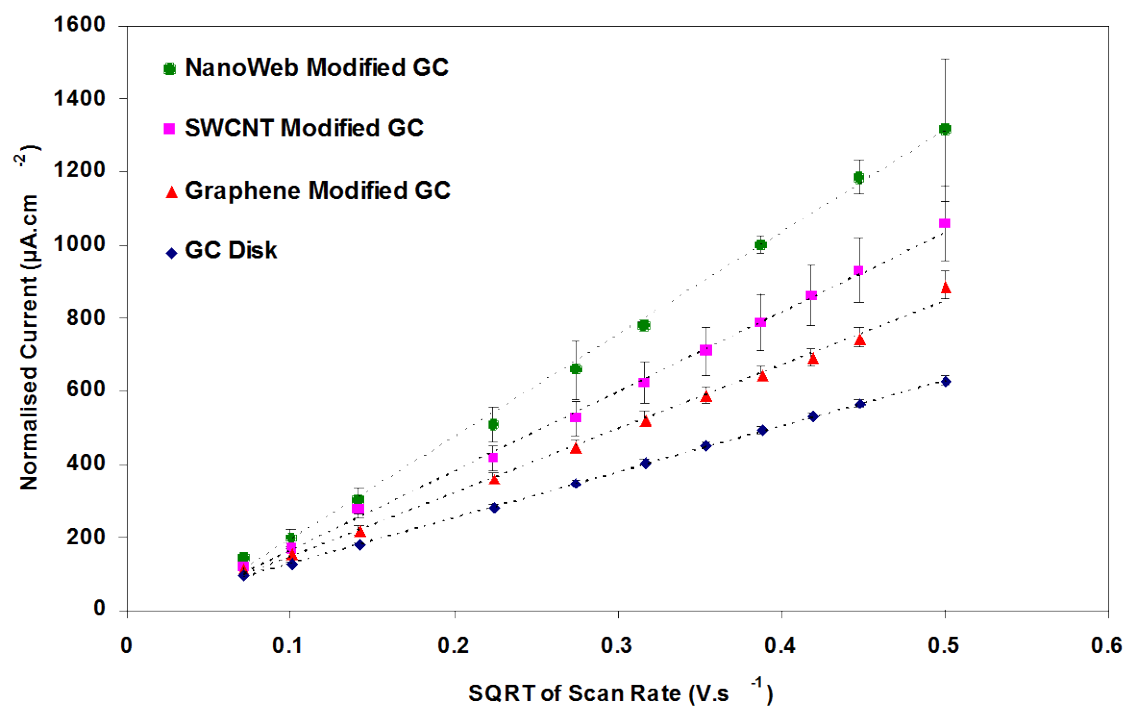
1. V. Flexer, N. Brun, R. Backov and N. Mano, *Energy and Environ. Sci.*, **2011**, DOI = C0EE00466A.
2. O. Courjean, F. Gao and N. Mano, *Angew. Chem. Int. Ed.*, **48**, **2009**, 5897.
3. F. Mao, N. Mano and A. Heller, *J. Am. Chem. Soc.*, **125**, **2003**, 4951.
4. N. Mano, H.-H. Kim, Y. Zhang and A. Heller, *J. Am. Chem. Soc.*, **124**, **2002**, 6480.

### Determination of Electrochemical Surface Areas

In order to determine the electrochemical surface area of different materials, cyclic voltammograms were recorded at different scan rates, after immersing the electrodes in deaerated solutions containing 2 mM ferrocenemethanol and 0.1 M phosphate-buffered saline. Figure S1 shows the CVs obtained using a NanoWeb modified glassy carbon plate electrode, while Figure S2 shows the Randles-Sevcik plot derived from the anodic peak currents for this as well as several other electrodes examined under identical conditions. The slopes of the Randles-Sevcik plots were then used to determine the electrochemical surface areas of the electrodes.



**Figure S1.** Cyclic voltammograms obtained using a NanoWeb modified glassy carbon plate electrode immersed in deaerated phosphate buffered saline solution, also containing 2.0 mM ferrocenemethanol. All potentials were recorded vs. a Ag/AgCl reference electrode. The anodic peak current derived from each of the CVs was used to construct the Randles-Sevcik plot shown in Figure S2.

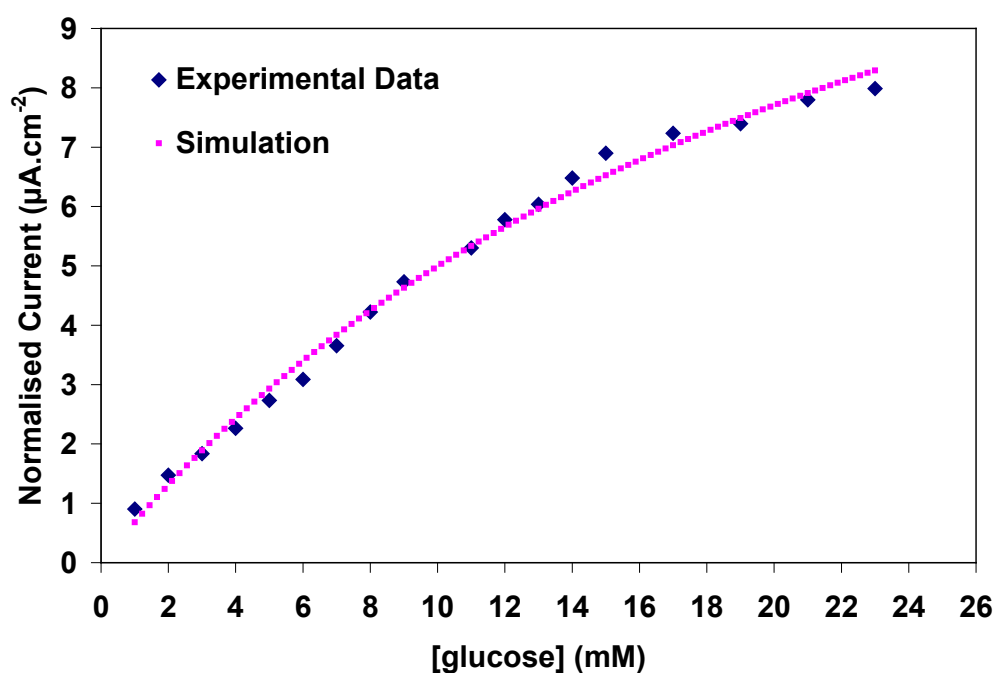


**Figure S2.** Randles-Sevcik plots derived from CVs obtained using a NanoWeb modified glassy carbon plate electrode immersed in deaerated phosphate buffered saline solution, also containing 2.0 mM ferrocenemethanol

### Determination of Electrochemical Capacitances

The capacitance of NanoWeb modified RVC and other electrode materials was determined from the results obtained by performing CV experiments after immersing the electrodes in 0.1 M phosphate-buffered saline. In each case the CVs displayed the rectangular shape expected for a double layer capacitor. The capacitance of each material was derived from the slope of plots of anodic current vs. applied scan rate.

**Evidence that the glucose oxidase immobilised in a Nafion film deposited on NanoWeb/RVC is still catalytically active.**



**Fig. S3** Normalised current/concentration profile obtained using a GOx/Nafion modified NanoWeb/RVC electrode after successive additions of 1 mM glucose. An applied potential of -0.42 V (vs. Ag/AgCl) was used. The line indicates the result of non-linear regression analysis.