

Electronic Supplementary Information:

Improved formation of electrically-deposited enzyme-embedded chitosan coatings onto carbon fiber microelectrodes

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Supplemental Method Information:

Carbon Fibers

The carbon fibers used in this work (grade 34-700s) have very similar mechanical, structural, and electrical properties as compared to the traditional Thornel® T-650 fibers. Tensile strength and tensile modulus are two mechanical properties of carbon fibers which provide insight into their structural integrity (and thus their molecular-level composition), are known to vary over several orders of magnitude, and can affect electrochemical performance.¹⁻³ T-650s have a tensile strength and tensile modulus of 37 Msi and 620 ksi (respectively) while the 34-700s have a tensile strength and tensile modulus of 34 Msi and 700 ksi (respectively).^{4,5} Of most critical electrochemical importance is the electrical resistivity of these fibers. Both fiber types have a reported electrical resistivity of 15 $\mu\text{Ohm}\cdot\text{m}$.^{4,6}

Benzoquinone Coating Solution:

As stated in the main work, the BQ-coating solution developed a reddish-brown color over time (Figure S1, panel E). Coating solutions containing only enzyme and chitosan maintained a stable yellow color due to the yellow color of GOX (panel A). Solutions containing only BQ also remained a pale yellow with time due to the yellow color of BQ (panel B). A red color developed within minutes in any solution that contained BQ and an available reactive amine (BQ with chitosan in panel C, BQ with GOX in panel D, and BQ with GOX and chitosan in panel E).

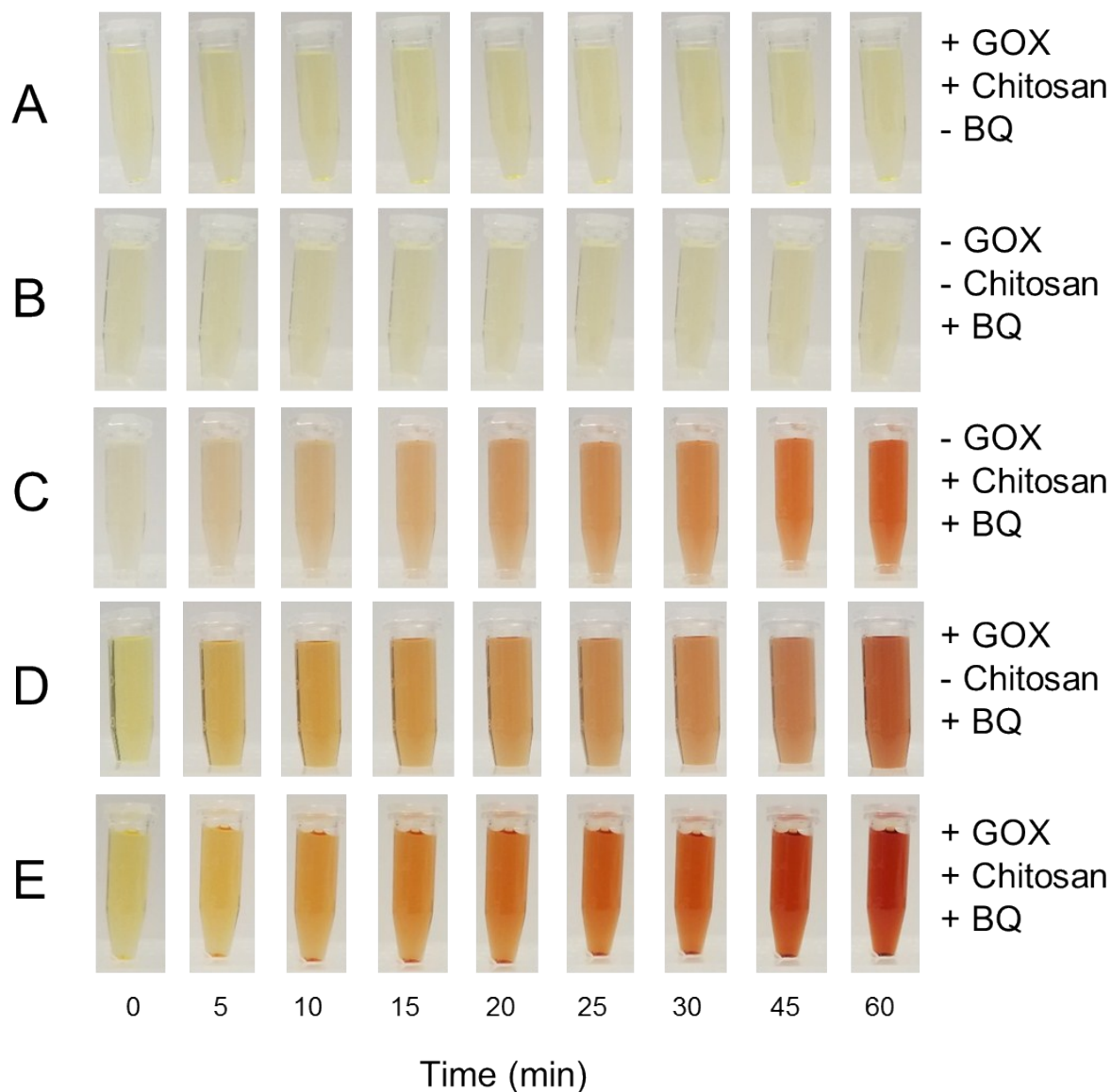


Figure S1. Color development in benzoquinone-containing coating solutions. Panels A-E contain time-lapse images of coating solutions in the presence (indicated by +) or absence (indicated by -) of the enzyme glucose oxidase (GOX) at 5 mg/mL, the polymer chitosan at 0.5% w/v, and the small molecule benzoquinone (BQ) at 20 mM. All solutions were aqueous with pH of 5.0, prepared as described in the main paper.

Slow-Scan Cyclic Voltammetry:

BQ undergoes a simple two proton, two electron reduction into hydroquinone while the electrochemical behavior of CAM is known to be more complex.⁷ Slow-scan cyclic voltammograms of BQ (Figure S2) and CAM (Figure S3) confirmed that the -1.4 V DC potential applied was sufficient to cause reduction of these species during the coating process.

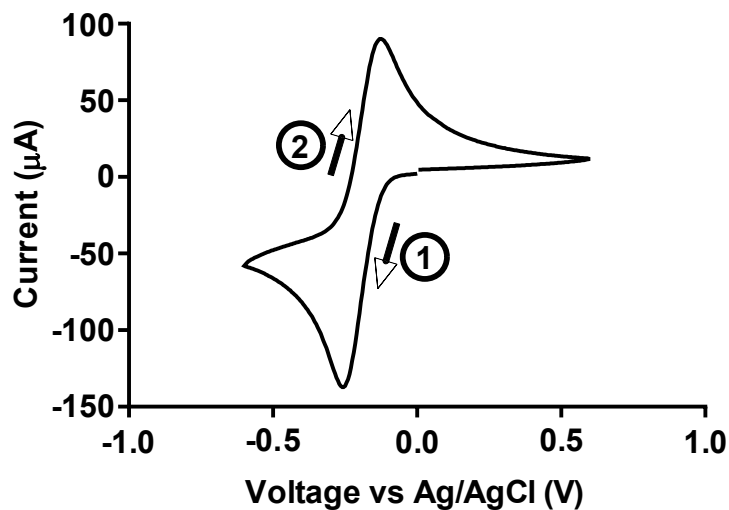


Figure S2. Cyclic voltammogram of BQ (0.04 mg/mL in 0.1 M KCl, pH 5.0) taken on a screen-printed carbon working electrode (20 mm²). The voltage was scanned from 0.0 V to -0.6 V to +0.6 V to 0.0 V at 100 mV/s, with the numbered arrows indicating the voltage sweep direction order.

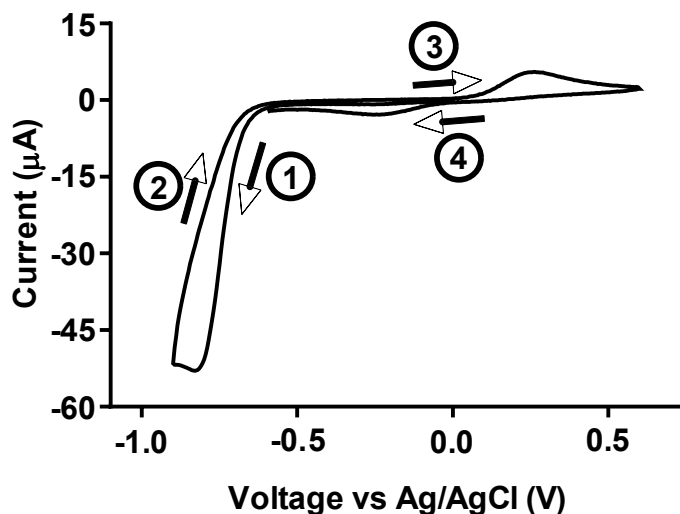
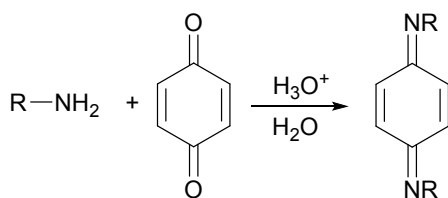


Figure S3. Cyclic voltammogram of CAM (0.11 mg/mL in 0.1 M acetate buffer, pH 4.62) taken on a screen-printed carbon working electrode (20 mm²). The voltage was scanned from 0.0 V to -0.9 V to +0.6 V to -0.6 V at 100 mV/s, with the numbered arrows indicating the voltage sweep direction order.

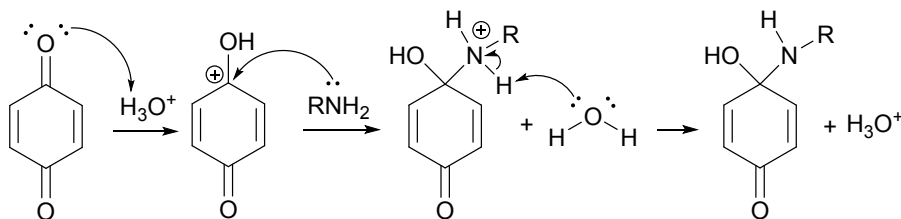
Hypothesized Cross-Linking Mechanism

It is hypothesized that a chemical reaction is taking place between benzoquinone and primary amines in solution, either those on the chitosan polymer and/or amine-containing residues (i.e. lysine) on outer side chains of GOX⁸, to produce a *p*-benzoquinone diimine crosslinked network. Imine formation is highly favored in mildly acidic (pH 4-5) aqueous solutions such as the one used for coating; a proposed mechanism for this process is shown below in Figure S4, derived from the well-characterized interaction of primary amines and carbonyl groups.⁹⁻¹²

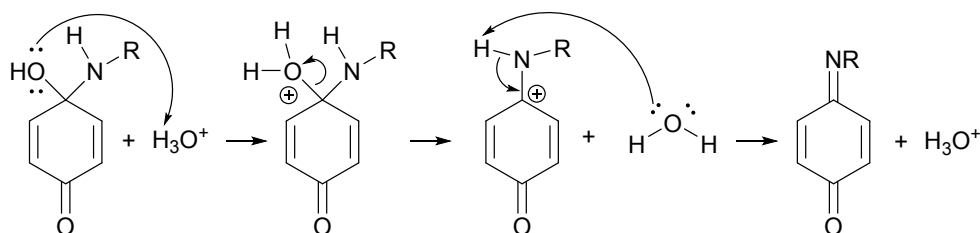
Overall Net Proposed Reaction



Step 1: Acid-catalyzed addition of an amine to a carbonyl



Step 2: Acid-catalyzed dehydration



Step 3: Repeat to form *p*-quinone diimine

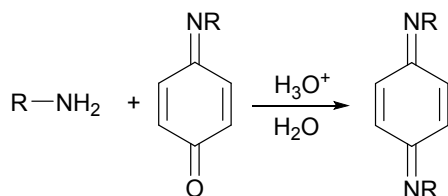


Figure S4. Proposed mechanism for the reaction between a primary amine and benzoquinone to produce a diimine. Diimine formation proceeds by addition of an amine to a carbonyl followed by dehydration in a mildly acidic solution. The R group can represent the rest of the chitosan polymer or GOX.

Film Morphology Studies

To investigate differences in film morphology, GOX/CHIT-CFMs produced traditionally and through the reduction of BQ or CAM were briefly soaked into a 0.2 M acetic acid solution (pH 2.7). Images of the electrodes before and after acetic acid submersion are shown below.

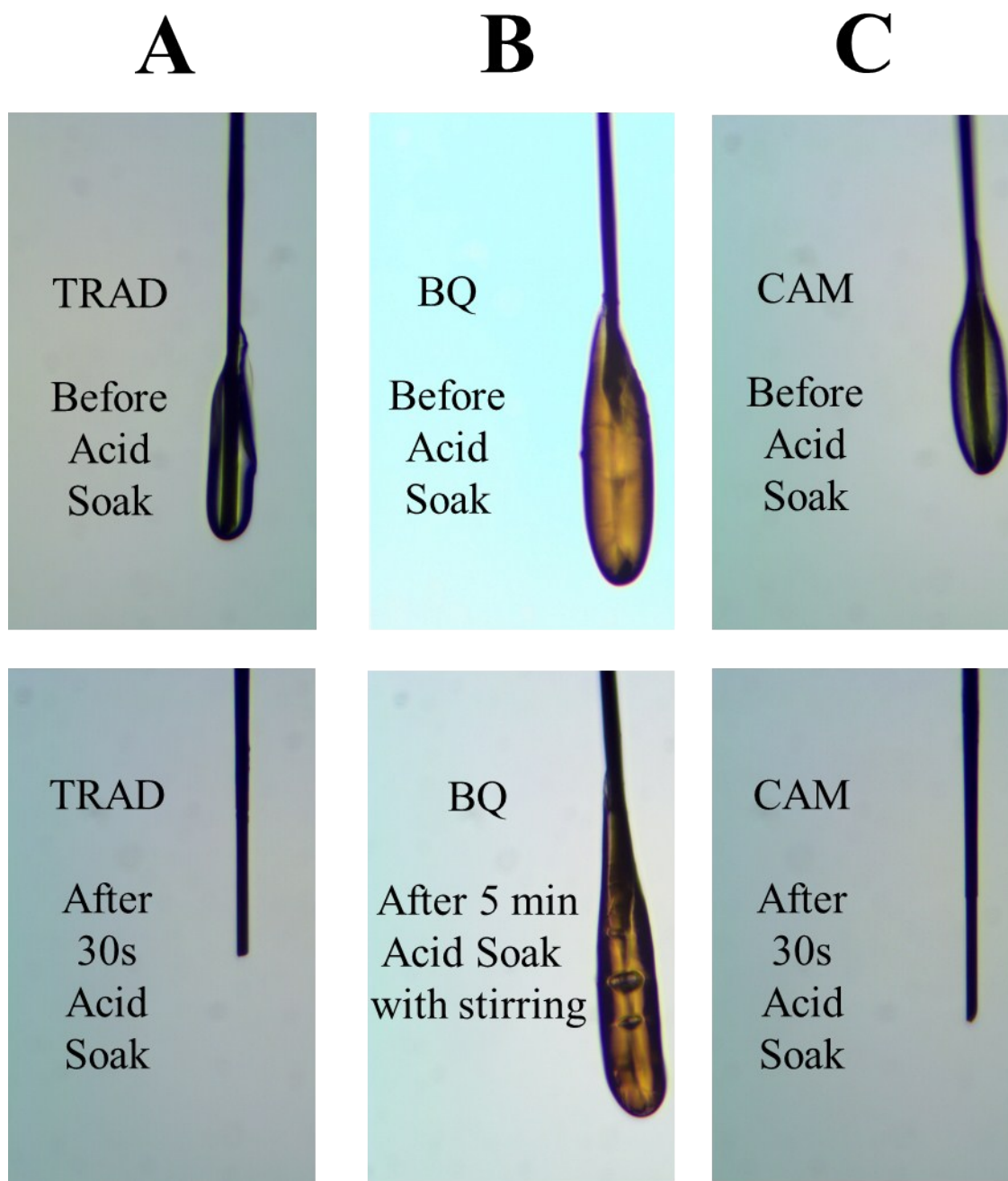


Figure S5. Acetic acid soak study for GOX/CHIT-CFMs produced by each of the three approaches. Panels A show an electrode produced by the traditional approach before (top) and after (bottom) a 30 second soak in a 0.2 M acetic acid solution. Panels B show an electrode produced through the reduction of BQ before (top) and after (bottom) a five minute soak in a 0.2 M acetic acid solution with stirring. Panels C show an electrode produced by the reduction of CAM before (top) and after (bottom) a 30 second soak in a 0.2 M acetic acid solution.

Supplementary Bibliography

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