Supporting Information

Programmable bioelectronics in a stimuli-encoded 3D graphene interface

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METHODS

Materials. All chemicals and solvents are reagent grade and used without further purification. Potassium dihydrogen phosphate (KH₂PO₄, ≥99.0%), dipotassium hydrogen phosphate (K₂HPO₄, ≥99.0%), potassium chloride (KCl, ≥99.0%), potassium ferrocyanide (K₃[Fe(CN)₆], ≥98.5%) and potassium ferricyanide (K₄[Fe(CN)₆], ≥99.0%, cholesterol (≥, cholesterol oxidase (ChEt) (≥50 units/mg, from Brevibacterium species), t-octylphenoxy polyethoxy ethanol (Triton X-100) (laboratory grade) amine-terminated poly(N-isopropylacrylamide) (Mn ~ 2,500) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification. Phosphate buffer saline (PBS, 0.1 M, pH 7.4) and ferri/ferrocyanide solutions were used as supporting electrolytes for all amperometric measurements. Aqueous
solutions were prepared with Milli-Q water (18.2 MΩ·cm) obtained by Millipore system (Billerica, MA, USA). Apo-pyrroloquinoline quinone dependent glucose dehydrogenase (apo-GDH) was from Roche Diagnostics. Pyrroloquinoline quinone (PQQ) was from Fluka. HEPES and D (+)-glucose monohydrate was from Applichem. Peroxidase from horseradish (HRP, 300 units/mg), 4-aminoantipyrene (4-AAP, ≥99.0%), hydrogen peroxide (H₂O₂, ACS reagent, 30 wt.% in H₂O), phenol (≥99.5%) for enzymatic assay were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Instrumentation.** Transmission electron microscopy (TEM) was performed using a G² Spirit/Biotwin (FEI-Technai, Hillsboro, OR, USA) with a working voltage of 120 kV and Scanning electron microscopy (SEM) was performed using LEO 155 Gemini (Zeiss, OR, USA). All voltammetric, amperometric and charging-discharging measurements were carried out using an Ivium Stat. XR electrochemical analyser (Eindhoven, Netherlands). Impedance measurements were carried out with an Autolab potentiostat-galvanostat (Utrecht, Netherlands). A three-electrode cell with gold working electrode, having 1.00 cm² surface area, and platinum wire auxiliary and Ag/AgCl (3 M KCl) reference electrodes were used in the voltammetric and amperometric measurements.

**Surface characterisation**

In order to prepare self-assembled enzyme-graphene structures for both enzymes, the graphene surface was functionalised by anionic surfactant to produce a negatively charged graphene surface in a well-dispersed solution. In a second step, enzymes were assembled on the functionalised graphene surface via electrostatic interactions.

Before assembling any enzymes on the graphene structure, scanning electron microscopy images at high magnifications were taken to reveal wrinkled structures and a low degree of aggregation in the graphene nanosheets before and after surface modification with negatively charged surfactant, sodium dodecyl benzene sulphonate (SDBS).
Figure S1. SEM images of graphene nanosheets before (a) and after surface modification (b).

Although the high resolution TEM image of a graphene-ChOx hybrid structure (Figure S2) shows good homogeneity and dispersion, it is difficult to resolve enzyme structures in these images. Here, we can assume that dark spots on graphene surface might be attributed to the enzyme.
Figure S2. High resolution TEM image of ChOx- immobilised graphene nanosheets.

The charge-transfer property of bare gold electrode was characterised by measuring electrochemical impedance (EIS) responses in 0.1 M PBS solutions containing 5mM Fe\([\text{CN}]_6\)^{3/-4-} and 0.1 KCl.
Figure S3. Impedance response of bare gold electrodes in 5 mM Fe[(CN)₆]⁴⁻ and 0.1 M PBS at 50 mV s⁻¹ vs Ag/AgCl reference electrode. The inset in is the equivalent circuit used to fit the data.

The Randles circuit was chosen to fit the impedance outputs (Figure S3). The result was shown as a Nyquist plot of spectra which contains semicircle and linear parts. The plot indicates both electron transfer limited (semi-circular portion) and diffusion processes (linear portion) at the same time. The charge transport resistance ($R_{CT}$) at the electrode surface can be quantified based on the diameter of the semi-circular part of plot and described by following equation:[¹]

$$Z(\omega) = R_s + \frac{R_{CT} + \sigma\omega^{-1/2}}{(C_d\sigma\omega^{1/2} + 1) + \omega^2 C_d^2 (R_{CT} + \sigma\omega^{-1/2})} - j \frac{\omega C_d (R_{CT} + \sigma^{-1/2}) + \sigma\omega^{-1/2} (\sigma\omega^{1/2} C_d + 1)}{(C_d\sigma\omega^{1/2} + 1) + \omega^2 C_d^2 (R_{CT} + \sigma\omega^{-1/2})}$$

where $R_s$ is the solution resistance, $C_d$ is the double layer capacitance, $\omega$ is the $2\pi f$, where $f$ is the frequency, and $\sigma$ is defined as:

$$\sigma = \frac{RT}{\sqrt{2} F^2 A \left( \frac{1}{\sqrt{D_o \sigma_o^* C_o^*}} + \frac{1}{\sqrt{D_k \sigma_k^* C_k^*}} \right)}$$
where \( A \) is the area of the electrode, \( D_o \) and \( D_R \) are the diffusion coefficients of oxidant and reductant, respectively. \( C_o \) and \( C_R \) represent the bulk concentrations oxidant and reductant, respectively. According to calculation, \( R_{CT} \) of bare gold electrode was found as 95 \( \Omega \).

In order to test the repeatability of each design, we cycled the system between two extreme points. The systems were switched between for four different states in both AND and OR gates. The states were changed by irradiating the electrode surface with UV light for 2 minutes and heating the reaction medium for 5 minutes. The results are shown in Figure S4a-d for the AND gate OR gate designs.

**Figure S4.** Repeatability tests of electrodes in the four different states in “AND” (a-b); and “OR” (c-d) gates.

**Reference:**