Redox Capacitor to Establish Bio-Device Redox-Connectivity

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Electronic devices process information and transduce energy with electrons, while biology performs such operations with ions and chemicals. To establish bio-device connectivity, we fabricate a redox-capacitor film from a polysaccharide (i.e., chitosan) and a redox-active catechol. We report that these films are rapidly and repeatedly charged and discharged electrochemically via a redox-cycling mechanism in which mediators shuttle electrons between the electrode and film (capacitance ≈ 40 F/g or 2.9 mF/cm^2). Further, charging and discharging can be executed under bio-relevant conditions. Enzymatic-charging is achieved by electron-transfer from glucose to the film via an NADPH-mediated redox-cycling mechanism. Discharging occurs by electron-donation to O_2 to generate H_2O_2 that serves as substrate for peroxidase-mediated biochemical reactions. Thus, these films offer the capability of inter-converting electrochemical and biochemical inputs/outputs. Among potential applications, we anticipate that catechol–chitosan redox-capacitor films could serve as circuit elements for molecular logic operations or for transducing bio-based chemical energy into electricity.

1. Introduction

Electronics offer incredible capabilities for energy transduction and information processing. However, electronics are not inherently well-suited for "extracting" chemical energy or information from biological or aqueous systems. Potentially, biology could provide mechanisms to facilitate bio-device coupling. In fact, there has been considerable effort to "connect" biology with electronics[1–3] with envisioned applications that include microbial[4–9] or enzymatic[10,11] fuel cells that transduce chemical energy into electricity, or to generate bio-based logic gates that convert solution-phase information into device-compatible output.[12–15]

In electronics, capacitors are common circuit elements both for energy transduction and signal processing. Electrochemical capacitors (ECs) are under intense study for electrical energy storage[16–18] and are categorized based on their energy storage mechanisms[19,20] Electric double layer capacitors (EDLCs) store energy through ionic charge separation at the electrode surface and offer high power densities but low energy densities. Redox-capacitors (also known as pseudo-capacitors) employ reversible surface redox reactions to store energy through Faradaic mechanisms and they generally offer higher energy densities but lower power densities.

In biology, the most familiar capacitor is the lipid bilayer membrane (capacitance ≈ 1 μF/cm^2) that stores energy in the form of an ionic charge separation across the insulating bilayer. The controlled discharging of this membrane is used for energy transduction (e.g., mitochondrial ATP synthesis) and signal processing (e.g., neuronal action potentials). While the capacitor properties of lipid bilayers are integral to life, it is challenging to exploit this approach in vitro. We are investigating an alternative bio-based capacitor that relies on reversible redox reactions (i.e., Faradaic processes). This capacitor is fabricated from a polysaccharide film and a redox-active organic moiety, catechol, that is common in biological and environmental systems.[21]

Our redox-capacitor is fabricated in two steps.[21–23] First, a thin film (≈300 nm after drying) of the stimuli-responsive film-forming aminopolysaccharide chitosan is electrodeposited[24–28] from solution onto a gold-coated silicon wafer. Second, the chitosan-coated electrode is electrochemically-modified with catechol. During this second step, **Scheme 1**a illustrates that catechol diffuses through the chitosan film, is anodically oxidized at the underlying gold, and the oxidation product covalently grafts to the chitosan film. While the anodic oxidation of catechol to *o*-quinone is well-established, the grafting reaction between *o*-quinone and chitosan is complex and likely involves multiple grafted species (e.g., monomers and oligomers).[23] Initial electrochemical studies demonstrated that the catechol-modified chitosan films possess unique properties: they are non-conducting but redox-active. In particular, the films are unable to directly exchange electrons with the underlying electrode but they can be repeatedly switched between oxidized and reduced states.[29] These properties are illustrated by the cyclic voltammograms provided in the Supporting Information (Figures S2 and S3).

Scheme 1b illustrates that charging is achieved electrochemically by the cathodic reduction of Ru^{3+} to Ru^{2+} and the subsequent transfer of electrons to the film–presumably converting oxidized quinones (Q state) to reduced catechols (QH_2 state). Electrochemical discharging (Scheme 1c) is achieved by anodic oxidation of Fc to Fc^+ and the subsequent transfer of electrons from the film–presumably converting catechol to quinone moieties. As noted, charging/discharging of the catechol–chitosan films requires a mediator-based redox-cycling

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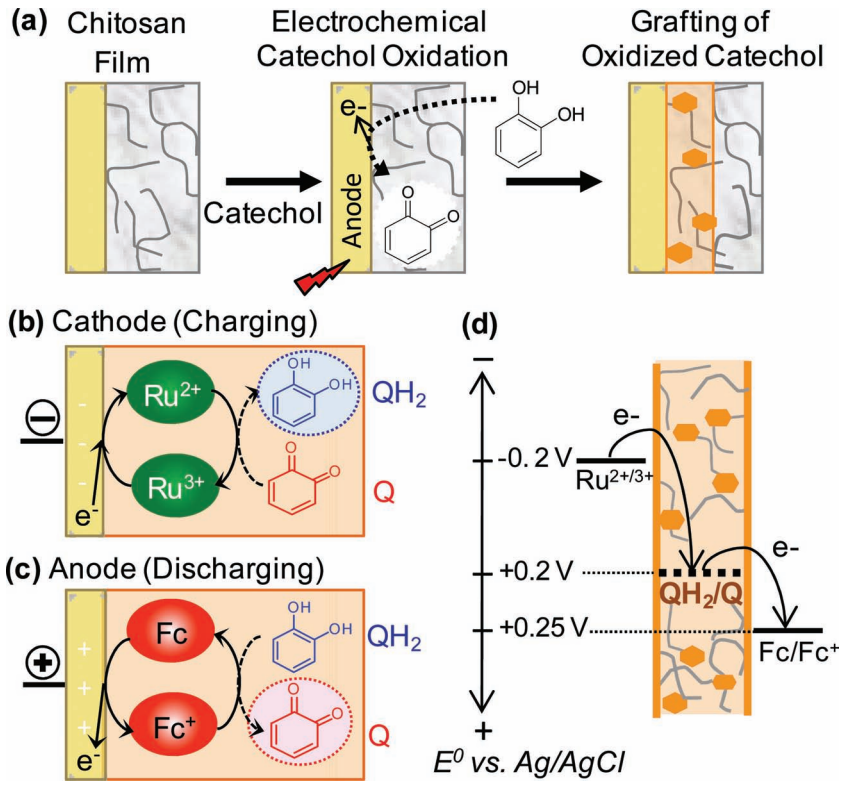


Scheme 1. Redox-capacitor films. a) Film fabrication by electrochemical-grafting of catechol to chitosan. b) Electrochemical redox-cycling to charge the film (i.e., to reduce the grafted moieties). c) Electrochemical redox-cycling to discharge the film (i.e., to oxidize the grafted moieties). d) Thermodynamics of electrochemical charging/discharging.

mechanism because the films are non-conducting and cannot directly exchange electrons with the underlying electrode.[29] Also, as illustrated in Scheme 1d, the electrochemically-mediated charging and discharging of the film is constrained by thermodynamics with electrons flowing in the direction of decreasing free energy or increasing (more positive) potential.

Here, we characterize the capacitor properties of these catechol-modified chitosan films using the electrochemically-mediated redox-cycling reactions of Scheme 1. Further, we report an enzymatically-catalyzed redox-cycling mechanism to charge the film and show that the films can be discharged by donating their electrons to O_2 to generate H_2O_2 which serves as substrate for a subsequent enzymatic reaction. Finally, we demonstrate that these films allow the inter-conversion of electrochemical/biochemical inputs/outputs to perform simple logic operations.

2. Results

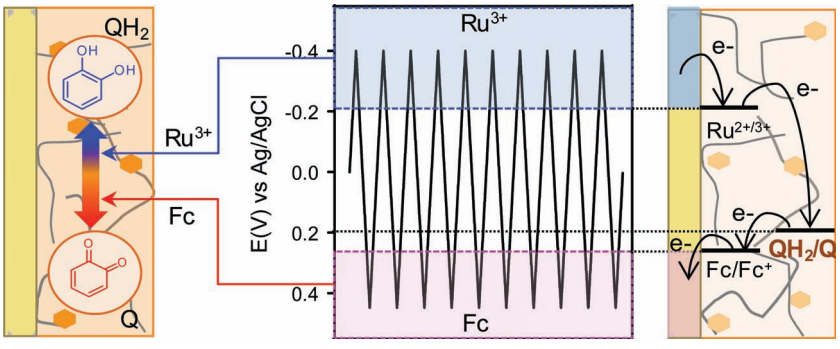
2.1. Redox Capacitor Activity

Initial studies characterized conventional redox-capacitor properties. We first compared the electrodes coated with either an unmodified chitosan film (control) or a catechol–chitosan film. Film-coated electrodes were immersed in a solution containing both Fc (25 μM) and Ru^{3+} (25 μM) and the input voltage was cycled between –0.4 and +0.4 V (vs Ag/AgCl with a Pt wire counter-electrode) as illustrated in **Scheme 2**. When the imposed reducing potential is less than –0.2 V, Ru^{3+} is electrochemically reduced allowing the redox-cycling reaction of Scheme 1b to charge the catechol–chitosan film. As the imposed electrode potential is cycled to positive potentials, residual Ru^{2+} is first oxidized (E > –0.2 V), and then Fc-oxidation occurs (E > +0.25 V) which enables the redox-cycling reactions of Scheme 1c to discharge the catechol–chitosan film.

The output currents shown at the right in **Figure 1**a are significantly different for the chitosan control film and the catechol–chitosan film (Figure S4 in the Supporting Information shows equivalent cyclic voltammograms). The chitosan film shows relatively small peak currents that presumably reflect the electrochemical reduction and oxidation of the mediators in the absence of interactions between the mediator and film.[30] The peak currents for the catechol–chitosan films are considerably amplified (vs. the control) due presumably to the redox-cycling reactions in the film.

In addition to the amplified peak currents, two additional observations for the catechol–chitosan film should be noted from Figure 1a. First, the amplification is observed despite relatively short imposed cycle times (30 sec) which indicates that charging/discharging is rapid. Second, the peak currents remain largely unchanged during the 10 cycles indicating that charging/discharging is repeatable.

A pair of experiments provide additional support for the hypothesis of mediated electrochemical charging/discharging.



Scheme 2. The redox-capacitor properties of the catechol-modified chitosan films was evaluated using a cyclic input-voltage in the presence of both electrochemical mediators. Film charging occurs at imposed potentials less than –0.2 V when Ru^{3+} reduction induces the redox-cycling reaction of Scheme 1b. Film discharging occurs at imposed potentials greater than +0.25 V when Fc oxidation induces the redox-cycling reaction of Scheme 1c.

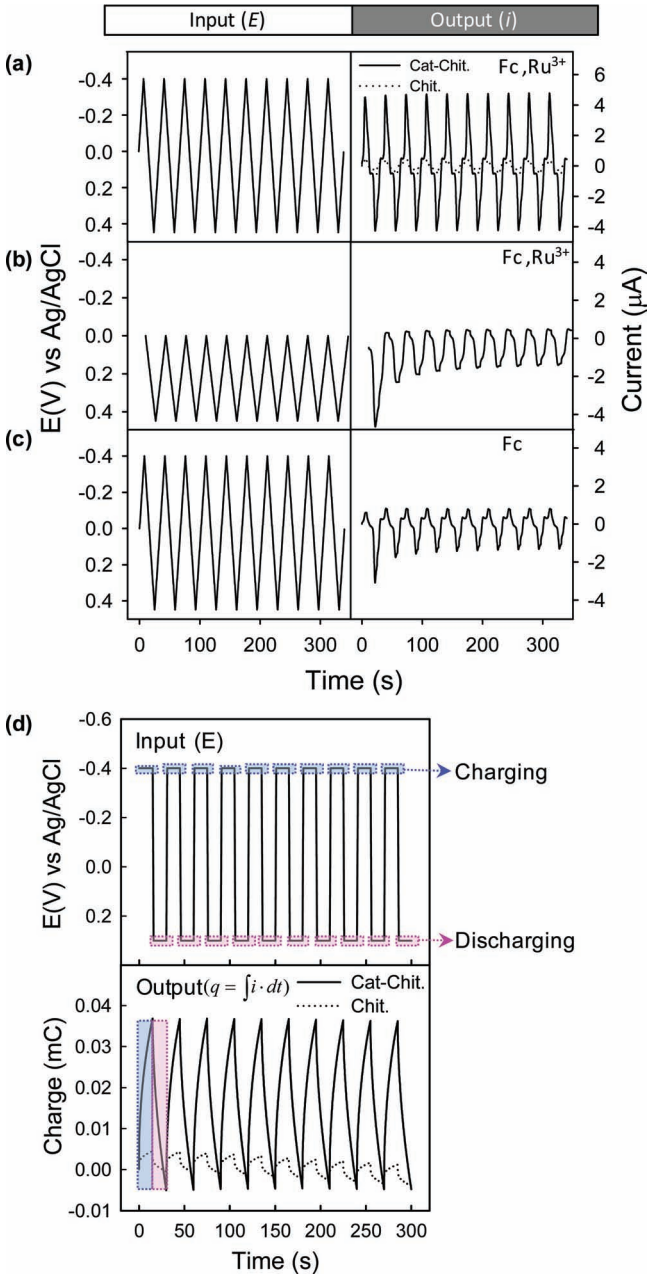


Figure 1. Electrochemical evidence for redox-capacitor activity. a) Voltage input (–0.4 to +0.4 V) and current output for film incubated with both mediators (Fc and Ru^{3+}; 25 μM). (b) Voltage input (0.0 to +0.4 V) and current output for film incubated with both mediators. c) Voltage input (–0.4 to +0.4 V) and current output for film incubated with only the Fc mediator. d) Voltage input (–0.4 to +0.3 V) and charge output for film incubated with both mediators (Fc and Ru^{3+}; 25 μM).

In Figure 1b, the catechol–chitosan film was immersed in a solution containing both Ru^{3+} and Fc and the imposed voltage was cycled between 0 and +0.4 V. In this case, the potential is never low enough to reduce Ru^{3+} for the redox-cycling to charge the film. The output in Figure 1b shows anodic currents under oxidizing potentials but minimal cathodic currents under reducing conditions (presumably these reducing currents are due to reduction of residual Fc^{+} formed during the oxidative sweep). This absence of substantial reduction peaks is consistent with a failure to charge the films under these conditions. Also consistent with a failure to charge the film is the observation that the peak oxidation current decreases with each subsequent cycle as the film is progressively depleted of electrons.

In Figure 1c, the catechol–chitosan film was immersed in a solution containing Fc (but not Ru^{3+}) and the imposed potential was cycled between –0.4 and +0.4 V. The output current in Figure 1c is similar to that in Figure 1b; large oxidation with minimal reducing currents, and a decrease in peak oxidation current with each subsequent cycle. These results indicate that a mediator (e.g., Ru^{3+}) is required to charge the catechol–chitosan film because it cannot directly exchange electrons with the electrode (i.e., the film is non-conducting).[29]

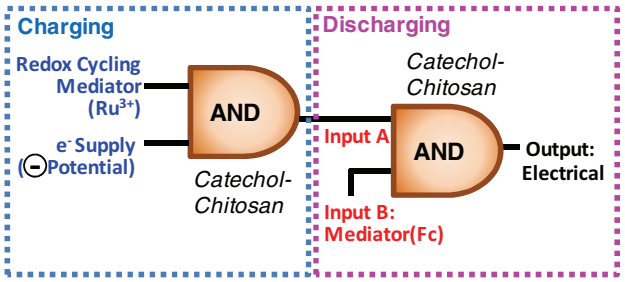
To quantify capacitance, we performed chronocoulometric measurements in which the imposed potential was cycled stepwise and the output charge transfer ($q = \int i dt$) was monitored. In this experiment, electrodes coated with either chitosan or catechol–chitosan were immersed in a solution containing both Fc (25 μM) and Ru^{3+} (25 μM) and the input potential was switched as illustrated in Figure 1d. Specifically, a cathodic charging potential (–0.4 V) was applied for 15 s after which it was switched to an anodic discharging potential (+0.3 V) for 15 s.

The output charge transfer during these charging/discharging steps is shown in Figure 1d. During the brief charging steps (15 s), Figure 1d shows that the catechol–chitosan coated electrode stores about 0.037 mC (1.2 mC/cm^2) of charge, while the chitosan-coated control electrode stores only 0.0046 mC (0.15 mC/cm^2). The cathodic charge (q) and voltage difference (dE = 0.4 V) are used to determine the capacitances (C = q/dE) of 2.9 mF/cm^2 (40 F/g) for the catechol–chitosan film, and 0.36 mF/cm^2 (7.7 F/g) for the chitosan film. The output response in Figure 1d also indicates that the discharging process appears to be complete as the charge accumulated during the individual charging step is depleted during discharging. The reversibility of charging/discharging was observed for 10 cycles (additional cycles were not studied).

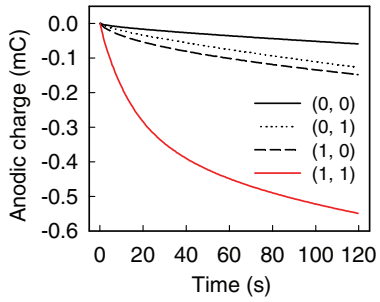
Figure 1 demonstrates that the catechol–chitosan film can be electrochemically-charged only when two requirements are met simultaneously; a source of electrons (sufficiently negative potential) *and* a mediator (Ru^{3+}) capable of the redox-cycling. Thus, electrochemical-charging of the catechol–chitosan film can be characterized as a simple AND logic gate as illustrated by the left-most box in **Figure 2**a. Similarly, Figure 2a illustrates that discharging can be characterized as an AND gate where the film must be charged to provide an electron source (input A) *and* it must be exposed to an oxidizing condition (input B). For electrochemical-discharging, input B is a sufficiently positive potential in the presence of a mediator (Fc) that can undergo the redox-cycling of Scheme 1c. The output from an electrochemically-discharged AND gate is the anodic current (i) or charge transfer (q).

To experimentally test this electrochemical logic gate we prepared films in either their charged (QH_2, input A = 1) or discharged (Q, input A = 0) states and then tested them by

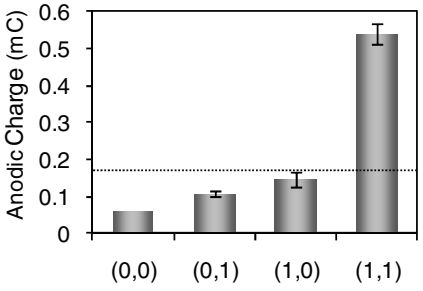
(a)



(b)



(c)



(d)

Input A (Charged)	Input B (Fc)	Output (Anodic Charge)
0	0	0
0	1	0
1	0	0
1	1	1

Figure 2. Electrochemical AND gates. a) Schematic that depicts electrochemical charging and discharging as simple logic operations. b) Electrical output (charge, q) associated with discharging of catechol-modified chitosan film. c) Summary of discharging output. d) Truth table for electrochemical discharging.

chronocoulometry at an applied potential of +0.5 V in solutions containing the Fc mediator (input B = 1) or lacking the Fc mediator (input B = 0). Figure 2b shows the anodic charge (output) over this 2 min chronocoulometric incubation: large anodic charge transfer is only observed if both inputs A *and* B are 1. Figure 2c summarizes these results and suggests a cutoff of 0.18 mC to assess whether the electrical output is either 0 ($q < 0.18$ mC) or 1 ($q \geq 0.18$ mC). Based on these results, the truth table of Figure 2d is constructed to show that the observed electrical output signals from the catechol–chitosan film can be characterized by a simple AND logic gate.

2.2. Enzymatic Charging

We next explored film charging/discharging under bio-relevant conditions. **Figure 3**a illustrates an enzymatic charging mechanism in which glucose dehydrogenase (GDH) catalyzes the transfer of electrons from glucose to the diffusible mediator NADPH that redox-cycles to charge the film. The left schematic in Figure 3b illustrates the thermodynamics for this enzymatic-charging. Experimentally, enzymatic charging is achieved by incubating a catechol–chitosan film (initially oxidized to Q state) for 15 min in a solution containing 10 mM glucose, GDH and $NADP^+$.

To quantify the number of electrons transferred to the film by enzymatic charging, we recovered and rinsed the film, and then analyzed it using an electrochemical "titration" method that has been previously described.[31] Briefly, the film is immersed in a solution containing 50 μM Fc and a constant anodic potential (+0.5 V) is applied to exhaustively discharge the film by the electrochemical redox-cycling reaction of Scheme 1c. The thermodynamic scheme for this "titration" is shown at the right in Figure 3b.

The electrochemical titration curves for various films are shown in Figure 3c. The analytical positive control is a catechol–chitosan film that was completely charged (QH_2 state) by electrochemical reduction (Scheme 1b) and then titrated. Figure 3c shows a large number of electrons are "discharged" from this positive control during the two-minute titration. The analytical negative control is a catechol–chitosan film that was completely discharged (Q state) by electrochemical oxidation (Scheme 1c). Figure 3c shows that comparatively few electrons are transferred during titration of this negative control film (presumably the observed electron transfer is due to Fc oxidation). Electrochemical titration for the experimental film that had been incubated with glucose, GDH and $NADP^+$ is also shown in Figure 3c. The titration results for the experimental film are similar to those for the positive control indicating that the film was charged during the 15 minute incubation.

Additional control experiments were performed by incubating films with 2 of 3 components of the enzymatic system. Figure 3c shows that all of these controls are similar to the negative control which indicates that all three components, the electron source (glucose) and redox-cycling components (GDH and $NADP^+$), are required to enzymatically-charge the films.

As illustrated in Figure 3c, we calculate the maximum number of electrons that can be transferred to the film (i.e., the redox capacity, $q_{Film,max}$) from the difference in charge transfer between the positive and negative controls after the 2 minute titration.[31] Similarly, the number of electrons transferred to the film during the various treatments (q_{Film}) was calculated as illustrated in Figure 3c for the experimental film treated with all 3 components of the enzymatic system. To facilitate comparison, the Faraday equation was used to convert q_{Film} to a molar basis (N_{Film}). Figure 3d shows a quantification of the results from Figure 3c.

In a final enzymatic-charging experiment, we incubated catechol–chitosan films (initially in Q state) for 15 min with GDH, $NADP^+$ and varying levels of glucose, after which the films were electrochemically titrated to determine the number of electrons transferred. Figure 3e shows that electron-transfer to the film increased monotonically with glucose. The saturation observed

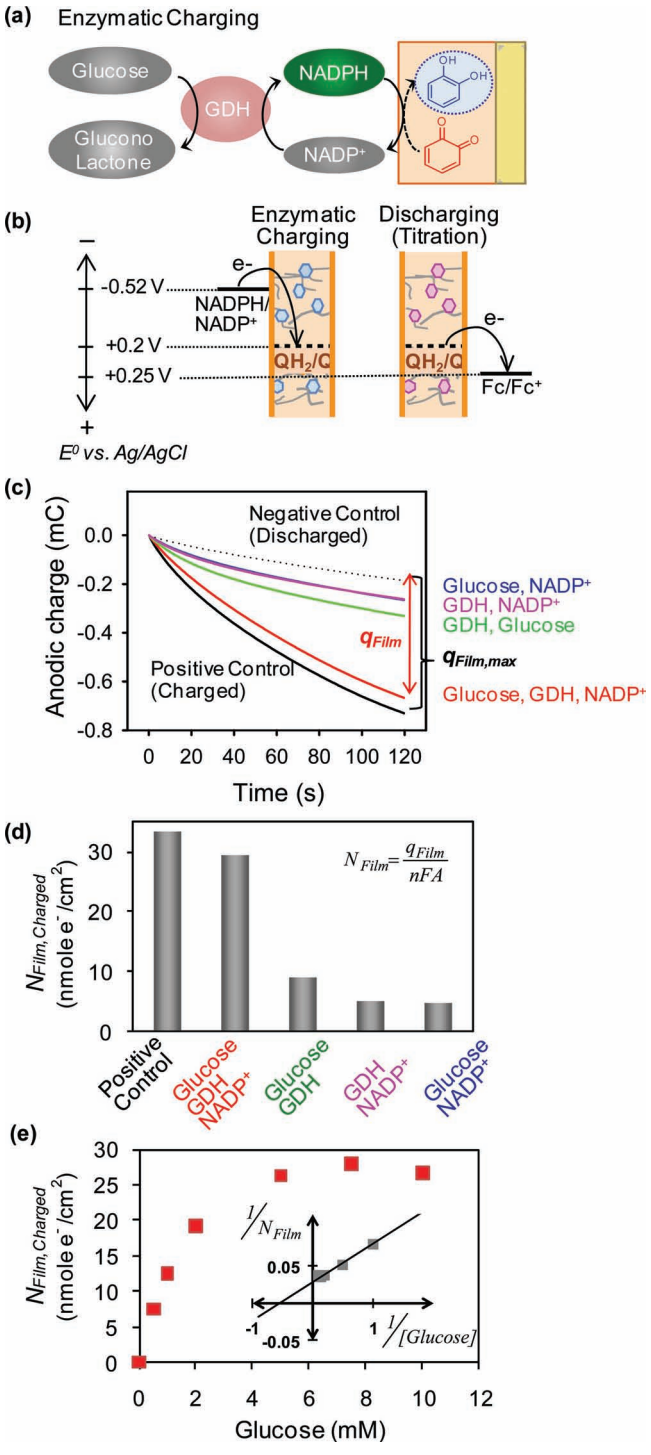


Figure 3. Enzymatic charging. a) Glucose dehydrogenase (GDH) catalyzed redox-cycling to charge the film. b) Thermodynamics of enzymatic charging and subsequent quantitative analysis by electrochemical discharging. c) Electrochemical "titration" curves. d) Quantification of film charging. e) Enzymatic charging is controlled by the electron source glucose (insert shows analogous Lineweaver-Burke plot).

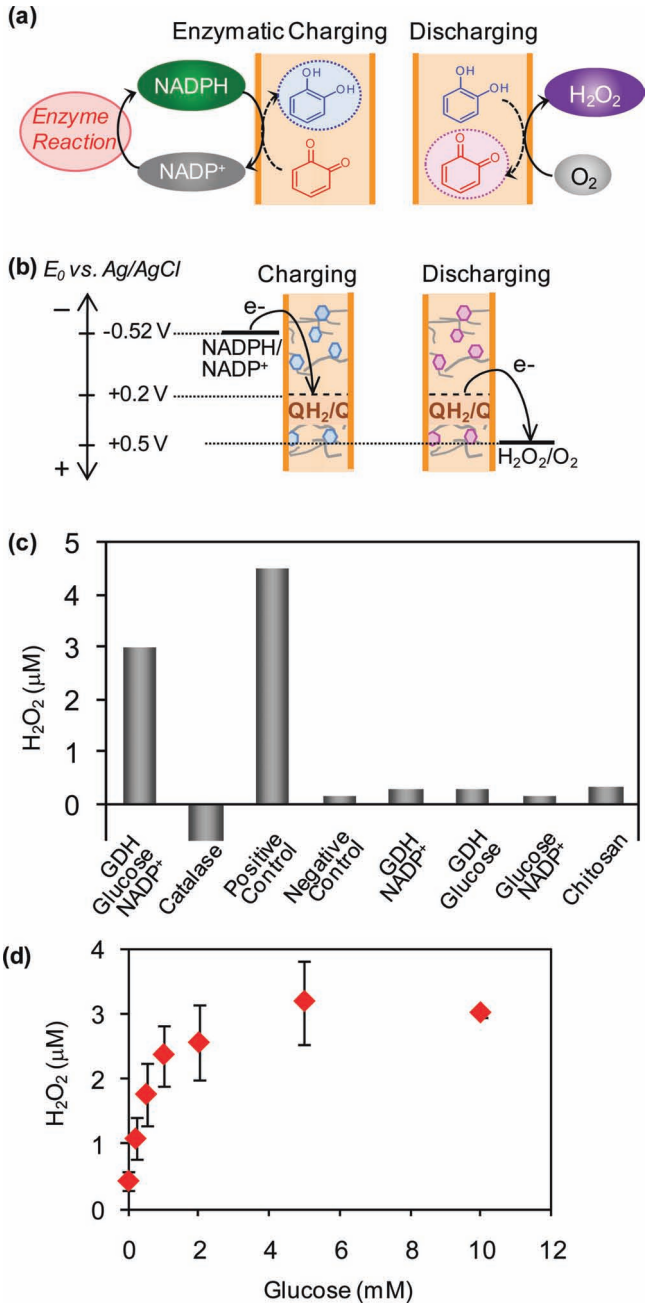
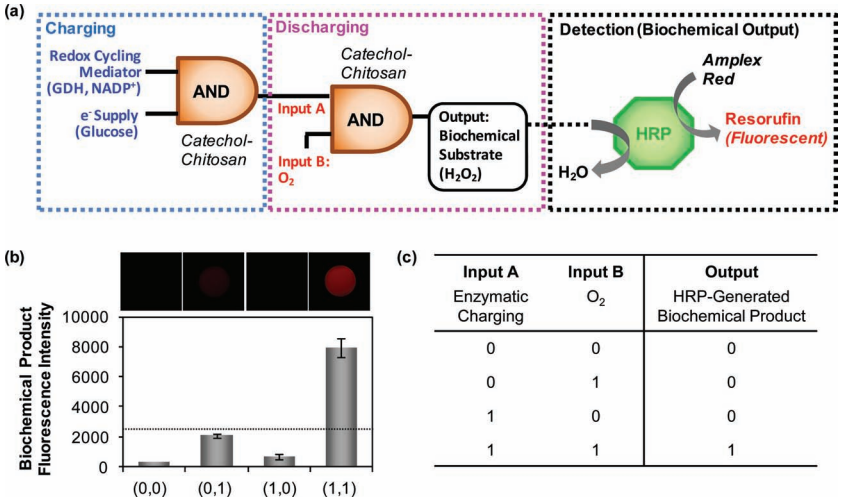


Figure 4. Discharging electrons to O_2 to generate reactive oxygen species (i.e., H_2O_2). a) Enzymatic charging followed by discharging to O_2. b) Thermodynamics of enzymatic charging and discharging to O_2. c) H_2O_2 generated when films were first enzymatically-charged and then discharged by incubation in air-saturated water for 15 min. d) Discharged H_2O_2 level is controlled by the electron source (glucose) during charging.

in Figure 3e appears to reflect the capacity of the film for accepting electrons, as the saturation value of ≈30 nmole cm^{-2} is comparable to the value observed in Figure 3d for the positive control. The insert in Figure 3e is a Lineweaver–Burke plot that allows estimation of the enzyme's half saturation constant (≈2 mM) which is comparable to values reported for GDH.[32]

2.3. Discharging for Reactive Oxygen Generation

Figure 4a illustrates that charged films can be discharged by donating their electrons to O_2 to generate reactive oxygen species (ROS). Figure 4b illustrates the thermodynamics associated



Input A Enzymatic Charging	Input B O_2	Output HRP-Generated Biochemical Product
0	0	0
0	1	0
1	0	0
1	1	1

Figure 5. Biochemical AND gates. a) Schematic of logic operations associated with enzymatic charging followed by discharging to provide the H_2O_2-substrate for a subsequent enzymatic reaction. b) Fluorescence output associated with the fluorogenic reaction of horseradish peroxidase (HRP). c) Truth table for the enzymatic AND gate.

with enzymatic charging and discharging. Experimentally, we first charged the catechol–chitosan film by 15 min incubation with glucose (10 mM), GDH and $NADP^+$. Next, the charged film was incubated for 15 min in air-saturated water (200 µL). Finally, the film was removed and the solution assayed for H_2O_2.[31] Figure 4c shows that 3 µM of H_2O_2 was generated from this experimental film. Incubation of an aliquot of this solution with catalase (20 U/mL for 30 min) shows a complete depletion of H_2O_2.

For comparison, Figure 4c shows results from various controls. A positive control film was electrochemically-charged to the QH_2 state (by the reaction in Scheme 1b) and then incubated in air-saturated water for 15 min. A negative control film was electrochemically-discharged to the Q state (by the reaction in Scheme 1c) and then incubated in water for 15 minutes. Figure 4c shows 4.5 and 0.1 µM H_2O_2 were generated by these positive and negative controls.

Additional controls in Figure 4c involved incubation of the catechol–chitosan films (initially in Q state) with 2 of 3 components of the enzymatic system. Low levels of H_2O_2 were generated by these controls. A final control in Figure 4c is an unmodified chitosan film initially incubated in the enzymatic charging solution. The low level of H_2O_2 generated by this chitosan control film is consistent with its lack of redox-activity.[30]

In the next experiment we examined the discharging of partially-charged films. In this case, we enzymatically-charged the films with varying levels of glucose and then discharged the electrons to O_2. Figure 4d shows that the films' H_2O_2-generation increased monotonically with glucose with an apparent saturation when high levels of glucose were used for charging.

Figure 3 demonstrates that the catechol–chitosan film can be enzymatically-charged by an $NADP^+$-mediated redox-cycling reaction. As illustrated by the left-most box in **Figure 5**a, enzymatic-charging can be characterized by an AND logic gate where both an electron source (glucose) *and* the redox-cycling components (GDH and $NADP^+$) must be present. In Figure 3, enzymatic-charging of the film was detected chronocoulometrically by anodically discharging the film in the presence of the Fc mediator. In Figure 4, enzymatically-charged films were discharged by donating their electrons to O_2 to generate H_2O_2. These results suggest that discharging could be described as an AND logic gate with either an electrical output (Figure 3) or a chemical output (Figure 4). In a final experiment, we examined whether the H_2O_2 output could drive an enzymatic reaction such that the AND gate would yield a biochemical output.

The central box in Figure 5a illustrates a logic gate where an enzymatically-charged film (input A) is exposed to O_2 (input B) to generate H_2O_2 and this chemical serves as the substrate for the horseradish peroxidase (HRP) catalyzed enzymatic reaction. Experimentally, a discharged film was either used directly (input A = 0) or enzymatically-charged for 15 min with glucose (10 mM) (input A = 1). These films were then removed from the charging solution and incubated in 200 µL of a solution that was either saturated with pure O_2 (input B = 1) or pure N_2 (input B = 0). Finally, we separated the resulting solution and incubated it in an individual well of a multi-well plate with horseradish peroxidase (0.2 U/ml) and the fluorogenic substrate amplex red as illustrated in the right-most box in Figure 5a.

Figure 5b shows fluorescence images of the individual wells after 30 min incubation in the HRP-amplex red assay solution. As expected, fluorescence is only observed if the films had been charged and then exposed to O_2. As suggested in Figure 5b, a fluorescence intensity cutoff of 2,600 could be used to generate the truth table in Figure 5c.

The biochemical logic operations in Figure 5 can be contrasted with a common alternative in which glucose oxidase directly transfers electrons to O_2 to generate H_2O_2 which can then be detected by a peroxidase-catalyzed reaction. What distinguishes the use the redox-capacitor film is that it allows information (i.e., electrons) to be stored for processing at a later time and in a different location: charging and discharging can be independently controlled. Further, the ability to accumulate and store electrons may provide a simple means to amplify outputs to enhance sensitivity.

3. Conclusions

In conclusion, we report that a fabricated catechol-modified chitosan film possesses redox-capacitor properties under biorelevant conditions. We suggest three aspects of this work are significant. First, the redox-properties are imparted to a biological film by the grafting of organic (i.e., catecholic) moieties that are also abundant in nature.[21,33] Thus, these results could provide a useful step toward the creation of soft electronics that are biocompatible, biodegradable and potentially even edible (chitosan and phenolics are commonly ingested).[34,35] Second, the films can be charged/discharged through either electrochemical or biochemical inputs/outputs. Thus, the films

can establish bio-device "redox-connectivity" that should facilitate efforts to integrate biology and electronics for information processing[12–15] and energy conversion.[5,7–9] Third, the redox-activities of these films may mimic the behaviors of natural phenolic/quinone materials. Phenolic materials are abundant in nature (e.g., humics, melanin and lignin) and are often reported to be sources,[36] sinks[37] and shuttles[38–43] for extracellular electron transfer (e.g., in soil) with importance for mineral cycling, anaerobic respiration and bioremediation. From a signaling perspective, biological electron "leakage" (e.g., from mitochondrial ubiquinone)[44–46] to O_2 yields ROS that serve as a signaling molecules for a range of biological phenomena.[47–56] These similarities in the activities of the catechol–chitosan films with the behavior of natural phenolic materials suggests that lipid bilayers may not be the only capacitor-type employed by biology for signal processing and energy transduction.

4. Experimental Section

The following were purchased from Sigma-Aldrich: chitosan, catechol, $Ru(NH_3)_6Cl_3$ (Ru^{3+}), 1,1′-ferrocenedimethanol (Fc), glucose dehydrogenase (GDH), and β-nicotinamide adenine dinucleotide phosphate sodium salt ($NADP^+$). The water (>18 MΩ) used in this study was obtained from a Super Q water system (Millipore). Chitosan solutions (1%, pH 5.6) were prepared by dissolving chitosan flakes in HCl to achieve a final pH of 5–6. The solutions of catechol and mediators (Fc and Ru^{3+}) were prepared in phosphate buffer (0.1 M; pH 7.0). Gold-coated silicon wafers were prepared using standard microfabrication methods.

Fabrication of the catechol-modified-chitosan film was performed in two steps. First, chitosan was electrodeposited by immersing the gold-coated electrode into a chitosan solution (1%, pH 5.6) and applying a cathodic voltage to achieve a constant current density (4 $A\text{-}m^{-2}$, 30 s) using a DC power supply (model 6614C Agilent Technologies) and a two electrode system (Pt foil anode). Second, the chitosan film was modified with catechol by immersing the chitosan-coated electrode into a catechol solution (5 mM, 0.1 M phosphate buffer, pH 7.0) and applying an anodic potential (+0.6 V, 5 min) in a three electrode system (CHI Instruments 6273C electrochemical analyzer with Ag/AgCl as a reference electrode and Pt wire as a counter-electrode).

Electrochemical charging of the catechol–chitosan film (to QH_2 state) was performed by immersing this film-coated electrode into 50 μM Ru^{3+} and applying a constant voltage of –0.4 V for 2 min using a three electrode system described above. Electrochemical discharging of the catechol–chitosan film (to Q state) was performed by immersing the film-coated electrode in 50 μM Fc and applying +0.5 V for 2 min.

Enzymatic charging of the catechol-modified-chitosan film was performed by incubating a discharged film (initial Q state generated by electrochemical discharging as described above) in 200 μL of GDH (5 U/mL), $NADP^+$ (2.5 mM), and glucose for 15 minutes. Discharging the film to O_2 was performed by incubating an enzymatically charged film in 200 μL of H_2O for 15 min and then analyzing the liquid for H_2O_2 by the ferrous mediated oxidation in the xylenol orange using standard assay kit (PeroXoquant Quantitative Peroxide Assay Kits, Piecrce, IL).[57] Horseradish peroxidase (HRP) reactions were performed with the Amplex Red reagent (Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit, Invitrogen, OR) and results were measured using a fluorescence plate reader (SpectraMax M2, Molecular Devices, CA).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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