Electronic Supplementary Information for:

Adaptive Nanowire-Nanotube Systems for On-Demand Bioelectrocatalytic Transformations

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I. Apparatus.

Voltammetric and amperometric measurements were performed using the µAutolab Potentiostat type II (Eco Chemie BV, Utrecht, Netherlands). The electrode assembly involved a 3 mm glassy carbon disk electrode of a thin-layer flow cell (MF-1095, Bioanalytical Systems Inc., W. Lafayette, IN) and a 2-cm high/1.25-cm i.d. cylindrical glass tube. The glass tube was glued onto the flow cell block, centered around the working electrode, to define the electrochemical cell. An electrical wire, connected to the bottom of the electrode, served as an electrical contact. The reference and counter electrodes were Ag/AgCl(3M KCl) (Model CHI111, CH Instruments, Austin, TX) and a 0.5 mm-diameter platinum wire, respectively. Switching the magnetic orientation of the functionalized Ni-Au-Ni nanowires on the electrode surface was achieved by rotating an external cube-shape

NdFeB/Ni-coated magnet (7/16"x7/16"x7/16", 12.4 kG) by 90 degree under the electrode.

II. Reagents.

Mercapto-succinic acid (MSA), 3-aminopropyl-triethoxysilane (APTES), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), β -nicotinamide adenosine dinucleotide sodium salt (NAD⁺), β nicotinamide adenine dinucleotide reduced disodium salt (NADH), hydrogen peroxide, dimethylformamide (DMF), sodium bicarbonate, polyethyleneimine (PEI). dehydrogenase (ADH alcohol from Saccharomyces cerevisiae, 314 U/mg, pI 5.4-5.8, K_M (ethanol) = 2.1mM) and alcohol oxidase (AOX from Candida boidinii, 1.55 U/mg, pI 5.4-5.8, K_{M} (methanol) = 3 mM) were obtained from Sigma-Aldrich. Ethanol (absolute 200 proof) was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY), methanol and nitric acid were recieved from EMD Chemicals Inc. (Gibbstown, NJ) and acetic acid was obtained from Mallinckrodt Baker, Inc (Phillipsburg, NJ). The phosphate buffer solution (0.1 M PBS, pH 7.4) was prepared by mixing proper amounts of monobasic and dibasic potassium phosphate (Aldrich) with nano-pure water (18.2 M Ω). All aqueous solutions were prepared using the phosphate buffer.

The gold plating solution (Orotemp 24 RTU) for the nanowires synthesis was obtained from Technic Inc. (Cranston, RI), while the copper and nickel plating solutions were prepared using $CuSO_4 \cdot 5H_2O$ and $NiCl_2 \cdot 6H_2O - Ni(H_2NSO_3)_2 \cdot 4H_2O - H_3BO_3$, respectively (obtained from Aldrich). Anodisc alumina membranes, with a pore size of 200 nm and thickness of 60 µm, were purchased from Whatman (Catalog no. 6809-6022; Maidstone, U.K.). Multiwall carbon nanotubes (CNT; 30±15 nm diameter, 1-5 μ m length) were obtained from Nano-Lab, Inc. (Newton, MA) and were purified prior to use.

III.1 Synthesis and Surface Modification of Nickel/Gold/Nickel Nanowires.

Triple-segment Ni-Au-Ni nanowires (~14 µm long and ~200 nm diameter) were prepared electrochemically using a membrane-template synthesis method.¹ The desired length of each of the three segments was deposited successively into the nanopores of the alumina membrane template via an electrochemical process, by controlling the electrodeposition charge. A gold film was first sputtered on one side of the template to serve as a working electrode for the electrochemical growth. The membrane was then assembled in a plating cell where aluminum foil provided the contact for the sputtered gold. An initial copper layer was electrodeposited from a 1 M CuSO₄·5H₂O solution at -1.0 V (vs. Ag/AgCl in connection to a Pt wire counter electrode) using a charge of 10 coulombs (C). Subsequently, nickel was plated at -1.0 V, from a solution containing 20 g L⁻¹ NiCl₂·6H₂O, 515 g L⁻¹ Ni(H₂NSO₃)₂·4H₂O, and 20 g L⁻¹ H₃BO₃ (buffered to pH 3.4) using a charge of 20C. The central gold segment was then plated at -0.9 V from the commercial gold plating solution using a charge of 5C. Finally, another nickel segment was electrodeposited in a manner similar to the preparation of the first nickel segment. After depositing the three (Ni-Au-Ni) segments the sputtered gold layer was removed by mechanical polishing using cotton tips, and the copper layer was dissolved in a 0.5 M CuCl₂·2H₂O solution (in 30% HCl). To ensure a complete separation of the nanowires, the membrane surface was polished (with 3 µm alumina slurry and cotton tips). The number of nanowires per membrane (ca. 2.5×10^9) was estimated based on

the geometric area of the polished membrane and its pores density (ca. 1.0×10^9 pores/cm²). The nanowires were then removed from the template by dissolving the alumina template in an agitated 3M NaOH solution for 30 min. The resulting nanowires were repeatedly washed with water to remove residual base and salts. After the washing step, the nanowires were collected by placing a small magnet on the side of the flask while decanting the water. The enzyme immobilization of the nanowires was performed according to a slightly modified the early procedure.² The Ni-Au-Ni nanowires were suspended in a solution of 3-aminopropyl-triethoxysilane APTES (10 mM in ethanol) and vortexed overnight. This resulted in coverage of the three segments. The nanowires were then washed several times with ethanol and with 0.1M sodium bicarbonate solution; subsequently 1 ml of a 20 mM EDC (in 0.2M acetic acid) solution was added and allowed to react overnight with the amino-terminated group of APTES at 4°C based on carbodiimide coupling reaction. Excess reagents were removed using a phosphate buffer 'wash' solution. To obtain a selective functionalization on Au segment, the APTES layer on that segment was replaced by a mercapto-succinic acid (MSA) coating (0.2M in ethanoic solution). For this purpose the nanowires were vortexed in the MSA solution overnight at room temperature and then washed twice with absolute ethanol and PBS. The resulting carboxylated segment was then functionalized first with polycationic PEI (10 mg/ml in PBS) by vortex mixing for 30 minutes.³ Subsequently, the nanowires were rinsed thoroughly with PBS and were added to a 200 µl of the enzyme solution (0.5 U/µl ADH in PBS or 0.5 U/µl ADH - 0.01 U/µl AOX in PBS) and incubated in the enzyme solution for 2 hours at 4°C. Finally, the enzyme-functionalized Ni-Au-Ni nanowires were washed repeatedly with PBS prior to use. Based on the formula of the maximum surface coverage previously described by Xu et. al.⁴ and the crystallographic data of specific enzymes^{5,6}, we estimated maximum surface coverages of the enzymes on the gold segment to be 0.34 ng/mm² for ADH alone, and 0.07 and 0.52 ng/mm² for ADH and AOX, respectively, in the mixed layer

III.2 Immobilization of CNTs on Glassy Carbon Electrode.

The CNT were first purified by dispersing 100 mg CNT in 100 ml of a concentrated nitric acid and sonicating at 60 °C for 2 hours. The solution was then incubated at 60 °C overnight. After the acid treatment, the CNT were washed repeatedly with nanopure water (18.2 M Ω) until the pH of the solution was neutral. Finally, the purified CNT were dried at 60 °C.⁷ For coating the CNT, 0.5 mg of purified CNT were added into 1 ml of DMF and the mixture was sonicated for 1 hour. A 5 µl droplet of the sonicated solution was placed directly onto a well-polished glassy electrode surface and was allows to dry under nitrogen for 30 min. This results in a surface coverage of ca. 35 µg CNT/cm². The electrode was rinsed thoroughly with DI water before the bioelectrocatalytic detection.

IV. Measurement Procedure.

Measurements were performed in a PB supporting electrolyte medium. Cyclic voltammograms were recorded at 10 mV s⁻¹ under quiescent conditions, with the nanowires in the 'horizontal', 'vertical', or 'off' positions. By positioning the magnet below the corresponding working electrode, the Ni-Au-Ni nanowires were attracted to the CNT modified electrode surface. Amperograms were recorded (usually at +0.45V) in the presence of the substrate and NAD⁺, during cyclic vertical-to-horizontal reorientations of the nanowires. Such switching was accomplished by

rotating the external magnet 90° below the electrode surface. The "off" position corresponded to the external magnet attracting the Ni-Au-Ni nanowires to the upper side wall of the cell, away from the working electrode area. All measurements were performed at ambient temperature (ca. 23 °C).

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