Electronic Supplementary Information for:

NAD-dependent dehydrogenase bioelectrocatalysis: the ability of a naphthoquinone redox polymer to regenerate NAD^+.

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Experimental

Materials

Unless specified otherwise, chemical supplies were purchased from Sigma Aldrich and used without any additional purification. NAD-GDH (E.C.L 1.1.1.47) was purchased from Toyobo USA Inc. (*Bacillus sp.,* recombinantly-expressed in *E. coli*) and used without any additional purification. Laccase (*Trametes versicolor*, E.C.: 1.10.3.2) was purchased from Sigma Aldrich and used without any additional purification. 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, -COOH functionalized) were purchased from Cheap Tubes, USA. Glassy carbon (GC) working electrodes (3 mm diameter) and saturated calomel (SCE) reference electrodes were purchased from CH Instruments, Inc.

Synthesis of naphthoquinone redox polymer

The naphthoquinone-functionalized linear poly(ethylenimine) redox polymer (1,2-naphthoquinone-4-glycidyl-LPEI, NQ-LPEI) was synthesized as previously reported.[7] Briefly, diisopropylethylamine (DIPEA, 1.2 mol. eq., 179 mg, 1.4 mmol) was added drop-wise to glycidol ((±)-glycidol, 10 mL, 151 mmol) and stirred at room temperature for 10 minutes. Next, 1,2-naphthoquinone sulfonic acid sodium salt (1 mol. eq., 300 mg, 1.15 mmol) was added slowly and stirred for an additional 10 minutes, to yield an epoxide-functionalized naphthoquinone derivative. Multiple extractions were performed with dichloromethane against a saturated brine solution, until the organic phase was essentially colorless. The organic fractions were combined and further extractions with chloroform against water were performed. These steps are necessary to remove most of the unreacted naphthoquinone, glycidol and DIPEA. The combined organic fractions were dried over magnesium sulfate and purified over silica gel with a diethylether:dichloromethane gradient. The purified product had an approximate molecular yield of ~24%.

LPEI (4 mol. eq.) was synthesized as previously reported, combined with the epoxide-functionalized naphthoquinone (1 mol. eq.) in stirred methanol (~15 mL) and stirred overnight at room temperature.[22] The solvent was removed *in vacuo* and ethyl acetate was used to wash any residual naphthoquinone precursor from the naphthoquinone-functionalized LPEI.

NQ-LPEI/NAD-GDH bioelectrodes

NQ-LPEI (84 µL, 10 mg mL^{-1} in water), NAD-GDH (36 µL, 10 mg mL^{-1} in water) and EGDGE (4.5 µL, 10% v/v in water) were vortex-mixed and 3 µL of this mixture was

applied to each GC working electrode that had previously been modified with 5 µL of a MWCNTs suspension (5 mg mL^{-1} in isopropanol, sonicated for 1 hour). For experiments performed on Toray paper electrodes, 10 µL of the NQ-LPEI/NAD-GDH/EGDGE mixture was applied to an electrode with a geometric surface area of 0.25 cm^2 (treated with paraffin wax) that had been pre-coated with 18 µL of the above MWCNTs suspension. Electrodes were dried for 2 hours under positive airflow at room temperature, and briefly rinsed immediately prior to testing.

Laccase biocathodes

Laccase biocathodes were prepared on Toray carbon paper, as previously reported.[23] Anthracene-modified MWCNTs (Ac-MWCNTs) were used to orientate the T1 copper centre of laccase towards the electrode architecture, affording direct electron transfer and direct bioelectrocatalytic reduction of O_2 to H_2O. Briefly, laccase (75 µL, 20 mg mL^{-1} in citrate/phosphate buffer (0.2 M, pH 5.5)) was added to Ac-MWCNTs (7.5 mg) and mixed by successive vortex/sonication steps. Tetrabutylammonium bromide-modified Nafion was added (25 µL, 20 mg mL^{-1}) and mixed once more by vortex/sonication. The resulting suspension was divided between 3x Toray paper electrodes with geometric surface areas of 1 cm^2 and dried under positive airflow at room temperature for 2 hours.

Enzymatic activity assays

Enzyme (10 U) was added to mixtures containing different concentration of substrate (from 0 to 100 mM of glucose) and 10 mM NAD^+ in 50 mM citrate-phosphate buffer pH 6.5 in a volume of 200 µL. The production of NADH was monitored at 340 nm (ε = 6220 M^{-1} cm^{-1}). The assay was also performed by monitoring the oxidation of 1,2-Naphthoquinone-4-sulfonic (NQS) by NADH at 450 nm (ε = 12000 M^{-1} cm^{-1}) in 50 mM citrate-phosphate buffer pH 6.5 in a volume of 200 µL. These assays were carried out with 0.5 mM NQS. For all assays, the initial rates of enzyme reaction were expressed in 1 µmol of substrate transformed in 1 min, which is defined as 1U.

EFC evaluation

EFCs prepared by combining NQ-LPEI/NAD-GDH bioelectrodes (as bioanodes prepared on 0.25 cm^2 Toray paper electrodes) with laccase biocathodes (prepared on 1 cm^2 Toray paper electrodes) were evaluated in citrate/phosphate buffer (0.2 M, pH 5.5) containing glucose (200 mM) and NAD^+ (10 mM). To avoid crossover of NAD^+/NADH at the biocathode, the anodic and cathodic chambers were separated by a Nafion membrane (NRE-212) that was preconditioned by soaking in 1 M H_2SO_4 overnight. EFC performance was determined galvanostatically by slowly ramping current drawn from the EFC (0.1 µA s^{-1}) until short-circuit.

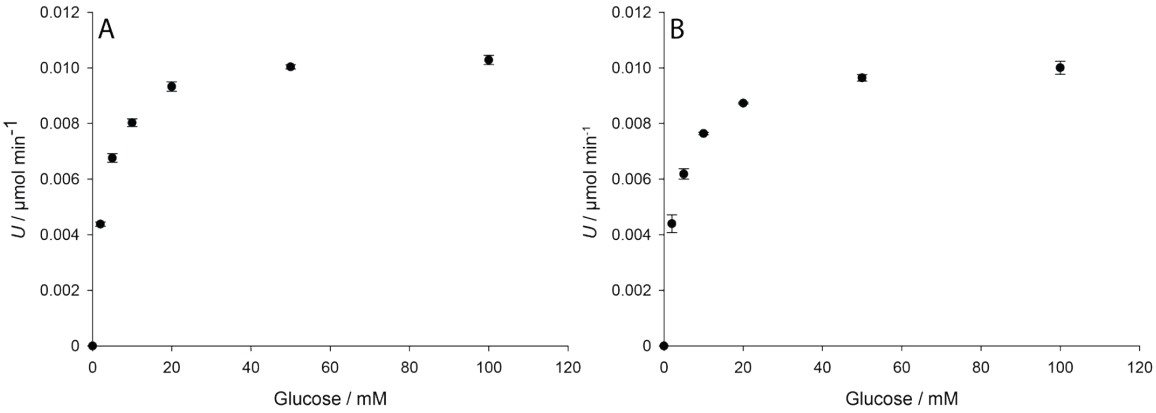

Figure S1 – Michaelis-Menten kinetics at pH 6.5 of NAD-GDH obtained by monitoring NADH production at 340 nm (A) and NQS reduction at 450 nm (B).