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Polymer-Immobilized, Hybrid Multi-Catalyst Architecture for Enhanced Electrochemical Oxidation of Glycerol

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Figure S5. GC spectra obtained after bulk electrolysis of MWCNT/TEMPO-LPEI/OxDc-modified electrode with glycerol

EXPERIMENTAL SECTION

Protocol S1. Chemicals and Materials

Sodium mesoxalate monohydrate, sodium tartrate dihydrate, sodium phosphate monobasic dihydrate ($NaH_2PO_4 \bullet 2H_2O$) and glycerol were purchased from Sigma (St. Louis, MO), while citric acid monohydrate, hydrochloric acid and sodium hydroxide were purchased from Fisher Scientific (Waltham, MA) and were used as received. 200 mM sodium phosphate buffer (pH = 7.0) and 150 mM citric acid-phosphate buffers (pH = 5.5) were prepared by dissolving the appropriate amounts of salts in ultrapure water (Milli-Q ultrapure water purification system). Ethylene glycol diglycidyl ether (EGDGE) was purchased from Polysciences, Inc. (Warrington, PA) and carboxyl-functionalized multi-walled carbon nanotubes (COOH-MWCNT) from Cheap Tubes Inc. (Cambridgeport, VT). TEMPO-LPEI was synthesized and purified in the lab (*vide infra*), while oxalate decarboxylase (OxDc)) was also expressed and purified in the lab (*vide infra*) and stored in -80°C until use.

Protocol S2. Synthesis and Purification of TEMPO-LPEI

Glycidyl-TEMPO was prepared using a procedure originally published by Song et al. (±)-epichlorohydrin (2.69 g, 0.032 mol) was dissolved into a solution of 50% wt/wt aqueous NaOH (10 mL) containing tetrabutylammonium hydrogen sulfate (0.09 g, 4 mol%). To the stirring mixture was added 4-hydroxy-TEMPO (1.00 g, 0.006 mol) and the reaction solution was stirred for 24 h at 25°C. The solution was then diluted with water and extracted with ethyl acetate. The organic portion was washed with brine and filtered through $MgSO_4$. The solvent was removed under reduced pressure. The product was purified by silica column chromatography (CH_2Cl_2 then MeOH); the first fraction off the column was determined to be 4-glycidyl-TEMPO, which was obtained as a red viscous liquid (1.01 g, 83% yield by mass balance). ^{1}H-NMR (400 MHz, CD_3OD): δ 3.77 (dd, 1H), 3.69 (m, 1H), 3.53 (dd, 1H), 3.08 (m, 1H), 2.75 (t, 1H), 2.58 (m, 1H), 1.92 (dd, 2H), 1.38 (td, 2H), 1.16 (s, 6H), 1.13 (s, 6H).

4-Glycidyl-TEMPO (0.106 g, 0.2 mol equivalents) was added to a stirring solution of LPEI (0.10 g) in methanol. The solution was stirred at room temperature for 24 hours and the solvent was removed under reduced pressure. The product was washed with dichloromethane to remove any remaining staring material. The purified product was obtained as an amber amorphous solid, and was determined by ^{1}H-NMR to be 20% substituted. ^{1}H-NMR (400 MHz, CD_3OD): δ 3.87 (m, 1H), 3.63 (m, 1H), 3.42 (m, 1H), 2.91 (br m, 1H), 2.70-2.87* (br m), 2.58 (br m, 1H), 1.92 (br m, 2H), 1.39 (br m, 2H), 1.17 (s, 6H), 1.13 (s, 6H). The substitution ratio of the polymer was determined by normalizing integrations to the TEMPO substituent and dividing the number of protons on the substituted repeat unit (4) by the total number of normalized backbone protons as follows:

$$\text{Substitution \%} = [4 / (\text{integration of LPEI backbone Hs, } \delta\ 2.70\text{-}2.87)] \times 100\%$$

Protocol S3. Expression and Purification of Oxalate Decarboxylase (OxDc) from *Bacillus subtilis*

Oxalate decarboxylase (OxDc) was expressed and purified as previously reported.[15] The strain, *E. coli* BL21(DE3) transformed with the plasmid pET9c-OxDc, was grown overnight at 37 °C in LB broth in the presence of 100 μg ml^{-1} kanamycin and chloramphenicol. The resulting starter culture was used to inoculate 2 L of LB broth and grown at 37 °C and shaken at 220 rpm until an OD_{600nm} value of 0.5 was reached. The cells were heatshocked at 42 °C for 12 min before the induction of the expression with the addition of 1 mM IPTG and 5 mM $MnCl_2$. The induced cells were incubated at 30 °C and shaken at 220 rpm for 4 h. Then, cells were harvested by

centrifugation and resuspended in 50 mM Tris-HCl (pH 7.0) containing 10 μM $MnCl_2$ (buffer A) and disrupted by microfluidizer method. The soluble fraction of the cell lysate obtained after centrifugation, was applied to a Q-Sepharose Fast Flow column (1 x 25 cm) equilibrated buffer A and elution was performed using a 500-mL linear gradient from 0 to 1 M NaCl. The fractions contained purified OxDc was pooled and concentrated. The OxDc solution was then dialyzed overnight against 50 mM phosphate buffer (pH 6.0) containing 100 mM NaCl and stored at -80 °C. A protein concentration of 3.4 mg ml^{-1} was measured with Pierce™ BCA protein assay kit (Life Technologies™, Carlbad, CA).

Protocol S4. Enzyme Activity Assays

OxDc activity in solution was evaluated by indirect enzymatic UV-Vis spectrophotometric assay using a 96-well plate and the Synergy HTX Multi-Mode Reader (BioTek). OxDc (60 μg) was added in 100 μl mixture containing different concentrations of oxalate (from 0 to 50 mM) in 150 mM citrate-phosphate buffer (pH 4.0). After 5 min, the reactions were quenched with 140 μl of K_2HPO_4 followed by the addition of 5 mM NAD^+. The levels of produced formic acid were established in a coupled assay with the addition of 1 U of formate dehydrogenase and the absorbance at 340 nm (ε = 6220 M^{-1} cm^{-1}) was recorded. The formate concentration was quantified by the Michaelis-Menten equation containing K_m and V_m of the formate dehydrogenase obtained in the same condition of this essay. The activity of immobilized OxDc was characterized using the same procedure, but by incubating glassy carbon electrodes modified by the immobilization of the same quantity of enzyme (60 μg; following the procedure described below), into wells containing different concentration of substrate.

Protocol S5. Electrode Surface Modification

Electrode surface modification was done on 3-mm glassy carbon (GC) electrodes (CH Instruments, Austin, TX) at 25°C. The electrodes were mechanically polished on a microcloth using a micropolish containing aluminum oxide in water (Buehler, Lake Bluff, IL). The electrodes were thoroughly rinsed with ultrapure water, air-dried and used for subsequent modifications. For the MWCNT/TEMPO-LPEI-modified electrodes, 80 μL of a 10-mg mL^{-1} TEMPO-LPEI solution (dissolved in 0.1 M HCl) was mixed with 2 mg COOH-MWCNT and vortexed for ~ 5 s. As a control, MWCNT/C_8-LPEI-modified electrodes were also fabricated in a similar way, but using 80 μL of a 10-mg/mL C_8-LPEI solution (dissolved in H_20) instead. To these solutions, 7.5 μL of 10 % (v/v) EGDGE solution (dissolved in H_2O) was added and the mixture was vortexed for ~ 5 s and sonicated for ~ 30 s. For the hybrid MWCNT/TEMPO-LPEI/OxDc, 50 μL of a 3-mg mL^{-1} enzyme solution was added to the polymer-MWCNT mixture prior to addition of EGDGE. The resulting mixtures were double coated onto the surface of a GC electrode by adding 3 μL of the mixture each time using a pipette tip, while making sure that the film is evenly distributed across the electrode surface. The MWCNT/TEMPO-LPEI-modified electrodes were air-dried and allowed to cure at 4°C for ~ 12 h prior to use.

Protocol S6. Electrochemical Measurements and Data Analysis

All electrochemical measurements were performed using cyclic voltammetry in a three-electrode setup (SCE reference electrode, platinum mesh counter electrode) on a CH Instruments 611C Electrochemical Work Station (CH Instruments, Austin, TX), employing a step potential of 0.001 V, a potential range of 0.00 – 0.80 V (vs. SCE), and a scan rate of 10 mV s^{-1}, unless otherwise specified. Equilibration CV runs (~ 5 - 10 cycles from 0.0 – 0.80 V (vs. SCE) at 50 mV s^{-1}) were always performed for the modified electrodes prior to actual CV measurements (typically done at 10 mV s^{-1}). All amperometric titrations were carried out using constant potential

amperometry with the potential held at 0.70 V (vs. SCE) in a 150-mM citric acid-phosphate buffer (pH = 5.5), unless otherwise stated. Data analysis was done employing the amperometric currents generated for the substrate-induced electrocatalytic oxidation, where the values were normalized to the electrode area (0.0707 cm^2) and expressed as current density ± SD in units of mA cm^{-2}. The error bars reported represent the standard deviation of measurements made on three electrodes and thus, represent sensor-to-sensor variability.

Protocol S7. Glycerol oxidation cascade and CO_2 detection

The 24-hour glycerol oxidation cascade was performed by bulk electrolysis (0.8 V *vs.* SCE) using MWCNT/TEMPO-LPEI/OxDc-modified Toray carbon paper electrodes in a sealed container (prepared under argon atmosphere in glove box) containing 50 mM glycerol in 150 mM citric acid-phosphate buffer, pH 5.5, at 25° C. Carbon dioxide (CO_2) released into the headspace after bulk electrolysis was detected via gas chromatography (GC) using a thermal conductivity detector (TCD). Headspace analysis was carried out by manual injection into the GC with a gas-tight syringe. A Trace™ 1310 GC equipped with Carboxen®-1010 PLOT column (30 m X 0.32 mm) coupled with a TCD was used to detect CO_2. The column temperature was programmed as follows: 35°C hold for 2 min, followed by a ramp to 250°C at a rate of 24°C/min. The injector temperature was 230°C (10:1 split) and the TCD temperature was set at 230°C. The carrier gas was helium with a flow rate of 1 mL/min.

SUPPLEMENTAL FIGURES

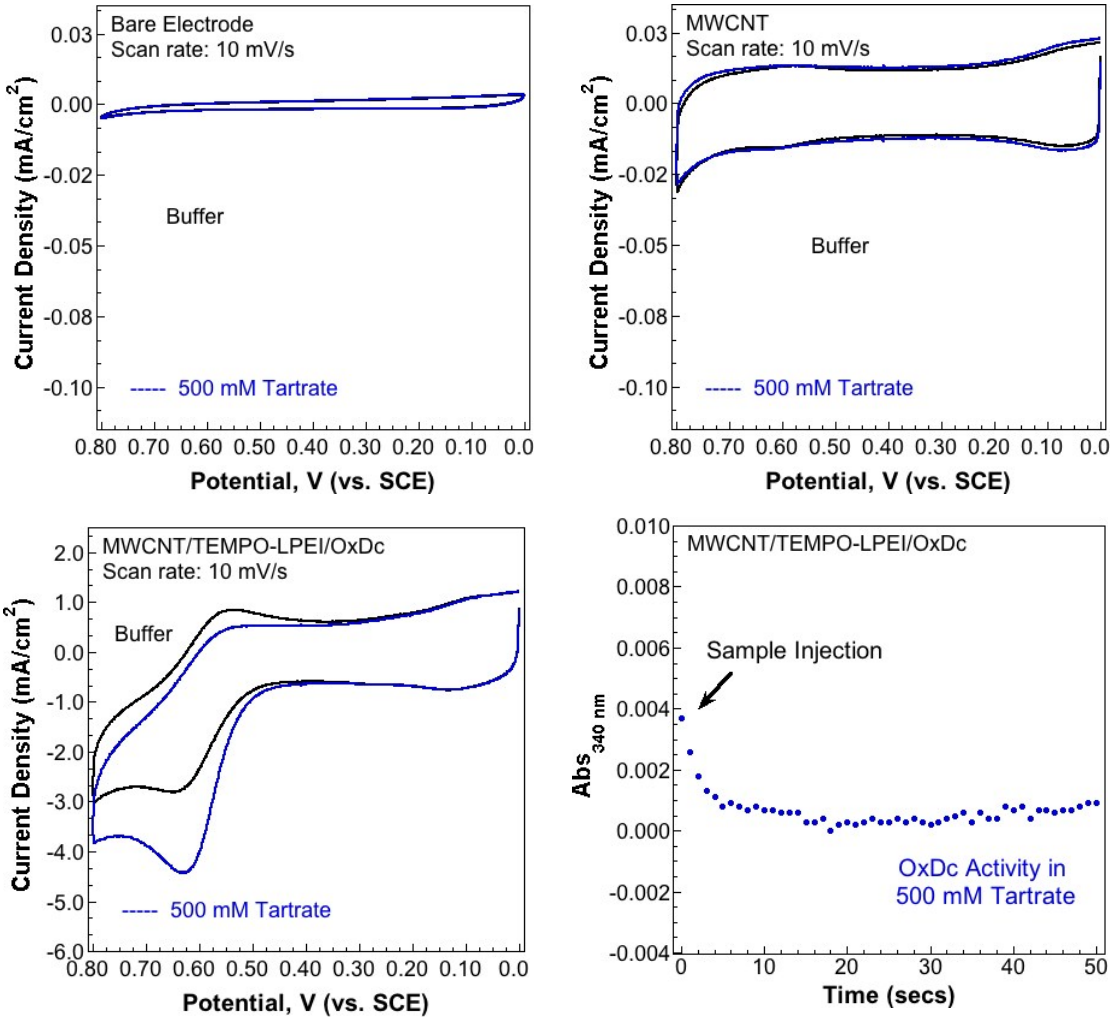

FIGURE S1. Catalytic activity of a bare electrode (*top panel, left*), immobilized MWCNTs (*top panel, right*), immobilized MWCNT/TEMPO-LPEI/OxDc (*bottom panel, left*) and immobilized OxDc (*bottom panel, right*) towards tartrate. Catalytic cyclic voltammograms (CVs) obtained for bare, MWCNT- and MWCNT/TEMPO-LPEI/OxDc-modified electrodes, as well as the kinetic OxDc activity curve obtained at 340 nm in the absence (*black curve*) and presence of 500 mM tartrate (*blue curve*).

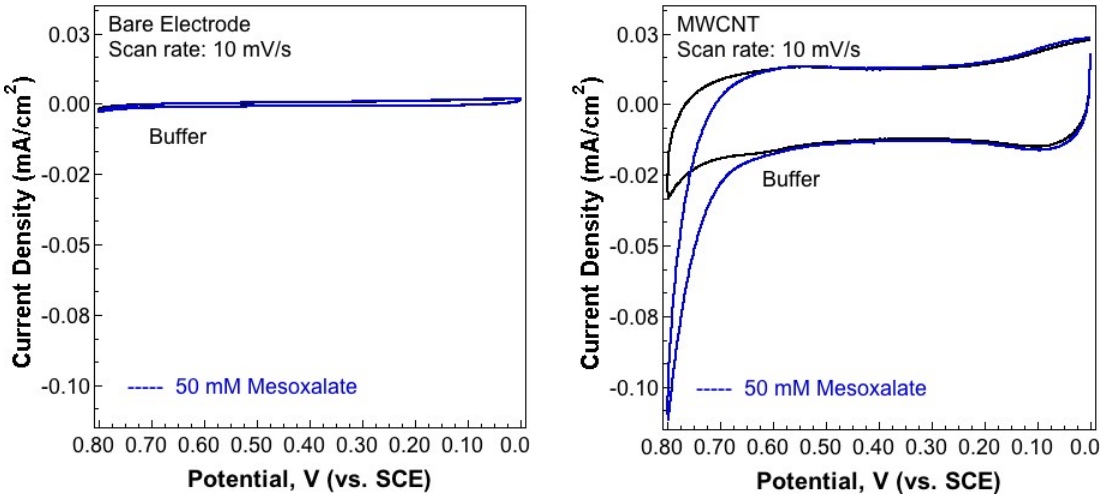

FIGURE S2. Catalytic activity of mesoxalate by immobilized MWCNTs. Catalytic cyclic voltammogram (CV) obtained for bare (*left*) and MWCNT-modified (*right*) electrodes in the absence (*black curve*) and presence of 50 mM mesoxalate (*blue curve*).

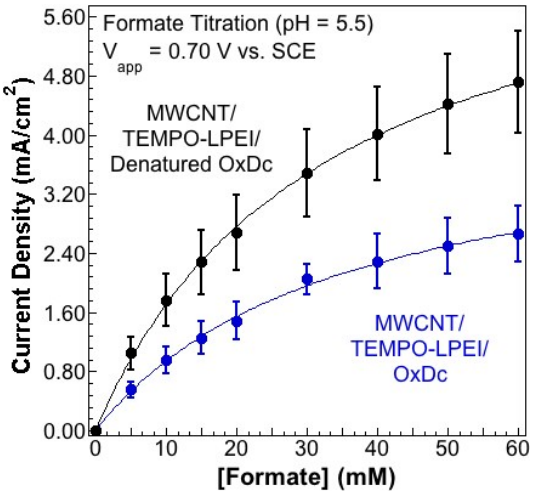

FIGURE S3. Catalytic oxidation of formate by immobilized MWCNT/TEMPO-LPEI/OxDc. Titration curves obtained for the active (*blue curve*) and denatured (*black curves*) forms of OxDc in the presence of formate.

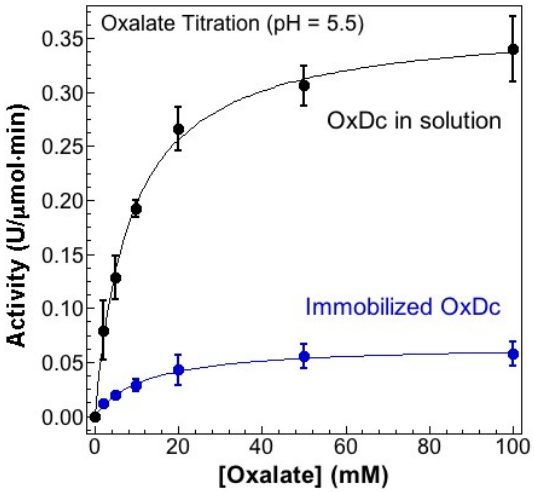

FIGURE S4. Kinetic titration curves obtained for solution-based OxDc (*black curve*) and surface-immobilized MWCNT/TEMPO-LPEI/OxDc (*blue curve*) in the presence of varying concentrations of oxalate. OxDc activity was measured at 340 nm using a UV-Vis spectrophotometer.

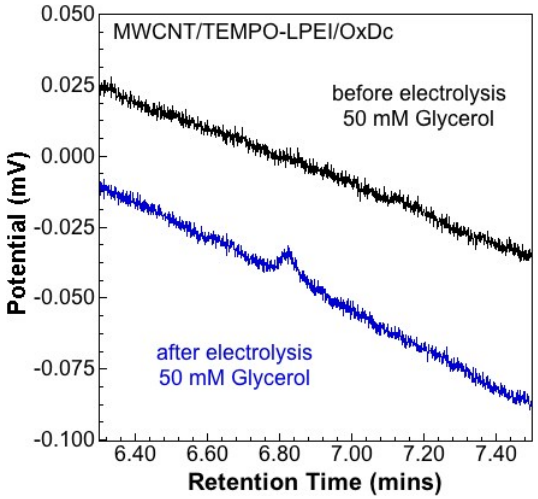

FIGURE S5. Gas chromatography spectra obtained for the MWCNT/TEMPO-LPEI/OxDc-modified electrode before (*black curve*) and after (*blue curve*) bulk electrolysis with 50 mM glycerol for 24 hours. The peak at ~ 6.80 mins indicates the presence of CO_2 (*blue curve*).