SUPPLEMENTARY INFORMATION for:
"Rational design of quinones for high power density biofuel cells", by
Ross D. Milton, David P. Hickey, Sofiene Abdellaoui, Koun Lim, Fei Wu, Boxuan Tan and Shelley D. Minteer

Materials

Unless otherwise stated, FAD-dependent glucose dehydrogenase (E.C.: 1.1.99.10, *Aspergillus sp.*, GLDE-70-1192) was purchased from Sekisui Diagnostics (UK) and used without any further purification. For comparison experiments, GDH was also purchased from Amano Enzyme Inc., Japan ("Amano 8", *Aspergillus oryzae*) and BBI Solutions USA (GLD1, *Aspergillus oryzae*). Bilirubin oxidase (*Myrothecium sp.*, E.C.: 1.3.3.5) was donated by Amano Enzyme Inc., Japan. Glucose oxidase (*Aspergillus niger*, E.C.: 1.1.3.4, ~200 U mg^{-1}) was purchased from Sigma Aldrich. Unless specified otherwise, all other chemical supplies were purchased from Sigma Aldrich. Ethylene glycol diglycidyl ether (EGDGE) was purchased from Polysciences, Inc. Toray carbon paper was purchased from Fuel Cell Earth (USA, TGP-H-060). Multi-walled carbon nanotubes (MWCNTs, -OH and –COOH modified) were purchased from Cheap Tubes. Glassy carbon working electrodes and saturated calomel reference electrodes were purchased from CH Instruments, Inc.

Synthesis of 1,2-naphthoquinone-4-glycidol (1)

Diisopropylethylamine (1.2 mol. equiv., 178.8 mg, 1.38 mmol) was added drop-wise to glycidol ((±)-glycidol, 10 mL, 151 mmol) and left to stir for 10 minutes at room temperature. Next, 1,2-naphthoquinone-4-sulfonic acid sodium salt (1 mol. equiv., 300 mg, 1.15 mmol) was added gradually and left to stir for a further 10 minutes. Brine solution was then added to the reaction mixture (100 mL) and the crude product was extracted repeatedly with dichloromethane (35 mL) until the organic fraction was colorless. The organic fractions were combined and the dichloromethane was removed *in vacuo*. The crude product was dissolved in chloroform (35 mL) and water was added (100 mL). The crude product was extracted further with chloroform (3x 35 mL total), and the combined organic fraction was reduced in volume and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude product was purified by silica gel chromatography with an diethylether:dichloromethane gradient (5:1 increasing to 1:1). The solvent was removed *in vacuo* to yield deep yellow crystals (62 mg, 0.27 mmol) – 23.4% yield (**Supplementary Fig. 5-7**).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.6 Hz, 1H, -CH), 7.87 (d, *J* = 7.8 Hz, 1H, -CH), 7.66 (t, *J* = 7.6 Hz, 1H, -CH), 7.55 (t, *J* = 7.6 Hz, 1H, -CH), 5.90 (s, 1H, -CH), 4.45 (dd, *J* = 11.3, 2.1 Hz, 1H, -O-CH_2-), 3.97 (dd, *J* = 11.3, 6.4 Hz, 1H, -O-CH_2-), 3.48 – 3.40 (m, 1H, -CH- epoxy), 2.97 (t, *J* = 4.4 Hz, 1H, -CH_2- epoxy), 2.79 (dd, *J* = 4.5, 2.5 Hz, 1H, -CH_2- epoxy).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.21 (C=O), 179.06 (C=O), 167.18 (C-O), 134.90 (CH), 131.57 (C), 131.53 (CH), 130.19 (C), 128.99 (CH), 124.72 (CH), 103.62 (CH), 70.28 (-O-CH_2), 48.87 (epoxy CH), 44.27 (epoxy CH_2).

ESI-MS (High Resolution): 253.0477 $[M+Na]^+$.

Synthesis of 1,4-naphthoquinone-2-glycidol (2)

Diisopropylethylamine (1.2 mol. equiv., 60 mg, 0.46 mmol) was added drop-wise to glycidol ((±)-glycidol, 6 mL, 151 mmol) and left to stir for 10 minutes at room temperature. Next, 1,4-naphthoquinone-2-sulfonic acid potassium salt (1 mol. equiv., 107 mg, 0.39 mmol) was added gradually and left to stir for a further 10 minutes. Dilute brine solution was then added to the reaction mixture (100 mL) and the crude product was extracted repeatedly with dichloromethane (35 mL) until the organic fraction was colorless. The organic fractions were combined and the dichloromethane was removed *in vacuo*. The crude product was dissolved in chloroform (35 mL) and water was added (100 mL). The crude product was extracted further with chloroform (3x 35 mL total), and the combined organic fraction was reduced in volume and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude product was purified by silica gel chromatography with diethylether. The solvent was removed *in vacuo* to yield faint yellow crystals (14 mg, 0.06 mmol) – 15.7% yield (**Supplementary Fig. 8-10**).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 7.99 (m, 2H, -CH), 7.78 – 7.62 (m, 2H, -CH), 6.18 (s, 1H, -CH), 4.27 (dd, *J* = 11.4, 3.0 Hz, 1H, -O-CH_2-), 3.96 (dd, *J* = 11.4, 5.8 Hz, 1H, -O-CH_2-), 3.42 (td, *J* = 6.3, 3.0 Hz, 1H, -CH epoxy), 2.93 (t, *J* = 4.4 Hz, 1H, -CH_2- epoxy), 2.77 (dd, *J* = 4.7, 2.6 Hz, 1H, -CH_2- epoxy).

^{13}C NMR (101 MHz, $CDCl_3$) δ 184.75 (C=O), 179.67 (C=O), 159.21 (C-O), 134.29 (CH), 133.40 (CH), 131.87 (C), 131.03 (C), 126.69 (CH), 126.14 (CH), 110.80 (CH), 69.94 (-O-CH_2-), 49.02 (epoxy CH), 44.61 (epoxy CH_2).

ESI-MS (High Resolution): 253.0482 $[M+Na]^+$.

Synthesis of naphthoquinone linear polyethyleneimine redox hydrogels (1,2-NQ-4-glycidyl-LPEI (NQ-4-LPEI) and 1,4-NQ-2-glycidy-LPEI (NQ-2-LPEI))

Linear polyethyleneimine (LPEI) was synthesized as previously reported.[1] NQ-4-LPEI was prepared by dissolving LPEI (4 mol. equiv. 46.3 mg, 1.1 mmol) in stirred methanol (~15 mL), followed by the addition of **1** (1 mol. equiv., 62 mg, 0.27 mmol). The reaction mixture was left to stir overnight at room temperature and solvent was removed *in vacuo*. Diethylether (~ 25 mL) was added to the polymer residue to remove any unreacted **1** (three washes of 1 hour each). The diethylether was decanted and the polymer was dried *in vacuo*, to yield NQ-4-LPEI (100 mg). Washing with diethylether recovered 7.3 mg of **1**, suggesting an approximate theoretical substitution of 22%; ^{1}H NMR integration of the resulting polymer estimated 21% substitution of the backbone. Using the substitution estimated from ^{1}H NMR, an average molecular mass of 91.3 g mol^{-1} was predicted for an average repeating unit of the NQ-4-LPEI polymer (**Supplementary Fig. 11-13**).

NQ-2-LPEI was synthesized by the same method, although ^{1}H NMR calculations predicted a substitution of approximately 5%. An average molecular mass of 53.6 g mol^{-1} was calculated (**Supplementary Fig. 14,15**).

Glucose dehydrogenase labeling with 1 and 2 (NQ-4-GDH and NQ-2-GDH)

Glucose dehydrogenase (30 mg) was added to a suspension of **1** or **2** (30 mg mL^{-1}, 1 mL) in pH 7.4 citrate/phosphate buffer (0.05 M) and stirred overnight at 4 ºC. Unreacted **1** or **2**, denatured protein and dissociated redox cofactor (FAD) were separated from the product (labeled enzyme) using centrifugal size exclusion spin columns (30 kDa molecular weight cut-off) at 4 ºC, until the filtrate was colorless (6 washes with citrate/phosphate buffer, pH 7.4, 0.05 M). The presence of unreacted **1** or **2** in the filtrate was confirmed by electrochemistry, demonstrating the possibility to remove unreacted **1** or **2** by this technique. The GDH-NQ was concentrated to approximately 30 mg mL^{-1}. GDH quantification and labeling is provided in the Supplementary Information (**Supplementary Fig. 16, 17 and Supplementary Table 1,2**).

Synthesis of 4-methoxy-1,2-naphthoquinone

The synthetic route used to prepared **1** and **2** was adapted (albeit low yielding) to further demonstrate the synthesis of **1** and **2**. Diisopropylethylamine (1.2 mol. equiv., 178.8 mg, 1.38 mmol) was added drop-wise to methanol (10 mL) and left to stir for 10 minutes at room temperature. Next, 1,2-naphthoquinone-4-sulfonic acid sodium salt (1 mol. equiv., 300 mg, 1.15 mmol) was added gradually and left to stir for a further 10 minutes. Next, methanol was removed *in vacuo*, and the crude product was extracted twice with dichloromethane (35 mL) against water (100 mL). The combined organic fractions were reduced in volume, dried over anhydrous magnesium sulfate, and the solvent was removed *in vacuo*. The crude product was purified by silica gel chromatography with diethylether:dichloromethane (3:1). The solvent was removed *in vacuo* to yield deep yellow crystals (12 mg, 0.06 mmol) – 5.5% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 7.6 Hz, 1H, -CH), 7.84 (d, *J* = 7.8 Hz, 1H, -CH), 7.67 (t, *J* = 7.6 Hz, 1H, -CH), 7.56 (t, *J* = 7.6 Hz, 1H, -CH), 5.95 (s, 1H, -CH), 3.99 (s, 3H, -O-CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.50 (C=O), 179.40 (C=O), 168.67 (C-O), 134.95 (CH), 131.97 (C), 131.51 (CH), 130.37 (C), 129.06 (CH), 124.73 (CH), 103.07 (CH), 56.81 (-O-CH_3).

ESI-MS (High Resolution): 211.0372 $[M+Na]^+$.

Synthesis of 2-*N*-ethylmethylamino-1,4-naphthoquinone

2-methoxy-1,4-naphthoquinone (1 mol. equiv., 100 mg, 0.53 mmol) was added to methanol (5 mL), *N*-ethylmethylamine was added drop-wise (4 mol. equiv., 126 mg, 2.13 mmol) and left to stir overnight at room temperature. The solvent was removed *in vacuo* and the crude product was purified by silica gel chromatography with diethylether. The solvent was removed *in vacuo* to yield deep red crystals (24 mg, 0.11 mmol) – 20.9% yield. ^{1}H and ^{13}C NMR and high-resolution mass spectrometry characterization is provided within the Supplementary Information.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 7.6 Hz, 1H, -CH), 7.93 (dd, J = 7.6, 0.6 Hz, 1H, -CH), 7.64 (ddd, J = 7.6, 2.0, 1.1 Hz, 1H, -CH), 7.56 (ddd, J = 7.5, 2.1, 1.0 Hz, 1H, -CH), 5.81 (s, 1H, -CH), 3.59 (q, J = 7.0 Hz, 2H, -N-CH_2), 3.05 (s, 3H, N-CH_3), 1.26 (dd, J = 7.4, 6.7 Hz, 3H, N-CH_2-**CH_3**).

^{13}C NMR (101 MHz, $CDCl_3$) δ 183.55 (C=O), 182.74 (C=O), 151.97 (C-O), 133.78 (CH), 132.78 (C), 132.64 (C), 131.88 (CH), 126.40 (CH), 125.30 (CH), 106.52 (CH), 49.21 (N-CH_2), 39.69 (N-CH_3), 12.89 (N-CH_2-**CH_3**).

ESI-MS (High Resolution): 216.1025 $[M]^+$, 238.0843 $[M+Na]^+$.

Synthesis of 1,4-naphthoquinone-2-LPEI (side product)

The side product obtained when reacting **1** and **2** with LPEI by direct substitution of NQ to the LPEI polymer was demonstrated by mixing 2-methoxy-1,4-naphthoquinone with LPEI in the absence of any additional reagents. LPEI (91.4 mg, 2.13 mmol) was initially dissolved in methanol (~20 mL) and 2-methoxy-1,4-naphthoquinone was added and left to react overnight. The solvent was removed *in vacuo* and the resulting red LPEI residue was washed with diethylether (~ 25 mL) to remove any unreacted NQ (three washes of 1 hour each). The diethylether was decanted and the polymer was dried *in vacuo*, to yield 1,4-naphthoquinone-2-LPEI (82.9 mg).

NQ-X-LPEI GDH bioelectrodes

Initially, NQ-X-LPEI (84 µL, 10 mg mL^{-1} in water) was mixed with GDH (36 µL, 30 mg mL^{-1} in water) and EGDGE (4.5µL, 10% v/v). For experiments performed on glassy carbon electrodes (3 mm diameter), 3 µL of this mixture was deposited onto electrode surfaces that had been previously modified with MWCNTs (5 µL of a MWCNTs dispersion, prepared at a concentration of 5 mg mL^{-1} in isopropanol). For experiments performed on carbon paper electrodes (Toray paper, cut to provide a geometric surface area of 0.25 cm^2), electrodes were modified with 18 µL of the MWCNTs dispersion, followed by the addition of 10 µL of the above NQ-X-LPEI/GDH/EGDGE mixture. All electrodes were allowed to dry overnight at room temperature and rinsed with water before use. Control electrodes prepared with the 1,4-naphthoquinone-2-LPEI side product were prepared by the same method.

NQ-GDH bioelectrodes

Glassy carbon electrodes were modified with MWCNTs prior to enzyme modification (as mentioned above), followed by the addition of pyrene butyricacid succinimidylester (10 µL, 10 mM in acetone). Absolute ethanol (1 µL) was added to NQ-GDH (15 µL, 30 mg mL^{-1}) and 5 µL was deposited onto the glassy carbon electrode and allowed to dry. Hydrophobically-modified C_8-LPEI was prepared as previously reported[2]. C_8-LPEI (28 µL, 10 mg mL^{-1}) was mixed with EGDGE (1.5 µL, 50% v/v) and was applied to the modified electrode surface (5 µL). The resulting NQ-GDH bioelectrodes were dried under positive airflow and allowed to stand overnight at room temperature. The NQ-GDH bioelectrodes were gently rinsed before use.

NQ derivate testing

The ability for the different NQ derivatives (**Fig. 2a**) to mediate glucose oxidation by GDH was evaluated by entrapping GDH onto glassy carbon electrodes that had been pretreated with MWCNTs (procedure above). Hydrophobically-modified

Nafion (tetrabutylammonium bromide-modified Nafion, TBAB-Nafion) was prepared as previously reported.[3] GDH was prepared in water (100 µL, 10 mg mL^{-1}) followed by the addition of 5 µL TBAB-Nafion and 10 µL of this mixture was deposited onto the electrode surface and dried under positive airflow. Cyclic voltammograms are reported within as **Supplementary Figure 18a-c**.

Bilirubin oxidase bioelectrodes

Bilirubin oxidase (BOx) bioelectrodes were prepared on carbon paper (Toray) as previously reported.[4] Anthracene-modified MWCNTs (7.5 mg) were added to BOx (75 µL, 20 mg mL^{-1}, pH 6.5 citrate/phosphate buffer, 0.2 M) and mixed by successive vortex mixing/sonication steps. TBAB-Nafion (25 µL) was added and one more vortex/sonication mixing step was performed. This mixture was then evenly divided between three electrodes that had been cut to have geometric surface areas of 1 cm^2. For carbon felt electrodes with 1cm^2 facial geometric areas, this procedure was scaled up: 37.5 mg of anthracene-modified MWCNTs, 750 µL of BOx (10 mg mL^{-1}) and 250 µL of TBAB-Nafion were combined. Aliquots of this mixture (330 µL) were then applied to each carbon felt electrode. All electrodes were dried under positive airflow.

Specific activities of GDH, as observed by naphthoquinone

1,2-naphthoquinone-4-sulfonic acid (sodium salt) was used to report the specific activities of GOx and GDH from different commercial sources. A molar extinction coefficient for oxidized 1,2-naphthoquinone-sulfonic acid was determined to be 111.1 M^{-1} cm^{-1} at pH 6.5 (citrate/phosphate buffer, 0.05 M) at 500 nm. GOx and BSA control experiments did not exhibit enzymatic activity.

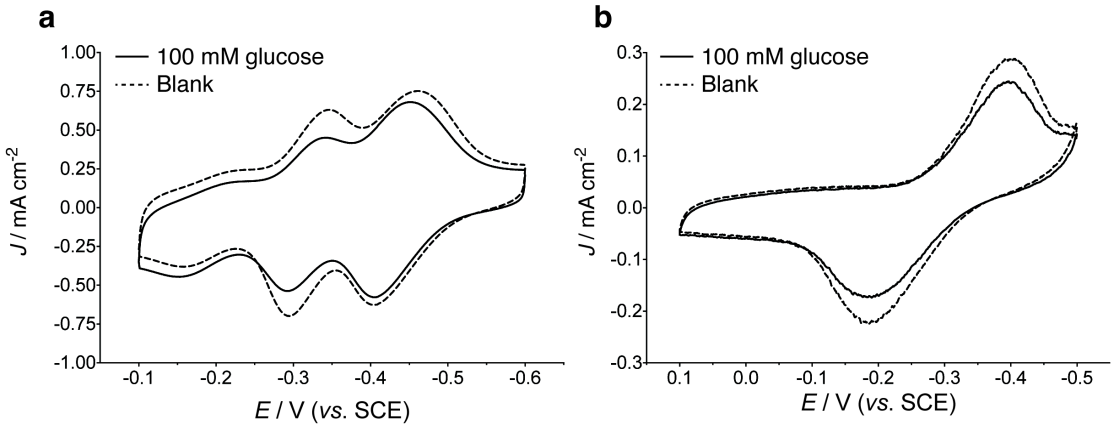
Enzymatic Fuel Cell Testing

Enzymatic fuel cell performance was evaluated galvanostatically by continually drawing current from the EFC at a slow gradient, until the potential difference of the EFC reached 0 V (short circuit). For NQ-4-LPEI GDH EFCs, currents were drawn with a ramp of 1 µA s^{-1}; EFCs based on NQ-GDH were evaluated at 0.1 µA s^{-1} do to their lower uncorrected anodic surface area. Blank EFCs demonstrated insignificant contributions from this technique.

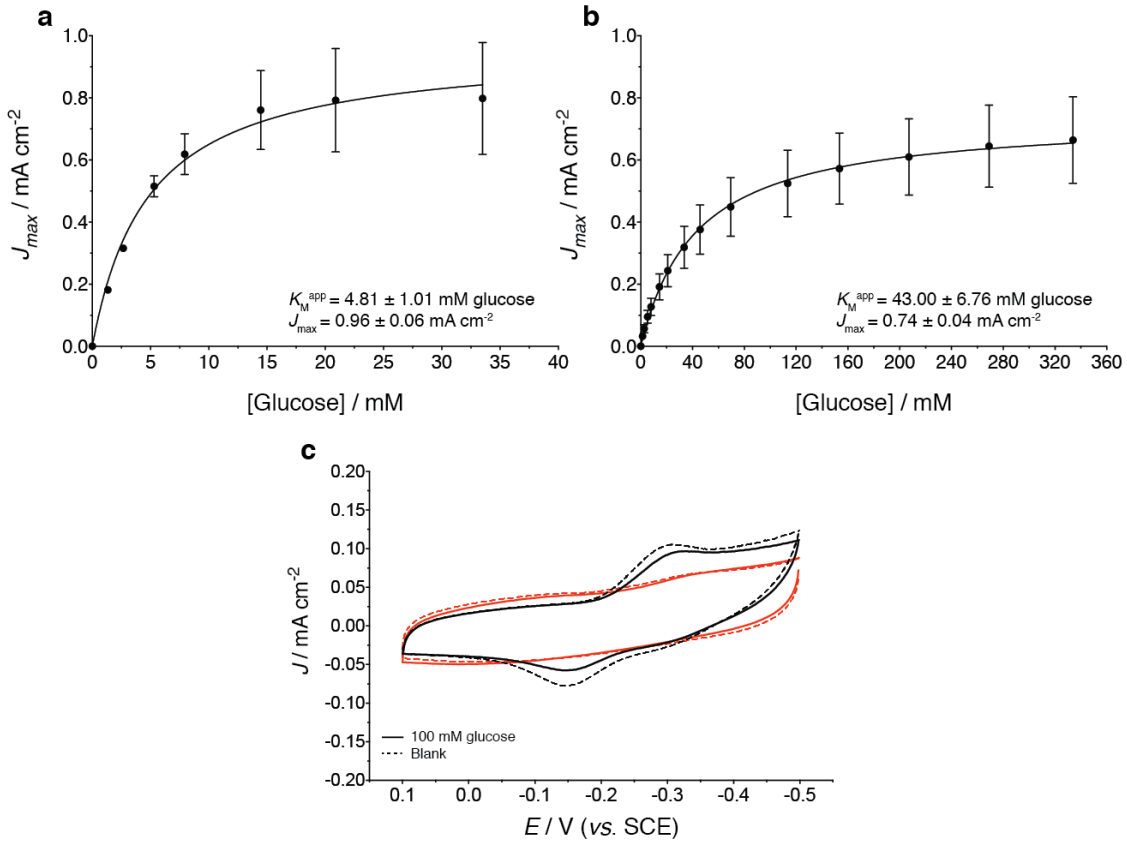
Confocal Laser Scanning Microscopy

Confocal laser fluorescence scanning microscopy was performed using a Nikon A1R confocal scanning laser fluorescence microscope, which was used to estimate the thickness of the NQ-4-LPEI redox hydrogel polymer. NQ-4-LPEI redox hydrogel polymer aliquots were prepared on ITO electrodes with the inclusion of 1 mM fluorescein as a fluorescent probe (excited at 488 nm), and electrochemically-conditioned prior to fluorescence experiments (cyclic voltammetry at 50 mV s^{-1}). Scanning in the z-direction at slices of 10 µm afforded an estimated film thickness of 210 µm, whereby slices that were of over 20% of the maximum observed intensity were designated as significant contributions towards the determination of the polymer film thickness.

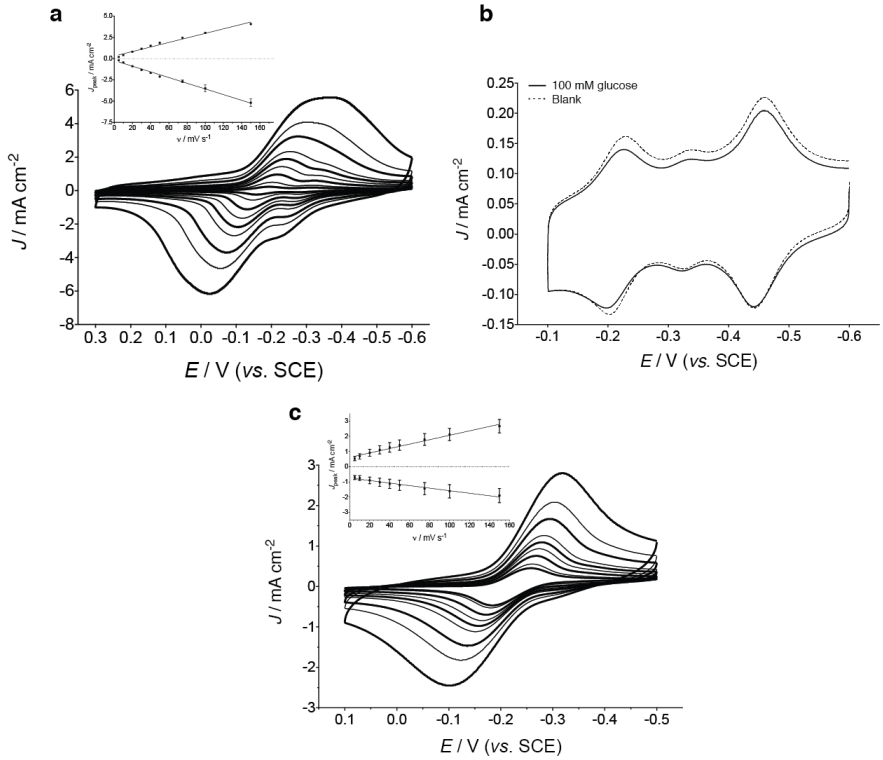
References
1. M. T. Meredith, D. Y. Kao, D. Hickey, D. W. Schmidtke and D. T. Glatzhofer, *J. Electrochem. Soc.*, 2011, **158**, B166-B174.
2. M. J. Moehlenbrock, M. T. Meredith and S. D. Minteer, *MRS Commun.*, 2011, **1**, 37-40.
3. T. Klotzbach, M. Watt, Y. Ansari and S. D. Minteer, *J. Membr. Sci.*, 2006, **282**, 276-283.
4. R. D. Milton, F. Giroud, A. E. Thumser, S. D. Minteer and R. C. T. Slade, *Chem. Commun.*, 2014, **50**, 94-96.



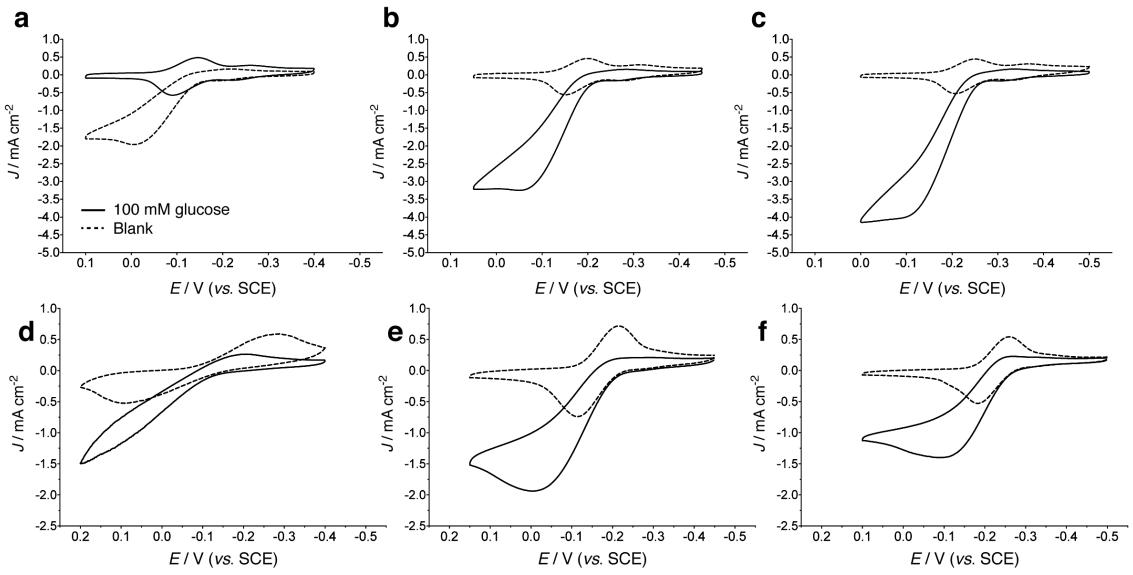
Supplementary Figure 1 – (**a**) Cyclic voltammograms of NQ-2-LPEI GDH bioelectrodes in the absence and presence of glucose, performed at a scan rate of 10 mV s^{-1} in citrate/phosphate buffer at pH 7.4. Electrodes were prepared on carbon paper (Toray) that was pretreated with carbon nanotubes. (**b**) Cyclic voltammograms of NQ-2-GDH bioelectrodes in the absence and presence of glucose, performed at a scan rate of 10 mV s^{-1} in citrate/phosphate buffer at pH 7.4. Electrodes were prepared on glassy carbon electrodes that were pretreated with carbon nanotubes.



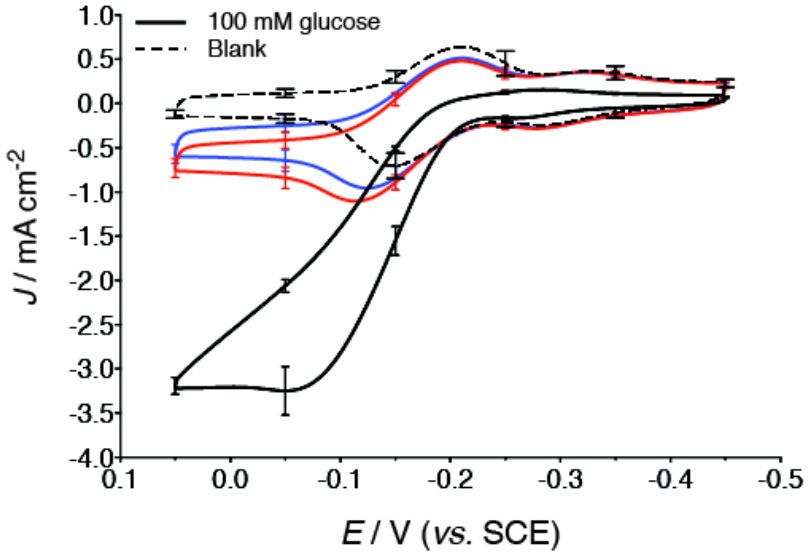
Supplementary Figure 2 – (**a**) Apparent Michaelis-Menten kinetics of NQ-4-LPEI GDH bioelectrodes, measured at 0 V (*vs.* SCE) at pH 6.5 (citrate/phosphate buffer, 0.2 M). Bioelectrodes were prepared on glassy carbon electrodes that were pretreated with carbon nanotubes. Experiments were performed in triplicate and errors bars represent one standard deviation. (**b**) Apparent Michaelis-Menten kinetics of NQ-4-GDH bioelectrodes, measured at 0 V (*vs.* SCE) at pH 6.5 (citrate/phosphate buffer, 0.2 M). Bioelectrodes were prepared on glassy carbon electrodes that were pretreated with carbon nanotubes. Experiments were performed in triplicate and errors bars represent one standard deviation. (**c**) Cyclic voltammograms of NQ-4-GDH control bioelectrodes in the absence and presence of glucose, performed at a scan rate of 10 mV s^{-1} in citrate/phosphate buffer at pH 7.4. Control bioelectrodes were prepared with unlabeled GDH (red) and bovine serum albumin (BSA) that had been labeled with NQ (NQ-4-BSA). Electrodes were prepared on glassy carbon electrodes that were pretreated with carbon nanotubes.



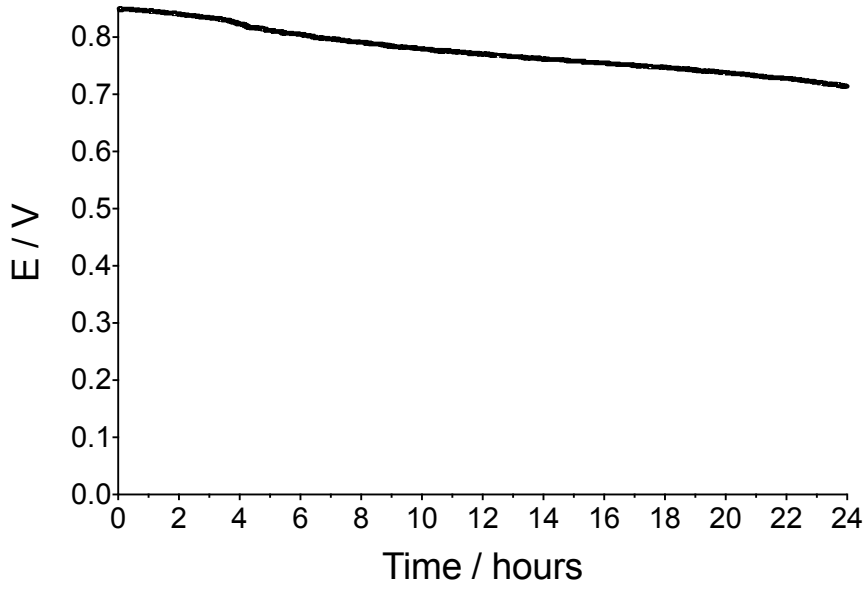
Supplementary Figure 3 – (**a**) Cyclic voltammograms of NQ-4-LPEI GDH bioelectrodes in citrate/phosphate buffer at pH 6.5, at scan rates between 5 and 150 mV s^{-1}. Experiments were performed in triplicate and error bars represent one standard deviation. The inset presents the dependence of peak current densities to scan rate. Electrodes were prepared on carbon paper (Toray) that was pretreated with carbon nanotubes. (**b**) Cyclic voltammograms of a NQ-LPEI GDH hydrogel prepared from the side reaction between 2-methoxy-1,4-naphthoquinone and LPEI without any additional reagents. Cyclic voltammograms were performed at 10 mV s^{-1} at pH 7.4 (citrate/phosphate buffer, 0.2 M) in the absence (dashed) and presence (solid) of 100 mM glucose. (**c**) Cyclic voltammograms of NQ-4-GDH bioelectrodes in citrate/phosphate buffer at pH 6.5, at scan rates between 5 and 150 mV s^{-1}. Experiments were performed in triplicate and error bars represent one standard deviation. The inset presents the dependence of peak current densities to scan rate. Electrodes were prepared on glassy carbon electrodes that were pretreated with carbon nanotubes.



Supplementary Figure 4 – (**a-c**) Cyclic voltammograms of NQ-4-LPEI GDH bioelectrodes in citrate/phosphate buffer at pH 5.5[a], 6.5[b] and 7.4[c], at a scan rate of 10 mV s^{-1}. Experiments were performed in triplicate and error bars represent one standard deviation. Electrodes were prepared on carbon paper (Toray) that was pretreated with carbon nanotubes. (**d-f**) Cyclic voltammograms of NQ-4-GDH bioelectrodes in citrate/phosphate buffer at pH 5.5[d], 6.5[e] and 7.4[f], at a scan rate of 10 mV s^{-1}. Experiments were performed in triplicate and error bars represent one standard deviation. Electrodes were prepared on glassy carbon electrodes that were pretreated with carbon nanotubes.

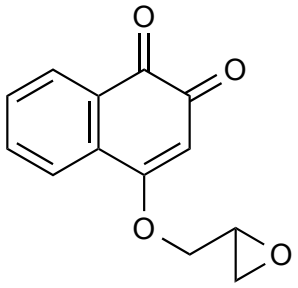


Supplementary Figure 5 – Cyclic voltammograms of NQ-4-LPEI GDH bioelectrodes prepared with GDH obtained from Sekisui Diagnostics UK ("Sekisui", black lines), Amano Enzyme Inc ("Amano", Japan, red lines) and BBI Solutions ("BBI", USA, blue lines). Sekisui GDH and Amano GDH were obtained from *Aspergillus sp.*, whereas BBI GDH was isolated from *Aspergillus oryzae*. All electrochemical experiments were performed at pH 6.5 (citrate/phosphate buffer, 0.2 M). Error bars represent one standard deviation (n = 3). Cyclic voltammograms were performed at 10 mV s^{-1}. Electrodes were prepared on carbon paper electrodes (Toray) that were pretreated with carbon nanotubes. Specific activities for Amano GDH and BBI GDH to 1,2-naphthoquinone-4-sulfonate were determined to be 589.6 ± 31.2 U mg^{-1} and 401.5 ± 4.9 U mg^{-1}, respectively, at pH 6.5 (experimental outlined below).



Supplementary Figure 6 – Stability as a function of potential difference over time for an EFC from which 10 μA cm^{-2} was continuously drawn. The EFC was operated in quiescent citrate/phosphate buffer (pH 6.5, 0.2 M) containing 100 mM glucose, with no additional gas purging.

Characterization of NQ derivatives

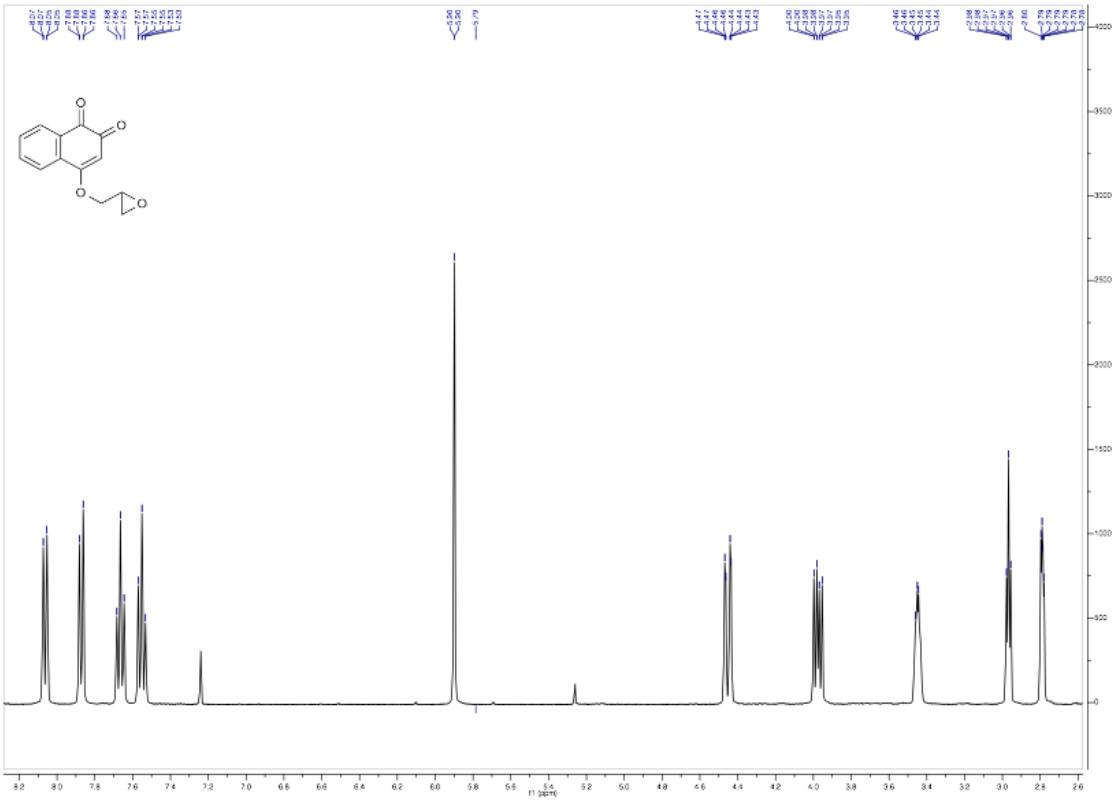


Supplementary Figure 6 - 1,2-naphthoquinone-4-glycidol

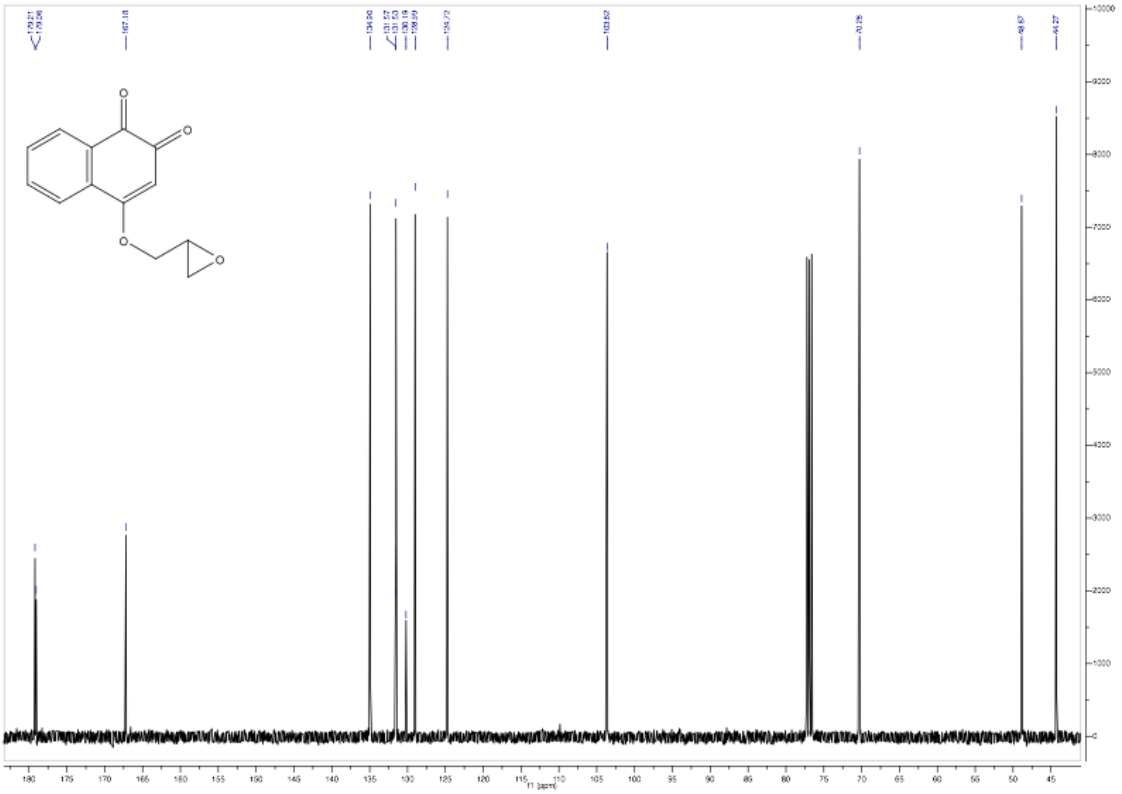
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.6 Hz, 1H, -CH), 7.87 (d, *J* = 7.8 Hz, 1H, -CH), 7.66 (t, *J* = 7.6 Hz, 1H, -CH), 7.55 (t, *J* = 7.6 Hz, 1H, -CH), 5.90 (s, 1H, -CH), 4.45 (dd, *J* = 11.3, 2.1 Hz, 1H, -O-CH_2-), 3.97 (dd, *J* = 11.3, 6.4 Hz, 1H, -O-CH_2-), 3.48 – 3.40 (m, 1H, -CH- epoxy), 2.97 (t, *J* = 4.4 Hz, 1H, -CH_2- epoxy), 2.79 (dd, *J* = 4.5, 2.5 Hz, 1H, -CH_2- epoxy).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.21 (C=O), 179.06 (C=O), 167.18 (C-O), 134.90 (CH), 131.57 (C), 131.53 (CH), 130.19 (C), 128.99 (CH), 124.72 (CH), 103.62 (CH), 70.28 (-O-CH_2), 48.87 (epoxy CH), 44.27 (epoxy CH_2).

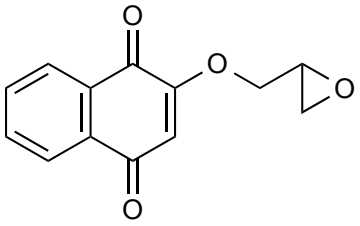
ESI-MS (High Resolution): 253.0477 $[M+Na]^+$.



Supplementary Figure 7 - ^{1}H NMR of 1,2-naphthoquinone-4-glycidol



Supplementary Figure 8 - ^{13}C NMR of 1,2-naphthoquinone-4-glycidol

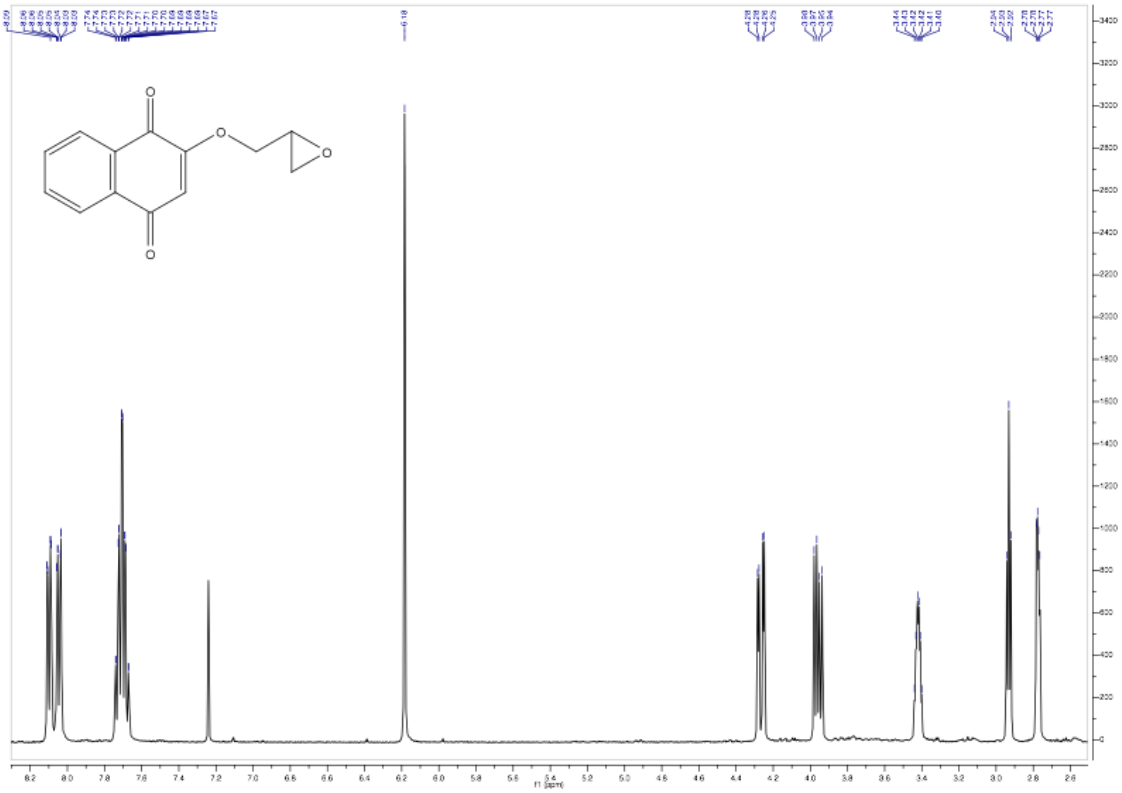


Supplementary Figure 9 - 1,4-naphthoquinone-2-glycidol

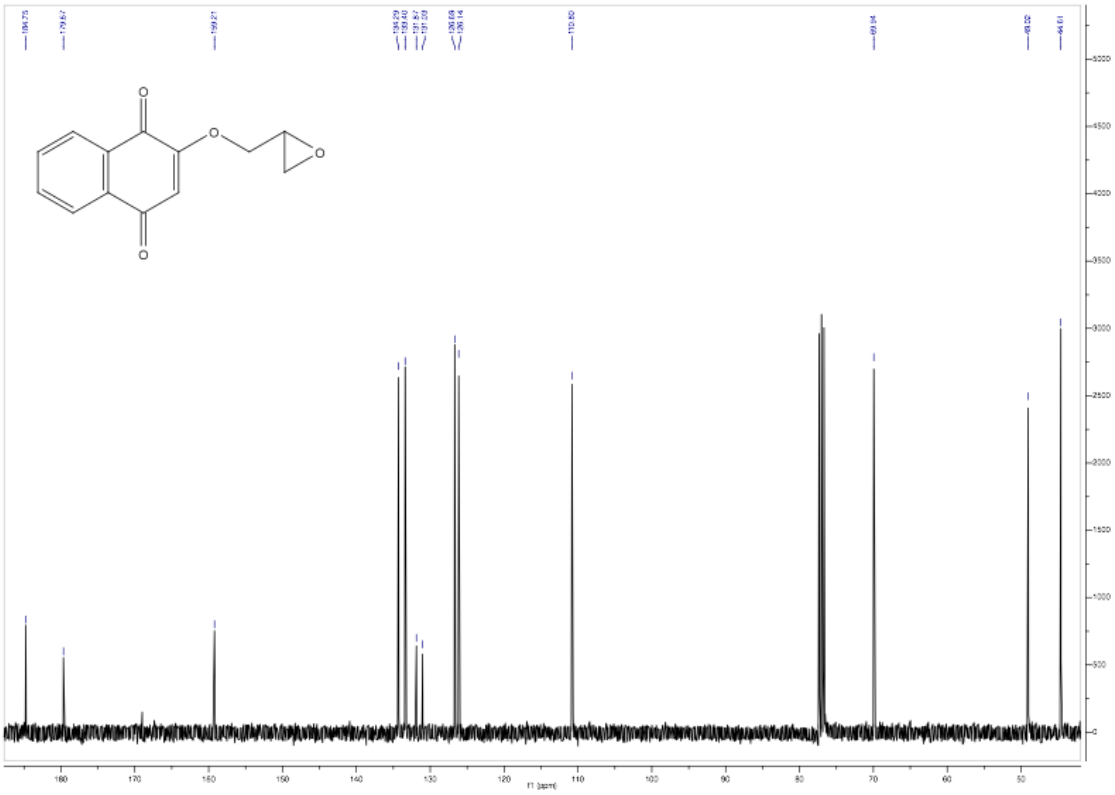
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 7.99 (m, 2H, -CH), 7.78 – 7.62 (m, 2H, -CH), 6.18 (s, 1H, -CH), 4.27 (dd, *J* = 11.4, 3.0 Hz, 1H, -O-CH_2-), 3.96 (dd, *J* = 11.4, 5.8 Hz, 1H, -O-CH_2-), 3.42 (td, *J* = 6.3, 3.0 Hz, 1H, -CH epoxy), 2.93 (t, *J* = 4.4 Hz, 1H, -CH_2- epoxy), 2.77 (dd, *J* = 4.7, 2.6 Hz, 1H, -CH_2- epoxy).

^{13}C NMR (101 MHz, $CDCl_3$) δ 184.75 (C=O), 179.67 (C=O), 159.21 (C-O), 134.29 (CH), 133.40 (CH), 131.87 (C), 131.03 (C), 126.69 (CH), 126.14 (CH), 110.80 (CH), 69.94 (-O-CH_2-), 49.02 (epoxy CH), 44.61 (epoxy CH_2).

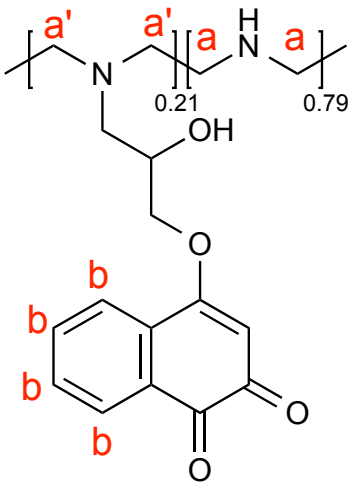
ESI-MS (High Resolution): 253.0482 $[M+Na]^+$.



Supplementary Figure 10 - ^{1}H NMR of 1,4-naphthoquinone-2-glycidol

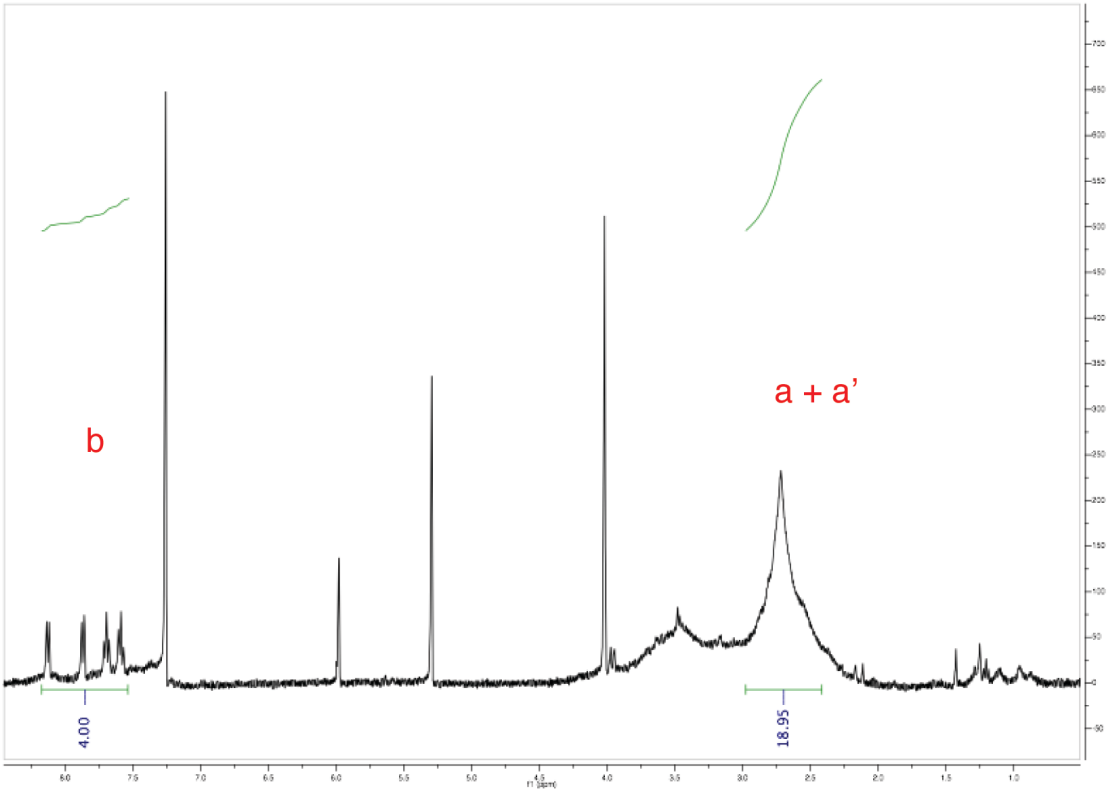


Supplementary Figure 11 - ^{13}C NMR of 1,4-naphthoquinone-2-glycidol



Supplementary Figure 12 - 1,2-naphthoquinone-4-glycidyl-LPEI (NQ-4-LPEI)

The substitution ratio of NQ to LPEI confirmed by ^{1}H NMR of NQ-4-LPEI.



Supplementary Figure 13 - ^{1}H NMR of NQ-4-LPEI ($CDCl_3$, 400 MHz)

$$\%\ substitution = \left(\frac{b}{a + a'}\right) \times 100 = \left(\frac{4}{18.95}\right) \times 100$$

Substitution = approximately 21%.

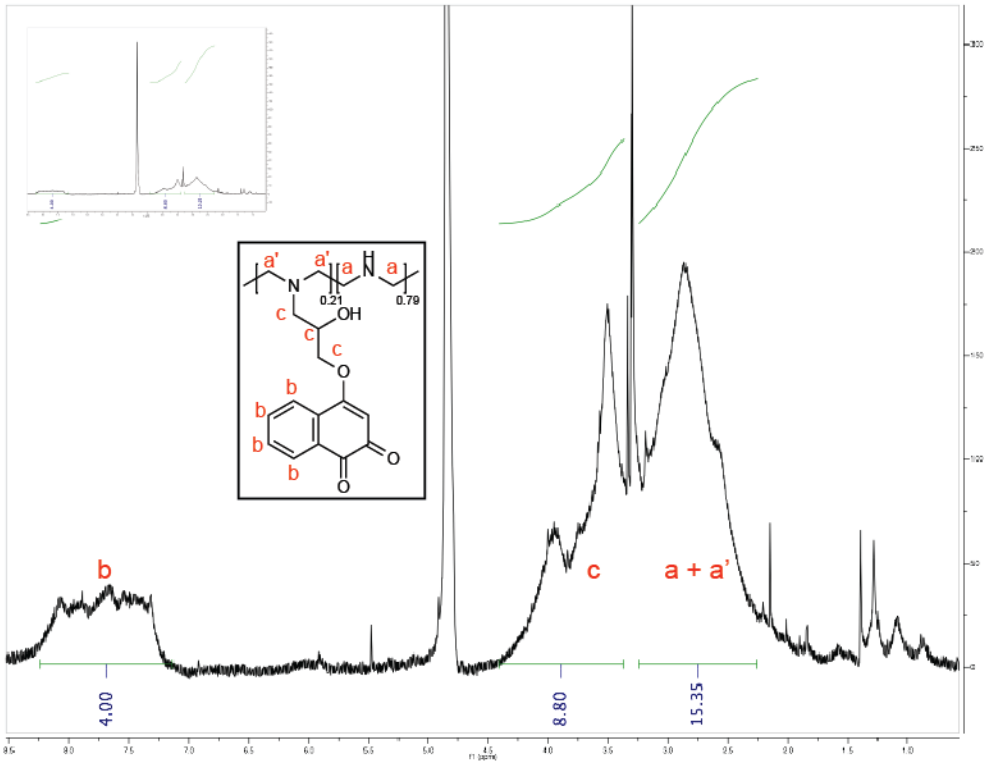
$$\text{Average molecular weight} =$$

$$\frac{(\%\ \text{units}_{\text{substituted}} \times \text{mol. weight of unit}) + (\%\text{units}_{\text{unsubstituted}} \times \text{mol. weight of unit})}{100}$$

$$= \frac{(21 \times 273.22) + (79 \times 43)}{(100)}$$

$$= 91.3\ \text{g mol}^{-1}$$

Initial NQ-4-GDH polymer characterization performed in deuterated chloroform resulted in well-resolved naphthoquinone peaks, although an anomalous peak was identified at ~4 ppm which was deemed too sharp in resolution to be part of the polymer backbone. As a secondary measure, the sample was dried and ^{1}H NMR was performed in deuterated methanol. This method resulted in lower peak resolution but confirmed NQ substitution of LPEI at the same ratio.



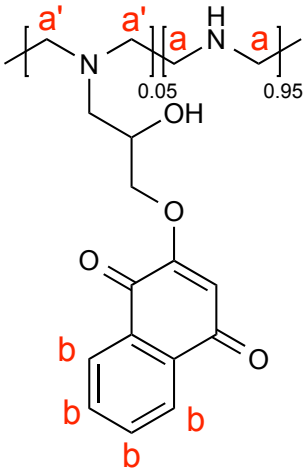
Supplementary Figure 14 - ^{1}H NMR of NQ-4-LPEI (CD_3OD, 300 MHz).

where:

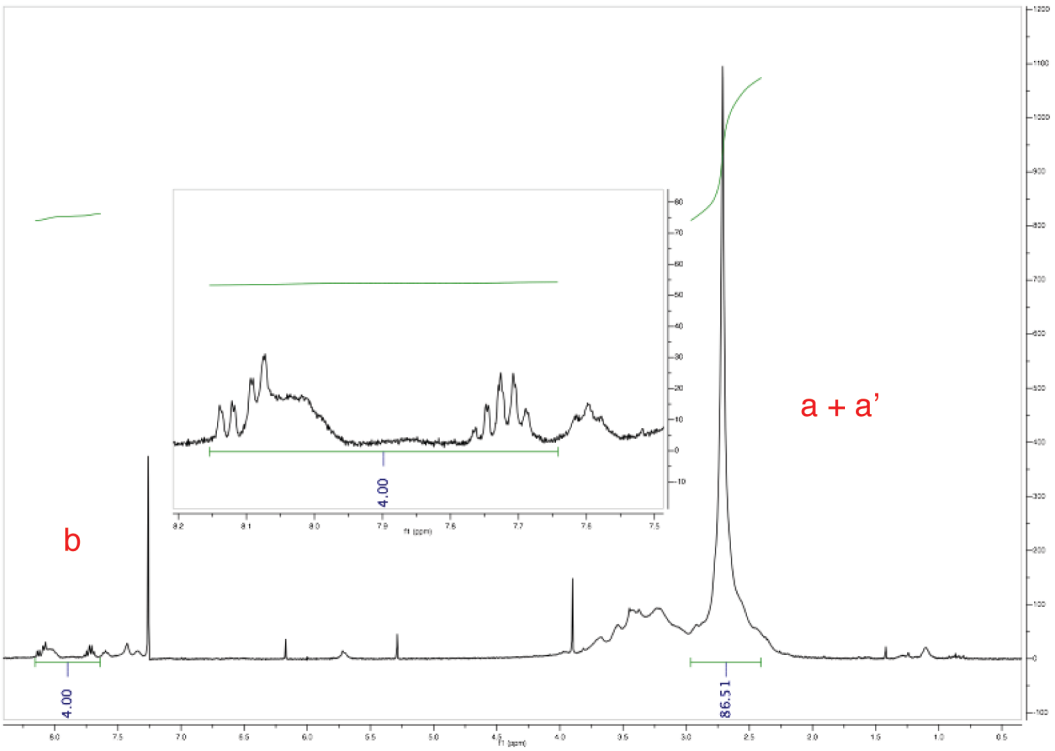
$$\%\ substitution = \left(\frac{b}{((a + a') + c) - 5}\right) \times 100 = \left(\frac{4}{19.15}\right) \times 100$$

Substitution = approximately 21%.

note = "5" accounts for 5 protons immediately adjacent to the LPEI backbone.



Supplementary Figure 15 - 1,4-naphthoquinone-2-glycidyl-LPEI (NQ-2-LPEI)

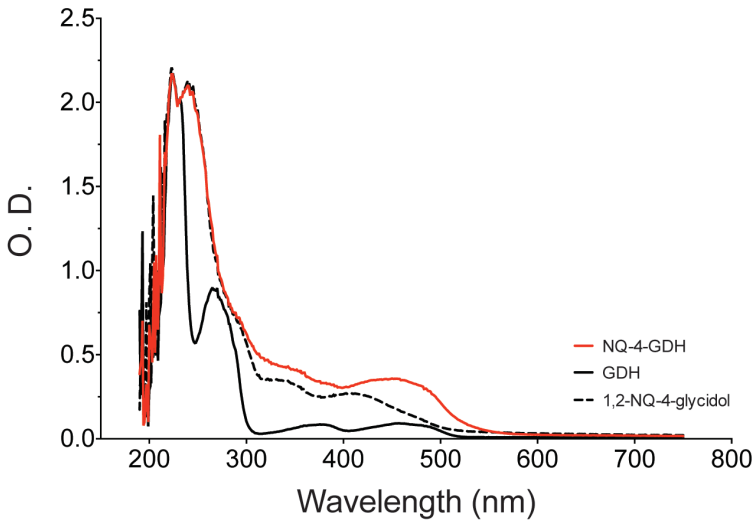


Supplementary Figure 16 - ^{1}H NMR of NQ-2-LPEI

Substitution = approximately 5%.

Average molecular weight = 53.6 g mol^{-1}.

Characterization and quantification of NQ-GDH labeling



Supplementary Figure 17 – Absorbance profiles of GDH, NQ-4-GDH and NQ-4-glycidol (**1**), in citrate/phosphate buffer (pH 6.5, 0.05 M). GDH was evaluated at 2 mg mL^{-1}. GDH-4-NQ was evaluated at 2 mg mL^{-1}. NQ-4-glycidol (**Compound 1**) was evaluated at 0.1 mM.

UV-Vis profiles of GDH, NQ-4-GDH and 1,2-naphthoquinone-4-glycidol determined that UV-Vis spectroscopy couldn't be effectively used to quantify the ratio of NQ to GDH.

Centrifugation to remove all unbound NQ from GDH yielded a suspension of protein modified with NQ. It was hypothesized that low solubility of NQ in water was responsible for protein precipitation. Thus, a bicinchoninic acid assay (BCA assay) was used to determine the presence of protein within the supernatant and precipitate of the suspension (Table 1).

Table 1 – Protein concentration of NQ-4-GDH

NQ-4-GDH	Concentration (mg ml^{-1})
Precipitate	1.71 ± 0.03
Supernatant	27.4 ± 1.9

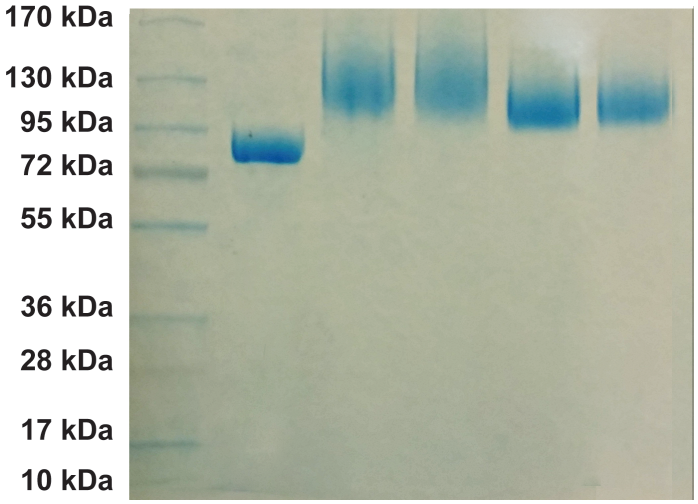
The BCA only provides an estimation of the protein concentration, since the BCA reagent is expected to quantify protein concentration by reacting with the same amino acid residues that are expected to be modified (partially) by 1,2-naphthoquinone-4-glycidol.

The specific enzymatic activities of enzyme within the supernatant and the precipitate of the suspension were also investigated, to confirm the presence of active protein within the precipitate (normalized to the previously determined protein concentration in both fractions. Dichloro*indo*phenol was used to report specific activities (Table 2).

Table 2 - Specific enzymatic activity of NQ-4-GDH

GDH	Specific activity (U mg^{-1})
GDH (unmodified)	748.17 ± 22.49
NQ-4-GDH (precipitate)	97.93 ± 5.72
NQ-4-GDH (supernatant)	251.48 ± 19.59

SDS-PAGE was also investigated as a possible procedure to probe any change in molecular weight of GDH that had been modified with NQ (**Supplementary Fig. 18**).



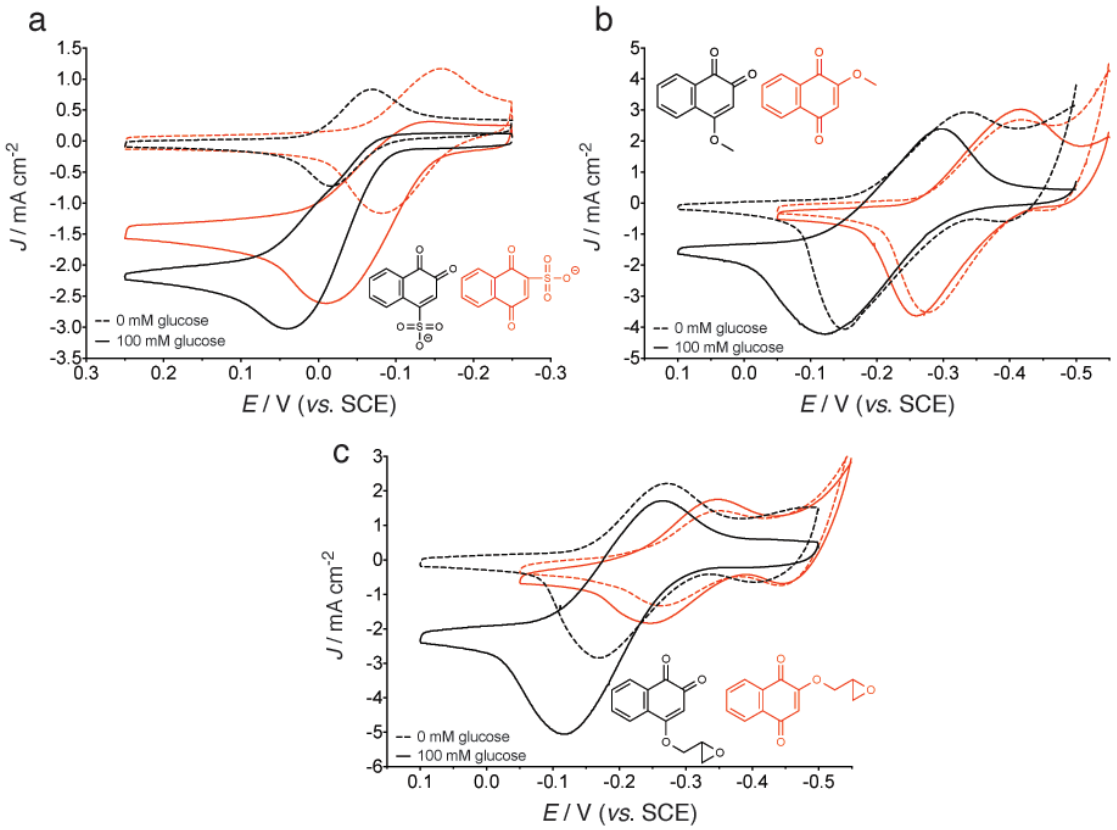
Supplementary Figure 18 – SDS-PAGE (10%) of (1) GOx, (2) "Amano" GDH, (3) "BBI" GDH, (4) "Sekisui" GDH and (5) NQ-4-GDH (NQ-labeled "Sekisui" GDH).

Analysis of the gel obtained from SDS-PAGE demonstrates a difference in molecular weight of GOx compared to all GDH samples. Further, it may also be possible to observe a small change in migration can be observed between crude GDH (lane 4) and NQ-4-GDH (lane 5).

Specific enzymatic activity of glucose oxidase

The specific enzymatic activity of GOx was also monitored by following the production of H_2O_2 with a peroxidase/ABTS assay and determined to be 324.6 4 ± 33.6 U mg^{-1} (pH 6.5, citrate phosphate buffer, 0.05 M).

Electrochemistry and mediated bioelectrocatalysis of NQ derivatives



Supplementary Figure 19 – (**a**) Cyclic voltammograms of GDH-modified glassy carbon electrodes in 2 mM naphthoquinone sulfonated derivatives with 0 and 100 mM glucose, operating at a scan rate of 10 mV s^{-1} in pH 7.4 buffer (citrate/phosphate, 0.2 M). GDH bioelectrodes were prepared as reported within the online methods of the main article. (**b**) Cyclic voltammograms of GDH-modified glassy carbon electrodes with 0 and 100 mM glucose, operating at a scan rate of 10 mV s^{-1} in pH 7.4 buffer (citrate/phosphate, 0.2 M). GDH bioelectrodes were prepared as reported within the online methods of the main article, although the electrodes were modified with the respective methoxy-naphthoquinone prior to the addition of GDH. The respective NQ was dissolved in dichloromethane (10 mg mL^{-1}) and a 5 μL aliquot was applied to the MWCNTs-modified glassy carbon electrode. (**c**) Cyclic voltammograms of GDH-modified glassy carbon electrodes with 0 and 100 mM glucose, operating at a scan rate of 10 mV s^{-1} in pH 7.4 buffer (citrate/phosphate, 0.2 M). GDH bioelectrodes were prepared as reported within the online methods of the main article, although the electrodes were modified with the respective epoxy-naphthoquinone (1,2-naphthoquinone-4-glycidol and 1,4-naphthoquinone-2-glycidol) prior to the addition of GDH. The respective NQ was dissolved in dichloromethane (10 mg mL^{-1}) and a 5 μL aliquot was applied to the MWCNTs-modified glassy carbon electrode.