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Reverse Engineering To Suggest Biologically Relevant Redox Activities of Phenolic Materials

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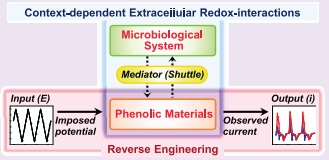
Supporting Information

ABSTRACT: Phenolics are among the most abundant redox-active organics in nature, but the intractability of phenolic materials (e.g., melanin) has precluded study of their biological activities and functions. Previous studies demonstrated that a model abiotic catecholic matrix can rapidly exchange electrons with biological oxidants and reductants without the need for enzymes. Here, a novel electrochemically based reverse engineering approach was employed to probe redox interactions between this model matrix and a population of bacteria. Specifically, this method employs redox-active natural products (e.g., pyocyanin) to shuttle electrons between the bacteria and the abiotic matrix, and imposed oscillating potential inputs to engage redox-cycling mechanisms that switch the matrix's redox state. The oscillating output currents were observed to be amplified, gated, and partially rectified, while the overall magnitude and direction of electron flow across the matrix depended on the biological and environmental context. These response characteristics support hypotheses that natural phenolic materials may be integral to extracellular electron transport for processes that include anaerobic respiration, redox signaling, and redox-effector action.



Phenolics are a diverse and complex group that includes small molecule natural products and biomacromolecular materials.[1] In some cases, phenolics are known to perform mechanical functions in biology: insects use low molecular weight phenolic cross-linkers to harden and seal their cuticle,[2] and marine mussels use the phenolic residues of their adhesive protein to adhere to surfaces.[3,4] Despite these examples, a comprehensive understanding of the biological activities and functions of phenolic materials is generally lacking. In particular, phenolics are probably the most abundant redox-active organics in nature, but the biological relevance of these redox activities are largely unknown. Paradoxically, clues to biological function may come from technological investigations of the "electronic" properties of nature-derived phenolics.[5,6] Forty years ago melanins were reported to possess (semi)-conducting properties,[7,8] and more recently, lignin derivatives were reported to improve the properties of conducting polymers.[9]

A common approach to infer the function of a complex technological system (e.g., an integrated circuit) is to study the response characteristics using reverse engineering methodologies. These methodologies often impose oscillatory inputs and analyze outputs using principles from control theory. Increasingly, reverse engineering is being applied for the top-down, systems-level study of complex biological systems[10,11] such as genetic,[12,13] metabolic,[14] and neuronal[15] networks. Key challenges to using reverse engineering for biology are imposing time-varying inputs and measuring time-varying outputs of variables that are biologically relevant.[16,17] We contend that redox biology is uniquely amenable to reverse engineering because electrochemical methods allow biologically relevant redox inputs to be imposed and outputs to be observed in real time.

Here, we developed an electrochemically based reverse engineering method to probe the biologically relevant and context-dependent redox interactions of a model catecholic matrix. As described previously (see Supporting Information), the model matrix is fabricated by grafting catechol moieties to a hydrogel film of the aminopolysaccharide chitosan.[18] This model matrix is permeable to small molecules (i.e., mediators), has redox activity consistent with catechol-quinone moieties (E° = +0.2 V vs Ag/AgCl), and is readily switched between reduced and oxidized states as illustrated in Scheme 1.[19,20] Importantly, this catecholic matrix is redox-active but non-conducting as electrons do not flow freely in response to an applied potential but must be transferred by soluble mediators (i.e., shuttles). Also important is that the catechol-chitosan matrix can exchange electrons with a diverse range of soluble mediators including biologically relevant oxidants and reductants.[20,21]

We report that the bacterial metabolite pyocyanin can transfer electrons from an anaerobically respiring microbial population to the matrix and that the matrix can transfer electrons to O_2 to induce a change in an *E. coli* intracellular H_2O_2-indicator protein. Next, we employed the electrochemically based reverse engineering method by imposing cyclic potential inputs in the presence of two redox-active natural products, pyocyanin (E° = −0.2 V) and the plant metabolite acetosyringone (E° = +0.5 V). The observed output currents displayed a surprisingly rich repertoire of response characteristics with the overall magnitude and direction of electron

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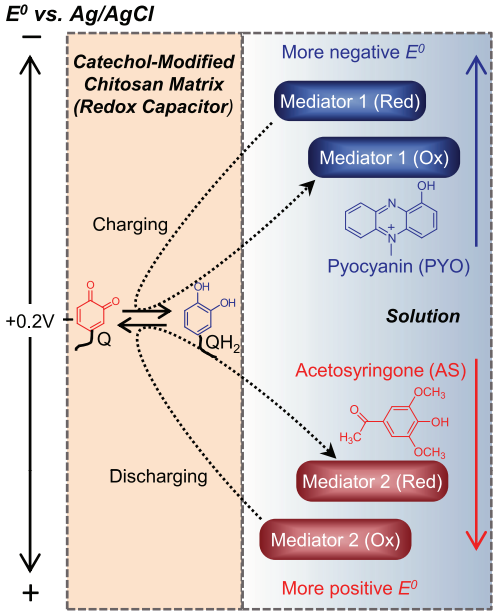
Scheme 1. Redox-Capacitor Properties of a Catecholic Matrix[a]



[a]The matrix is charged by accepting electrons from one mediator, stores electrons as reduced (QH_2) moieties, and is discharged by donating electrons to a second mediator.

"flow" across the matrix varying with the biological and environmental context. The broad biological implications of these results are discussed.

RESULTS AND DISCUSSION

Mediated Electron Transfer from Bacteria to the Matrix. Scheme 1 illustrates that the catechol-chitosan matrix is charged by the conversion of moieties from oxidized to reduced states;[20] presumably *o*-quinones (Q) to catechols (QH_2). The charged matrix can store electrons in this reduced (QH_2) state and thus serves as a redox capacitor. Figure 1a hypothesizes that matrix-charging can be achieved by a redox-cycling mechanism in which the pyocyanin mediator accepts electrons from a cell and transfers them to the matrix. Figure 1a also shows the thermodynamics for electron transfer from various intracellular redox couples to pyocyanin and then to the film.

Figure 1b illustrates that a dual-film system was prepared to provide evidence that pyocyanin can transfer electrons from a microbial population to the catechol-chitosan matrix. The first film, the catecholic matrix, was fabricated in two steps: electrodeposition of the stimuli-responsive aminopolysaccharide chitosan followed by electrochemical grafting of the redox-active small molecule catechol.[1] The second film, a model biofilm, was prepared by electrodepositing *E. coli* with alginate on top of the catechol-chitosan matrix.[22] This "(cat-chit)-biofilm" assembly served as the working electrode in a 3-electrode system that was immersed in LB medium supplemented with pyocyanin (50 μM). Before measurements were taken, N_2 was bubbled into the medium and then the working electrode was biased to 0 V (vs Ag/AgCl) and the current was monitored. The middle curve in Figure 1b shows the anodic current associated with the electrochemical oxidation of pyocyanin for this (cat-chit)-biofilm.

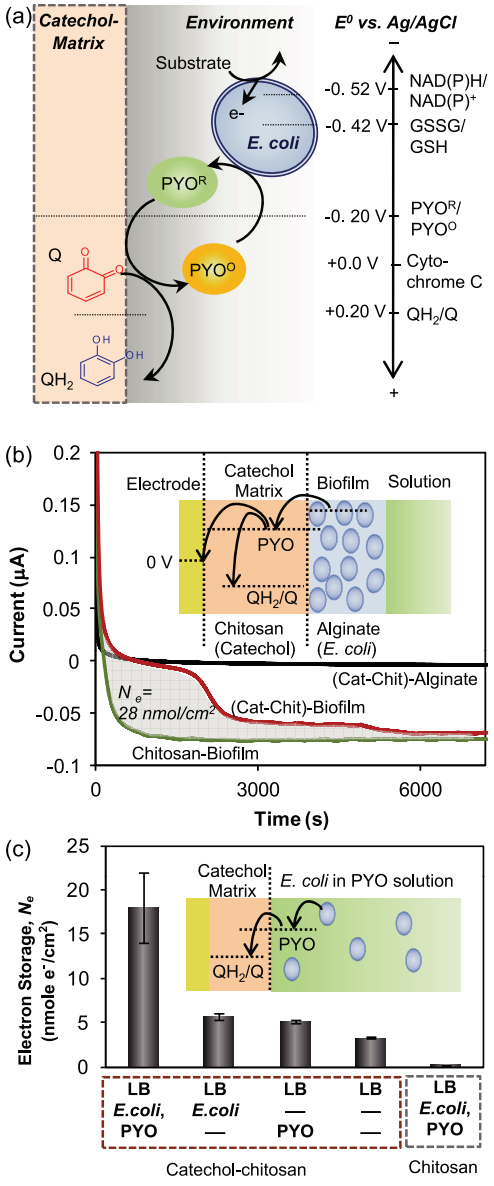


Figure 1. Microbiological charging of the catechol matrix under O_2-free conditions. (a) Schematic of pyocyanin-mediated electron transfer from bacteria to the matrix. (b) Chronocoulometric evidence of the matrix's redox capacitor properties; it "intercepts" electrons being transferred from the biofilm to the anode. (c) Electrons transferred to the catechol matrix during a 10-min incubation.

There are two controls for Figure 1b. The first control is an alginate film (without *E. coli*) deposited onto a catechol-chitosan matrix. This "(cat-chit)-alginate" control lacks biological activity and should be unable to reduce pyocyanin. As expected, Figure 1b shows negligible anodic currents for pyocyanin reoxidation. The second control is an *E. coli*-containing alginate biofilm deposited on top of a chitosan film (without grafted catechols). Results for this "chitosan-biofilm" control show that a steady state anodic current of 0.074 μA (1.6 μA cm^{-2}) is rapidly achieved. This observation indicates that pyocyanin is reduced by the biofilm and the reduced pyocyanin diffuses across the chitosan film and is reoxidized by the electrode.

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The capacitor properties of the catechol-chitosan matrix is assessed by comparing the behavior of the (cat-chit)-biofilm with the chitosan-biofilm (the bottom 2 curves in Figure 1b). Assuming the biofilms have the same pyocyanin-reducing-abilities, then differences in output currents should reflect catechol-chitosan's capacitor properties. At short times, the catechol-chitosan matrix is expected to "intercept" electrons being transferred from the biofilm to the electrode by the pyocyanin redox-cycling mechanism of Figure 1a; thus anodic currents should be suppressed. At long times, after the catechol-chitosan matrix has been fully charged, pyocyanin-mediated electron transfer between the biofilm and electrode should be unimpeded; thus oxidative currents should be the same for the (cat-chit)-biofilm and the chitosan-biofilm. These short-time and long-time expectations are observed in Figure 1b, while the shaded area between the two curves estimates a redox-capacity of 28 nmol electrons cm^{-2} for the catechol matrix, which is consistent with previous measurements.[20,21]

In a separate study, we examined the catechol-chitosan matrix's ability to accept electrons from a microbial community by immersing a matrix-coated electrode in an anaerobic suspension of *E. coli* (optical density 3) in LB medium containing pyocyanin (300 μM). After incubating for 10 min, the matrix-coated electrode was removed from the suspension, and the extent of charging was determined using a previously described chronocoulometric method.[21] Figure 1c shows that the 10-min incubation with *E. coli* and pyocyanin resulted in the transfer of 17 nmol electrons cm^{-2} to the matrix. Controls in which the matrix-coated electrodes were incubated in solutions lacking *E. coli*, pyocyanin, or both showed little matrix charging. Finally, Figure 1c shows that a control chitosan-coated electrode (without grafted catechol) possesses negligible electron-accepting activity (unmodified chitosan lacks redox activity). In summary, the results in Figure 1 demonstrate that the catechol-chitosan matrix can interact with a microbial community through a pyocyanin-mediated redox-cycling mechanism that transfers electrons from the microbe to the matrix.

Electron Transfer from the Matrix To Induce Microbial Response. In previous studies, we demonstrated that catechol-chitosan matrices could be discharged in the presence of air to generate reactive oxygen species (ROS).[21] Figure 2a illustrates an overall H_2O_2-generating reaction, while the dotted lines indicate that the reaction mechanism is more complex and less well-characterized than the simple 2-electron transfer reaction shown.[23−26]

Presumably, this matrix-generated ROS could interact with neighboring biological systems. To provide evidence for such a redox interaction, we charged a catechol-chitosan matrix by a 10-min incubation in an *E. coli* (OD of 1) suspension containing pyocyanin (100 μM), transferred the charged matrix to air-saturated H_2O (200 μL) for 15 min, and then tested aliquots of the resulting solution chemically and biologically.

Chemical analysis involved assaying aliquots for H_2O_2 using horseradish peroxidase (Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit, Invitrogen, OR). Figure 2b shows that ~13 μM of H_2O_2 was generated by the spontaneous discharging of the catechol-chitosan matrix. Controls in which the catecholic matrix was incubated without *E. coli*, pyocyanin, or both showed low levels of H_2O_2 generation. A final control in Figure 2b is an unmodified chitosan film incubated with both *E. coli* and pyocyanin for 10 min; negligible H_2O_2 was measured for this control. These results confirm that catechol-chitosan

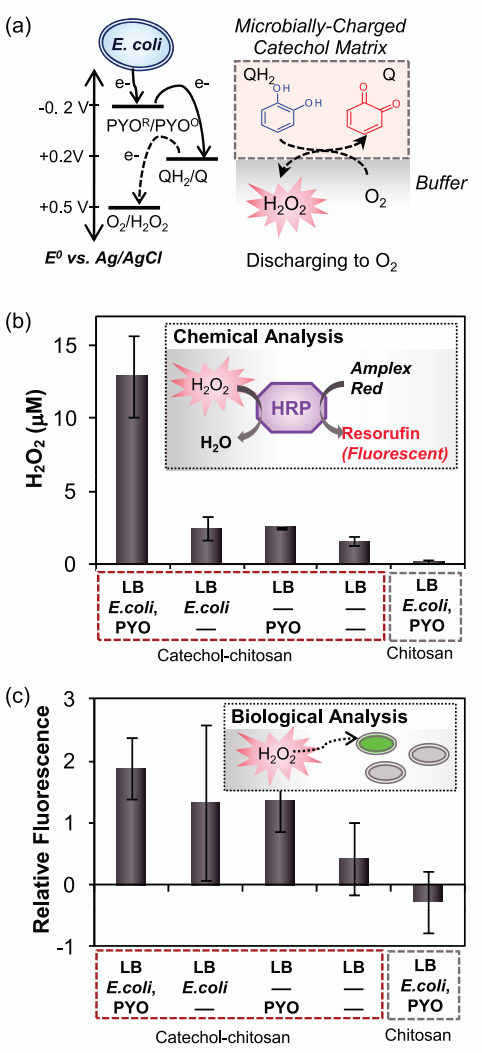

Figure 2. Discharging of the matrix by O_2 generates reactive oxygen species (ROS) and induces a biological response. (a) Schematic. (b) Chemical analysis of H_2O_2 generated from the microbially charged films. (c) Biological response of intracellular H_2O_2-responsive HyPer protein.

matrices that have been charged by accepting electrons from a microbial community can store these electrons and discharge them to O_2 to generate ROS.

Biological analysis involved incubating the H_2O_2-containing aliquots with *E. coli* expressing HyPer protein that undergoes a rapid H_2O_2-responsive conformation change that can be detected fluorescently.[27] The results in Figure 2c show a similar trend in the biological fluorescence response as observed in the chemical analyses. These results provide evidence that electron transfer from the film (in the form of ROS) can affect biological systems. (The Supporting Information provides details of Hyper-expressing *E. coli*, a standard curve of fluorescence vs H_2O_2, and additional experimental support that film discharging elicits a biological response.)

Oscillating Input and Output to Matrix under Abiotic Conditions. Scheme 1 illustrates that two separate mediators from solution can diffuse into the matrix and engage independent redox-cycling mechanisms to either charge or discharge the matrix. The results in Figure 1 indicate that

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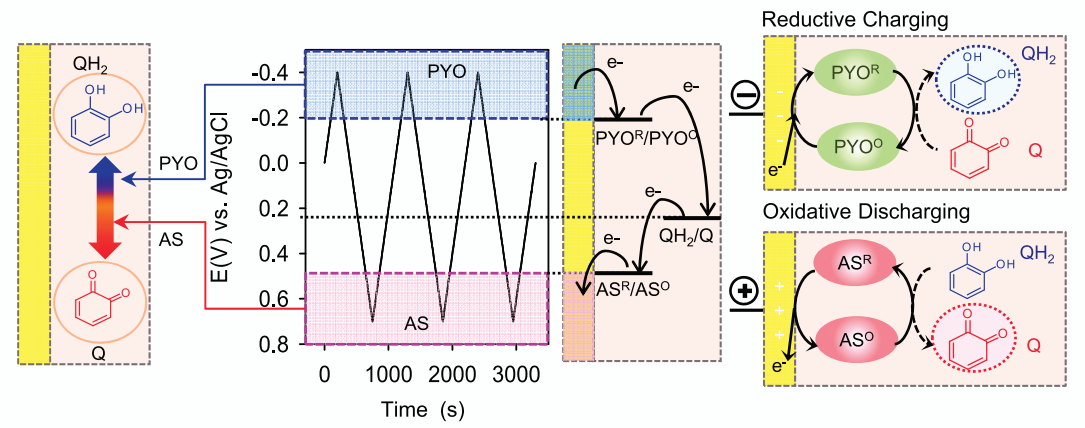
Scheme 2. Electrochemical Redox-Cycling Reactions under O_2-Free Conditions[a]



[a]Reactions with the natural products pyocyanin (PYO) and acetosyringone (AS) can charge and discharge the catechol-chitosan matrix under O_2-free conditions.

pyocyanin (PYO) can serve as the electron-donating mediator for matrix charging, while previous studies suggest the natural phenol acetosyringone (AS) can serve as the electron-accepting mediator for matrix discharging.[21] Scheme 2 suggests that these redox-active natural products can also exchange electrons with the electrode and thus could engage matrix-electrode redox-cycling reactions for charging and discharging. Scheme 2 further illustrates that the electrochemically mediated charging and discharging of the matrix is controlled by the imposed potential and thus cyclic potential inputs can drive cyclic charging and discharging reactions. We tested several features suggested in Scheme 2.

One feature suggested in Scheme 2 is that the pyocyanin mediator can charge but not discharge the matrix. Experimentally, we prepared a matrix-coated electrode, incubated it in the presence of the charging mediator (50 μM pyocyanin), and generated the cyclic voltammogram (CV) in Figure 3a. This CV shows large reducing currents but minimal oxidation currents. Controls with chitosan-coated electrodes (without grafted catechol) and matrix-coated electrodes incubated without pyocyanin show small currents for both oxidation and reduction. The observation that the catechol-chitosan matrix *amplifies* peak currents for pyocyanin reduction compared to controls is consistent with a redox cycling of this mediator to charge the matrix. The observation of large reducing but small oxidation currents, or a *partial rectification*, is consistent with an inability of pyocyanin to discharge the matrix.

A second feature of Scheme 2 is that electrochemical discharging of the matrix requires a second mediator with an E° > +0.2 V. Figure 3b compares CVs for matrix-coated electrodes incubated with two mediators (50 μM each): pyocyanin for charging and either ferrocene dimethanol (Fc)[19] or acetosyringone (AS)[21] for discharging. As expected, the matrix amplifies output currents (both for reduction and oxidation) compared to the control electrode coated with chitosan (without grafted catechol). Thus, a second mediator with a more positive redox potential (either Fc or AS) can discharge the catechol matrix.

A separate observation from Figure 3b involves the discharging potential. When Fc is used, the oxidative discharging current is drawn when the potential exceeds ca. +0.1 V. In contrast, when AS is used, the discharging current is not drawn until the potential exceeds ca. +0.4. This discharging behavior results because (i) a mediator is required to discharge the film, and (ii) discharging is only initiated when this mediator is oxidized. Because the redox potential of AS ($E^\circ \approx$ +0.5 V) is larger than that of Fc ($E^\circ \approx$ +0.25 V), a larger applied potential is required to initiate the redox-cycling discharging mechanism. The important point is that despite a large thermodynamic driving force, the film cannot be discharged until the mediator is oxidized. Thus, the mediator *gates* oxidative discharging.

A third feature suggested in Scheme 2 is that the independent redox-cycling mechanisms for charging and discharging allow cyclic input potentials to be used to generate "steady" output currents. To test this feature we added a matrix-coated electrode to a solution containing both pyocyanin and AS (50 μM each) and imposed the cyclic input potential illustrated in Figure 3c. The output current in Figure 3c is amplified compared to output from an electrode coated with chitosan (without grafted catechol). Further, the output currents appear steady over the time-course of the experiment. In addition, we integrated the current to yield the output charge ($Q = \int i\, dt$), and the Q vs t plot shows that the two redox-cycling mechanisms allow amplification of the output for the matrix-coated electrode vs the chitosan-coated control. Also, the upper Q vs t curve shows a net transfer of electrons from the electrode to the matrix when the matrix-coated electrode was contacted with only pyocyanin (without a discharging mediator). We estimate that over this 240-s experiment 24 nmol electrons cm^{-2} have been electrochemically transferred to the matrix by this pyocyanin-mediated charging reaction.

In summary, Figure 3 shows information-rich response characteristics associated with the redox interactions for this "simple" abiotic system composed of two redox-active natural products, the catechol-chitosan matrix and cyclically imposed potential inputs.

Context-Dependent Redox Interactions. The catechol-chitosan matrix along with the two mediators undergo a network of interactions that vary depending on the localized microbial and environmental redox activities. In particular, Scheme 3 shows that pyocyanin can "sample" redox information in its environment while the catechol-chitosan matrix "processes" this redox-information. To illustrate this capability, we incubated the matrix-coated electrode with both pyocyanin and AS under various redox conditions and probed

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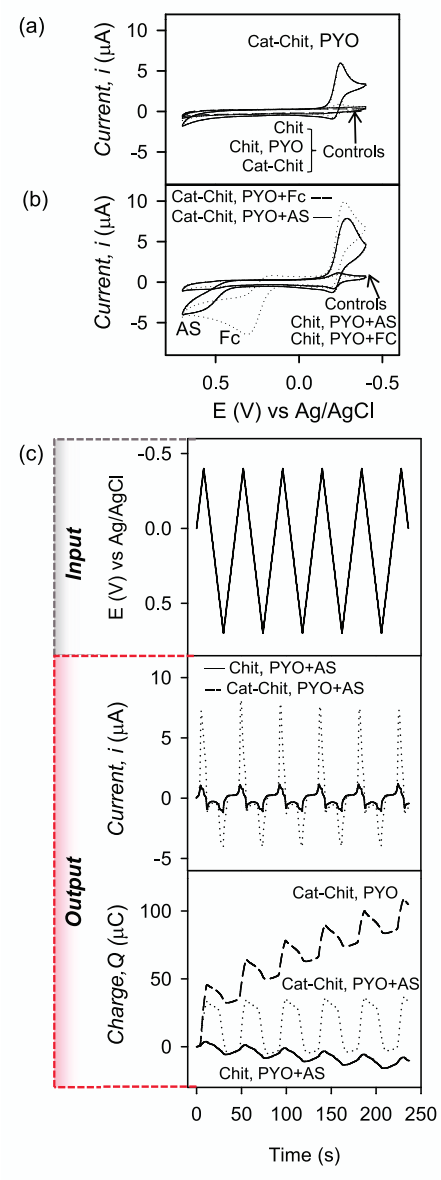
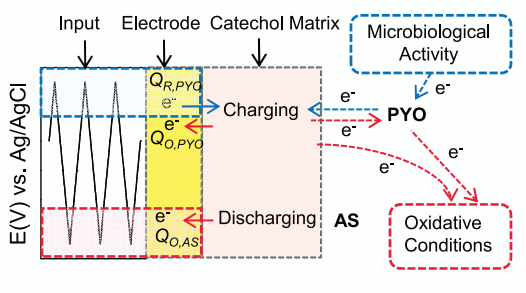


Figure 3. Redox-response characteristics of an abiotic system composed of catechol-chitosan matrix plus two redox-active natural products (i.e., mediators). (a) CVs for the matrix with pyocyanin show amplification and partial rectification of charging currents (relative to controls). (b) CVs for the matrix with two mediators show that discharging currents are gated by the mediator (ferrocene dimethanol, $E^{\circ}_{FC} \approx +0.25$ V; acetosyringone, $E^{\circ}_{AS} \approx +0.5$ V). (c) Input potential and output current and charge transfer ($Q = \int i\, dt$) curves.

the system using cyclic potential inputs. (Control studies performed with a chitosan-coated electrode are provided in Supporting Information.)

Figure 4 shows a schematic of the experimental system, CVs, and input/output plots for four redox conditions. The first condition is a non-interacting condition that involved incubation under abiotic, N_2 conditions in which the mediators did not interact with their environment (i.e., pyocyanin could neither accept electrons from a biological source nor donate them to an alternative oxidant). This non-interacting (Abiotic, N_2) condition serves as a baseline control. As expected, the Q vs t curve for this non-interacting case shows the output Q oscillates around a value of zero—the reducing current

Scheme 3. Network of Redox Interactions[a]



[a]Pyocyanin "samples" its redox-environment, and the catechol matrix "processes" this redox information.

associated with matrix charging is balanced by the oxidative discharging currents.

In the second condition (Biotic, N_2), metabolically active *E. coli* was added to the system under anaerobic conditions. These bacteria reduce pyocyanin, and the reduced pyocyanin can either charge the catechol matrix or diffuse through the matrix to be electrochemically oxidized. Figure 4 shows that these redox interactions perturb the output compared to the non-interacting control. Specifically, a peak current is observed for pyocyanin's electrochemical oxidation that is apparent in both the CV and i vs t curves. Further, the Q vs t curve displays a downward slope indicating a net transfer of electrons to the electrode for this (Biotic, N_2) condition.

In a third condition (Abiotic, O_2), the matrix-coated electrode was incubated under aerobic conditions without *E. coli*. When present, O_2 significantly perturbs output because O_2 can accept electrons from (i) the electrode (for cathodic potentials less than −0.2 V), (ii) pyocyanin,[28] (iii) the capacitor film,[21] and (iv) bacteria (if present). Consistent with expectations for this (Abiotic, O_2) condition, the CV and i vs t curves in Figure 4 show enhanced pyocyanin reducing currents; presumably electrochemically reduced pyocyanin can donate its electrons to oxygen. Also expected is the upward slope of the Q vs t curve, which indicates the net transfer of electrons from the electrode for this (Abiotic, O_2) condition.

In the final condition (Biotic, O_2), the matrix-coated electrode was exposed to metabolically active *E. coli* under aerobic conditions. Under this condition, the reducing activities of the bacteria are partially balanced by the oxidative activities of O_2. As expected, Figure 4 shows that the CV, i vs t and Q vs t curves for the (Biotic, O_2) condition are intermediate between the (Biotic, N_2) and (Abiotic, O_2) conditions.

Correlations from Reverse Engineering. The results in Figure 4 indicate that pyocyanin and AS engage independent redox-cycling mechanisms with the catecholic matrix and that a cyclic potential input can be imposed to generate "steady" cyclic outputs. Oscillating inputs are commonly imposed in technology to acquire information of complex dynamic phenomena (e.g., impedance spectroscopy). In this study, we demonstrate that results collected at a single frequency are rich with information on redox interactions. Specifically, we imposed cyclic input potentials to probe how mediator currents across the catechol-chitosan matrix vary depending on the redox context of the local environment.

To "extract" redox information and establish correlations, Figure 5a shows that we examined three regions of the CV. The first region is associated with the electrochemical reduction of

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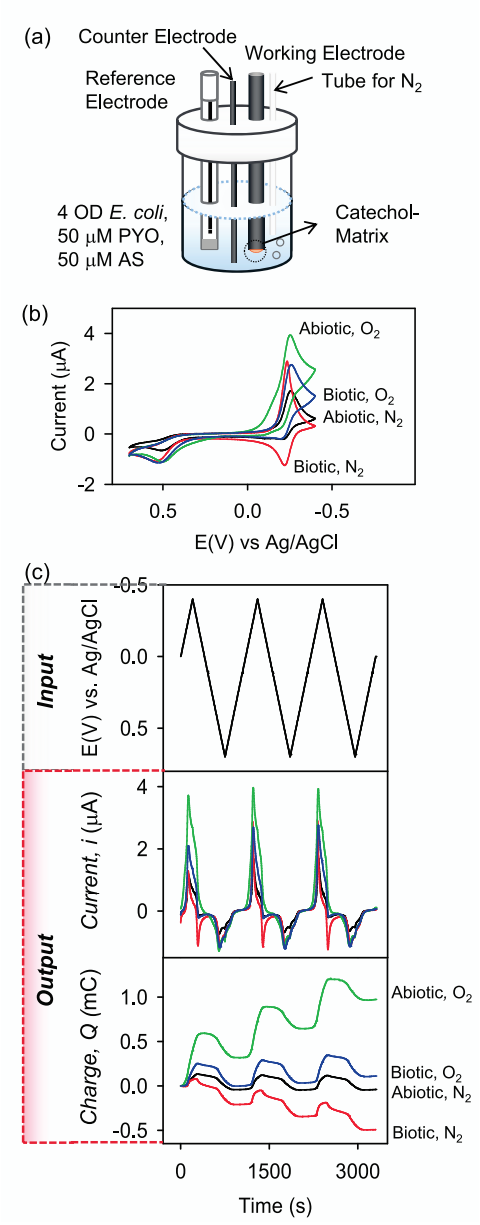

Figure 4. Context-dependent response of matrix-mediator system. (a) Schematic of experimental system. (b) CVs for four experimental conditions: aerobic (O_2), anaerobic (N_2), biotic (with *E. coli*), and abiotic (without *E. coli*). (c) Cyclic potential input (E vs t) and output current (i vs t) and output charge (Q vs t) curves for the four conditions.

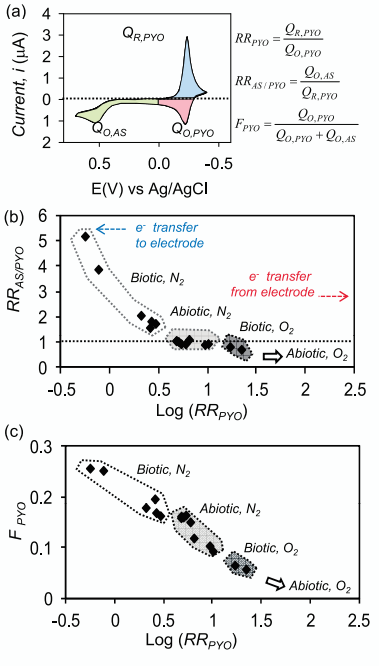

Figure 5. Correlations illustrating the matrix's response characteristics to localized redox conditions. (a) Parameters calculated from the CVs were used to correlate data. (b) Correlation between rectification ratios (*RR*s). (c) Correlation of the fraction of the total oxidation attributed to pyocyanin (F_{PYO}).

pyocyanin, and this charge transfer is designated $Q_{R,Pyo}$. The second region is associated with the electrochemical oxidation of pyocyanin and is quantified as $Q_{O,Pyo}$. As discussed, the catechol-chitosan matrix offers rectification properties, and one correlating parameter defined in Figure 5a is the apparent rectification ratio RR_{PYO}. The third region in Figure 5a is associated with acetosyringone oxidation ($Q_{O,AS}$).

Under conditions where pyocyanin rectification is complete and there are no environmental redox activities, then pyocyanin reduction should be balanced by acetosyringone oxidation ($Q_{O,AS} \approx Q_{R,Pyo}$). For this non-interaction case, the second rectification ratio defined in Figure 5a, $RR_{AS/PYO}$, should be 1. In agreement with this expectation the $RR_{AS/PYO}$ for the (Abiotic, N_2) condition in Figure 4 was calculated to be ca. 0.95. We performed additional experiments under analogous non-interacting conditions in which the catechol-chitosan matrix was exposed to environmental conditions that lacked redox activities (e.g., killed bacteria or latex beads under N_2 conditions). The dotted line in Figure 5b illustrates $RR_{AS/PYO} \approx 1$ for these non-interacting conditions (Abiotic, N_2). (Details of these additional non-interacting conditions are provided in Supporting Information.)

In the (Biotic, N_2) condition in which *E. coli* was incubated under anaerobic conditions, pyocyanin's electrochemical oxidation ($Q_{O,Pyo}$) is increased and its reduction ($Q_{R,Pyo}$) is decreased, and thus this perturbation lowers the calculated RR_{PYO}. Further, the decrease in pyocyanin reduction is accompanied by an increase in acetosyringone oxidation ($Q_{O,AS}$). Thus, the data at the upper left in Figure 5b for this (Biotic, N_2) condition are consistent with expectations that microbiological activities lead to a net transfer of electrons to the electrode. (As indicated in Supporting Information, results with *Pseudomonas aeruginosa* under N_2 conditions are also incorporated in Figure 5.)

The (Abiotic, O_2) condition resulted in large pyocyanin reducing currents ($Q_{R,Pyo}$), negligibly small pyocyanin oxidizing currents ($Q_{O,Pyo}$), and a very large RR_{PYO} that was too large to calculate. In the final (Biotic, O_2) condition, the reducing activities of the bacteria are partially balanced by the oxidative activities of O_2. Figure 5b shows this (Biotic, O_2) condition is distinguished from the (Biotic, N_2) condition by an increase in RR_{PYO} and a decrease in $RR_{AS/PYO}$.

A more intuitive correlating parameter defined in Figure 5a is the fractional oxidation current attributed to pyocyanin (F_{PYO}). In principle, pyocyanin "samples" its redox environment; it is reduced by bacteria and oxidized by O_2. This information is "reported" to the electrode as a combination of oxidation and

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reduction currents. The catechol-chitosan matrix serves to amplify and distort this output in ways that facilitate interpretation. For instance, if pyocyanin undergoes oxidation with its environment, then there is proportionally less electrochemical pyocyanin oxidation and the output trends to the lower right in Figure 5c. In contrast, if pyocyanin is reduced by its environment, there is proportionally more electrochemical oxidation and the data trends toward the upper left in Figure 5c

The correlations in Figure 5 support two general conclusions. First, electrochemical methods are uniquely suited to perform systems-level reverse-engineering studies in redox biology. Figure 5 shows that redox signatures characteristic of a system ($Q_{R,Pyo}$, $Q_{O,Pyo}$, and $Q_{O,AS}$) can be analyzed to assess context-dependent interactions. Second, the catecholic matrix's ability to participate in electron transfer reactions significantly alters these redox signatures (e.g., Figure 3b), which should enable a probing of putative biological activities of phenolic materials.

Biological Relevance of Experimental Observations. The reverse engineering of biological systems aims to allow the function of complex networks to be inferred from a study of their response characteristics.[10,11] Here, we used an electrochemically based approach to probe a model catecholic matrix and observed a surprisingly rich repertoire of output responses to various biologically relevant redox inputs. In the following, we suggest potential biological relevance for these observed response characteristics.

Results with pyocyanin (Figure 1) indicate that redox-active metabolites allow electrons to be transferred from biology to the matrix. Pyocyanin and related phenazines are secreted by *Pseudomonas* bacteria, which are capable of colonizing a wide range of niches. For plants and animals, *Pseudomonas* is an opportunistic pathogen and pyocyanin is a virulence factor that presumably accesses the host's reducing equivalents and disrupts the host-cell redox state.[29,30] *Pseudomonas* also inhabits soil, and the ability of phenazines to export electrons may provide a mechanism for anaerobic respiration. Potentially, redox cycling of these phenazines would enhance anaerobic respiration,[31] and the phenolic components of soils (i.e., humics) could serve as an electron sink.[32,33] There is considerable current interest in understanding such extracellular electron transfer processes to inform technological efforts for soil remediation[34] and possibly even to facilitate the development of microbial fuel cells and bioelectrosynthesis.[35−37]

Acetosyringone (AS) is a phenolic natural product, and we observed that, once oxidized, AS can accept electrons to discharge the catechol matrix (Figure 3). We oxidized AS electrochemically, but in nature AS is oxidized enzymatically (e.g., by laccase or peroxidase). AS and related phenolic small molecules have been suggested to be diffusible mediators used by fungi for delignification and are being considered for technological delignification processes.[38] AS is also involved in plant-pathogen recognition.[39] One intriguing observation is the report that AS is produced by tobacco upon infection by *Pseudomonas syringae* and AS is subsequently consumed during the plant's oxidative burst.[40] While the relevance to our current study is unclear, the possibility exists that the 3 components of our abiotic system are also present in this plant-pathogen example; the plant contributes the AS mediator, *P. syringae* contributes the phenazine mediator, and lignin may be deposited at the plant-pathogen interface as part of the plant's defense response.[41]

Both the phenazine pyocyanin and the phenolic AS are redox-active, and each is believed to participate in host−pathogen interactions. Recent research with phenazines is demonstrating that redox-active natural products can perform various biological functions that range from signaling to inducing oxidative stress.[28,42] Interestingly, redox-active phenolics appear to be broadly used by plants to perform signaling/effector defense functions. Salicylic acid is a well-known internal signaling molecule that elicits the plant's systemic acquired resistance to pathogens,[43] and various secreted phenolics are reported to mediate activities between organisms. For instance, the root exudate catechin appears to be toxic to soil bacteria[44] and other plants.[45] Potentially, the redox and biological activities of these natural products are interrelated,[42,46] and possibly the molecule's information/action depends on its redox state (oxidized or reduced). Results from the current study indicate that the catechol-chitosan matrix can alter these molecules' redox state—pyocyanin can be oxidized and AS can be reduced. Thus, our results suggest that phenolic matrices in nature (e.g., the humic component of soil) may intercede in the flow of redox information and alter redox-effector actions.

While the suggestion that redox-active natural products transmit information may be speculative, the ability of the catechol-chitosan matrix to generate reactive oxygen species (ROS; Figure 2) provides a direct link to established biological redox-signaling mechanisms. There is growing evidence that H_2O_2 serves as a long-distance ROS-signaling molecule in biology.[47−49] The results with the catechol matrix suggest that phenolic materials in nature may provide a means to generate redox signals at locations outside the cell and without the need for enzymes or direct contact with the cell membrane.

Biology uses low levels of ROS for signaling, while higher levels of ROS are used to perform effector functions (e.g., in host−pathogen interactions). Interestingly, both plants and insects can generate extracellular phenolic matrices as part of their innate immunity, lignin in the plant apoplast[41] and melanin in the insect hemolymph.[50] Traditionally, these matrices have been considered to be a means of "walling-off" infection, but it is also possible that such matrices allow targeted redox cycling and ROS generation at the site of infection.[51] Interestingly, some pathogens also appear to generate their own melanin to perform a putative protection function (e.g., inactivation of host-generated ROS).[52]

The results in Figure 5 indicate that the flow/fate of electrons that interact with the catechol-chitosan matrix depends on the localized environment, which may be analogous to the context-dependent "benefits" of natural phenolic materials. For instance, a charged matrix is poised to donate electrons to quench free radicals (a potentially beneficial effect) but also to generate ROS (a potentially detrimental effect). Such context dependency makes it difficult to classify phenolics as beneficial anti-oxidants or detrimental pro-oxidants. This difficulty is illustrated by the previously cited example of melanins, which are virulence factors for fungal pathogens[53] but defense compounds of insects. Similar context-dependent pro- and anti-oxidant activities have been reported for neuromelanins[54] and food phenolics.[55]

Finally, an intriguing analogy that emerges from this work involves a comparison of the catechol-chitosan matrix with lipid membranes. The CVs in Figure 3a shows that incubation of the catecholic matrix with pyocyanin leads to a rectified current. The addition of a second mediator (Figure 3b) allows currents

to flow in the opposite direction by a redox-cycling mechanism that is "gated" by this mediator's redox potential ($E°$). These observations are reminiscent of rectifying and voltage-gated ion channels that are integral to information processing in neurons. For the neuron, the lipid bilayer serves as the capacitor to separate ionic charge, while the voltage gate controls the flow of ions across this membrane. In an analogous manner, the catechol-chitosan matrix is a redox capacitor, and voltage gating controls the mediated flow of electrons. While an analogy between ionic and electronic (i.e., redox) currents is intriguing, the biological relevance is unknown.

Conclusions. Attempts to reverse engineer biology often employ omic-type data to infer the regulatory networks that a biological system uses to transduce input cues into phenotypic behaviors. We developed an electrochemically based reverse engineering approach to probe a model catecholic matrix that possesses minimal capabilities; the grafted moieties can be readily switched between redox states (Q vs QH_2) at a single redox potential $E°$. We observed that interactions between this abiotic matrix and two redox-active metabolites yielded a surprisingly rich repertoire of responses that varied depending on the biological and environmental context. Essentially, the matrix is a concentrated and interacting source/sink of electrons that is capable of transducing various redox-input cues. An even richer set of responses could be envisioned if the system had greater capabilities, i.e., if matrices possessed multiple moiety types (with differing $E°$ values) and numerous redox-active metabolites were present to interact with these matrices. We suggest that such matrices may already exist (humics) or may be purposefully synthesized (lignins and melanins) and that biological systems may secrete various redox-active metabolites as "sensors/actuators" to probe the matrices' stored redox information or to access the matrices' electrons to induce effector actions (ROS generation). Thus, this study suggests that phenolic materials provide a "dense open access redox medium" for biology to monitor its environment and launch offensive/defensive actions.

EXPERIMENTAL SECTION

The catechol-modified polysaccharide matrix is fabricated in two steps as previously described (see Supporting Information).[19−21] First, a film of the aminopolysaccharide chitosan is electrodeposited by inducing its sol−gel transition at the cathode (using 1% (w/v) chitosan, pH 5.6 and 6 A m^{-2}, 30 s). Second, catechol is grafted by immersing the film-coated electrode in a catechol solution (5 mM, pH 7.0) and biasing the underlying electrode to oxidize the catechol (+0.6 V, 3 min). The oxidation product (e.g., *o*-quinone) grafts to chitosan.

In some experiments, a biofilm was electrodeposited by (i) immersing the catechol matrix in a suspension containing the Ca^{2+}-responsive polysaccharide alginate (1% (w/v)), insoluble $CaCO_3$, and bacteria (*E. coli* or *P. aeruginosa*) and (ii) electrodepositing the alginate by biasing the underlying electrode to achieve a constant anodic current (6 A m^{-2}, 2 min).[22] The dual film "(catechol-chitosan)-biofilm" was rinsed and incubated in LB medium that was supplemented with 10 mM $CaCl_2$ (to prevent dissolution of the Ca^{2+}-alginate biofilm).

Experiments were performed using a 3-electrode system with a gold working electrode, Pt wire counter electrode, and Ag/AgCl reference. Microbial activity was provided by either a biofilm or bacterial suspension (*E. coli* or *P. aeruginosa*). Electrochemical measurements were made with a CHI workstation (CHI 6273c).

ASSOCIATED CONTENT

Supporting Information

Additional Information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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