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Spotlight on Applications

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Catechol-Based Capacitor for Redox-Linked Bioelectronics

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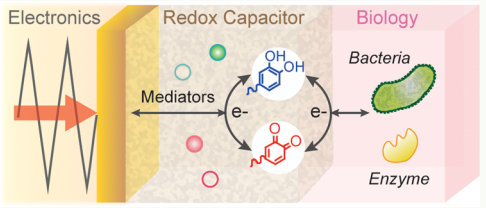
ABSTRACT: A common bioelectronics goal is to enable communication between biology and electronics, and success is critically dependent on the communication modality. When a biorelevant modality aligns with instrumentation capabilities, remarkable successes have been observed (e.g., electrodes provide a powerful tool to observe and actuate biology through its ion-based electrical modality). Emerging biological research demonstrates that redox is another biologically relevant modality, and recent research has shown that advanced electrochemical methods enable biodevice communication through this redox modality. Here, we briefly summarize the biological relevance of this redox modality and the use of redox mediators to enable access to this modality through electrochemical measurements. Next, we describe the fabrication of a catechol−chitosan redox capacitor that is redox-active but nonconducting and thus offers a unique set of molecular electronic properties that enhance access to redox-based information. Finally, we cite several recent studies that demonstrate the broad potential for this capacitor to access redox-based biological information. In summary, we envision the redox capacitor will become a vital component in the integrated circuitry of redox-linked bioelectronics.



KEYWORDS: *catechol, chitosan, mediators, redox biology, redox capacitor*

INTRODUCTION: COMMUNICATION MODALITIES

Microelectronics transformed our lives by enabling information to be coded and transmitted in electromagnetic radiation. This modality enables us to communicate nearly instantaneously to nearly anyone in the world at nearly any time. There is great interest in extending these communication capabilities from our technological world to our biological world: to create bioelectronic devices that can observe and actuate biological systems in meaningful ways. A key challenge for bioelectronics is that biology does not typically communicate using electromagnetic radiation, and thus, modalities must be available to bridge this technology−biology communication gap. Figure 1a illustrates three common signaling modalities that biology uses for molecular-based communication.[1]

Ionic Electrical Modality. Biology is well-known for its use of an electrical modality associated with the flow of ions across membranes. This ionic electrical modality allows rapid signaling at a global level and is integral to communication in the nervous and neuromuscular systems. From a bioelectronics perspective, this ionic electrical modality is remarkably easy to access through convenient electrode based instrumentation (Figure 1b). In fact, decades before the biotech revolution, electrodes were being used by neurobiologists to observe how nerve cells communicated through action potentials and by clinicians to generate electrocardiograms (EKGs) that characterize the health of a complex biological system (the cardiovascular system). In fact, this ionic electrical modality has been so easy to access that defibrillators can be used by minimally trained persons to reprogram life-threatening dysrhythmias.

Molecularly Specific Modalities. In addition to the rapid, globally acting ion-based electrical modality, biology also communicates using molecularly specific modalities. Typically, in these examples, cell-surface receptors "recognize" an extracellular molecular signal with "lock-and-key" specificity, and receptor−ligand binding triggers intracellular signal transduction mechanisms that cue biological responses (e.g., to open ion channels or alter gene expression). Such molecularly specific modalities are ubiquitous in biology and include: (i) short-range neurotransmitter-based communication across the synaptic junctions that separate nerve cells; (ii) intermediate-range hormonal signaling among cells within an organism (e.g., through the endocrine system); and (iii) long-range pheromone-based communication between plants. Developing technological approaches to engage biodevice communication through such molecularly specific modalities is technically challenging, as illustrated by the difficulties in designing new drugs, targeting the delivery of nanoparticle therapeutics, or the selective detection of molecular biomarkers. The classic bioelectronic success for such selective detection is the electrochemical glucose biosensor (Figure 1c)

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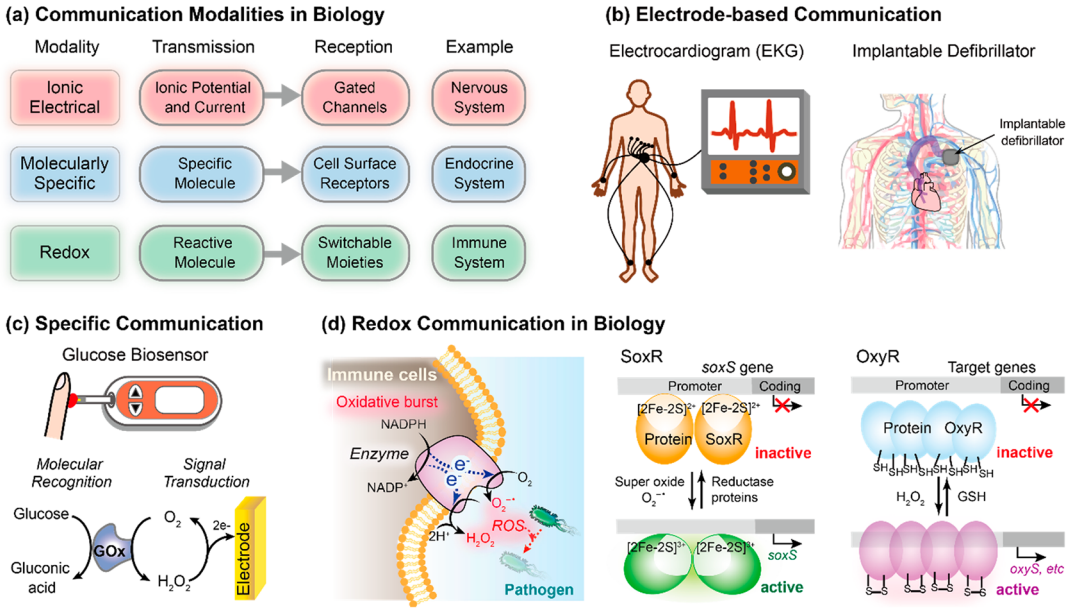

Figure 1. Modalities to span biodevice communication. (a) Three molecularly based modalities of biological communication. (b) Biology's ionic electrical modality is rapid, acts globally, and has been comparatively easy to access technologically using electrodes for detection and actuation. (c) Molecularly specific modalities are common in biology but have been more challenging to access technologically: the enzyme-based glucose sensor is a successful bioelectronics example for accessing chemically specific information simply and in near-real-time. (d) Redox is a third biological communication modality that shares features of biology's electrical and molecular modalities. Adapted with permission from ref 2. Copyright (2019) IEEE.

that uses an enzyme to selectively recognize this sugar and generate a product (e.g., H_2O_2) that can be electrochemically transduced into an electrical output.

Redox Modality. Emerging biological research indicates that biology also uses a third, redox, modality for signaling.[1,3,4] Traditionally, this redox modality was understood in terms of immune defense responses in which an oxidative burst transferred electrons to O_2 to generate reactive oxygen species (ROS; Figure 1d) that are toxic to invading pathogens and also potentially damaging to the host (e.g., such inflammation and ROS are believed to be linked to oxidative stresses associated with various human diseases).[5] The individual ROS have different lifetimes and thus can act over different length scales, and some ROS can cross cell membranes (e.g., NO and H_2O_2) and have been shown to serve as molecular signals for cell−cell communication. However, this redox modality has features that differ considerably from molecularly specific signaling modalities in that the redox signals are chemically reactive, appear to act more globally, and are recognized through mechanisms that are atomically (vs molecularly) specific. For instance, Figure 1d illustrates that one ROS-based stress response in *E. coli* "recognizes" superoxide ($O_2^{-\bullet}$) by oxidation of the iron−sulfur prosthetic group of the SoxR transcription factor, while another stress-response "recognizes" a different ROS (i.e., H_2O_2) through a sulfur switch (cysteine thiols that are oxidized to disulfides) of the OxyR transcription factor.[6−8] Further, it appears that gradients in ROS are integral to guiding the host's wound healing responses, while recent research suggests inflammation and ROS may serve to link communication between the immune and nervous systems, the brain and gut, and the microbiome and host.[9,10]

ENLISTING MEDIATED ELECTROCHEMICAL PROBING TO BRIDGE BIODEVICE COMMUNICATION

From a bioelectronics standpoint, the redox modality has both electrical and molecular features. The electrical features reflect the fact that this modality involves the flow of electrons through reduction and oxidation reactions. The molecular features reflect the fact that free electrons do not generally exist in aqueous solution and the flowing electrons of this redox modality must be shuttled by molecular carriers.[2] For instance, biology uses the reduced NADPH species to provide the diffusible source of electrons for biosynthesis. Important from a technological perspective is that the electrical features of this redox modality are accessible to simple electrode-based electrochemical instrumentation.

Electrode-based measurements offer the traditional benefits of simplicity, speed, and sensitivity, and such measurements were integral to the early advances in electrophysiology. However, electrode-based measurements for communication through the redox modality will be intrinsically more complex than the measurements of biology's ionic electrical modality. Specifically, measurements of redox-based information will likely require electron transfer across the electrode−solution interface, and such electrochemical reactions require direct contact between the electrode and the solution being measured. This electron transfer requirement suggests that measurements will be invasive and that communication will be limited to comparatively small length scales (on the order of 10^{-3} m if diffusion is the operative molecular transport mechanism). Additional limitations for measurements that require electron transfer across the electrode−solution interface are that some redox-active molecular species can have significant kinetic barriers for donating or accepting electrons, and the electrochemically measured currents may reflect the

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net reactions of multiple molecular species, and thus, details of which molecules are being observed may be lost. Despite these limitations, we believe electrochemistry provides exciting opportunities to enlist redox to bridge biodevice communication.

Redox Probing and the Sonar Analogy. Figure 2a illustrates that our approach to access redox-based information

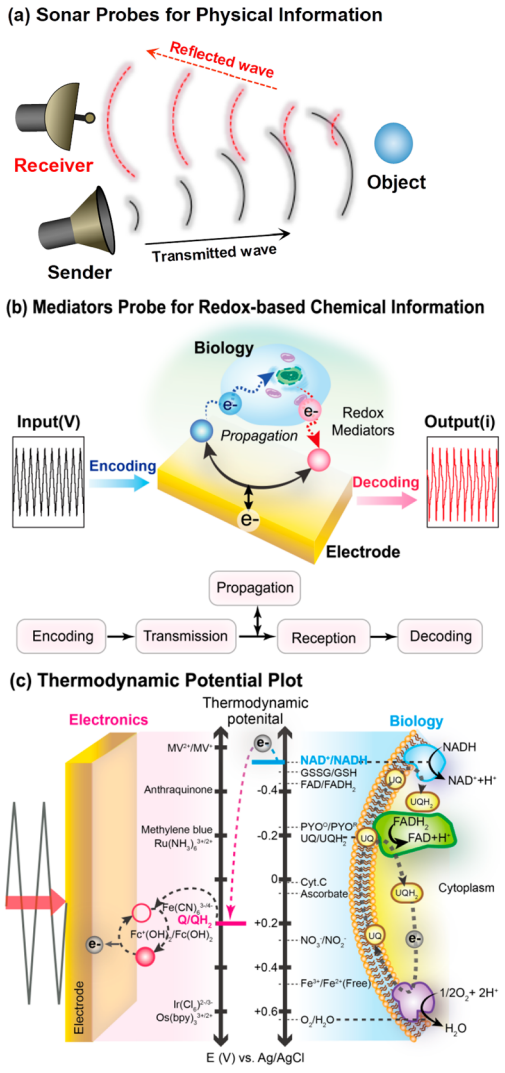

Figure 2. Analogy between sonar and the use of mediators to probe for redox information. (a) Sonar probes for physical information in an environment. (b) Mediators probe for redox-based chemical information in a local environment. Adapted with permission from ref 2. Copyright (2019) IEEE. (c) Thermodynamic potential plot suggests that electrochemically-excited mediators can interact with biological oxidants/reductants in a redox potential-dependent manner.

is approximately analogous to sonar's probing for physical information. For instance, sonar uses a transceiver device that codes and transmits a signal (i.e., a pressure wave) that propagates through the medium and physically interacts with objects in the environment in understandable ways to generate a reflected wave that is received and decoded by the device. Analogously, Figure 2b shows that we use the electrode as a transceiver that codes and transmits a redox signal that propagates into and chemically interacts with the local environment in understandable ways to generate a response that is received and decoded by the electrode. To generate our redox transmission, we purposefully add diffusible mediators that serve as the propagating redox signal that can be reversibly switched between oxidized and reduced states (i.e., typically, the mediators are bistable redox switches). The imposed electrical voltage provides the input that sets the mediator's redox state, and this redox state is altered through oxidation/reduction interactions in the local environment (i.e., these mediator reactions serve to access the redox information on the local environment). This redox information is received by the electrode when the mediator diffuses back and exchanges electrons with the electrode. Importantly, we believe this use of electrochemistry exploits a powerful but underutilized, capability of electrochemistry: the transduction of redox-based chemical information into an electronic format that is readily analyzable through advanced information-science and signal-processing methods.

Figure 2c illustrates a biological perspective for the sonar analogy. The left-hand side of this schematic illustrates that various diffusible mediators can be electrochemically excited by switching their redox state at an electrode, while the right-hand side of this schematic illustrates various biological electron carriers (i.e., oxidants and reductants) that mediate electron flow within and between cells. The thermodynamic potential scale suggests the possibility of selecting mediators with appropriate thermodynamic and kinetic properties to interact with (i.e., exchange electrons with) biological oxidants and reductants. A technological example illustrating the use of mediators to promote electron exchange between cells and electrodes is the microbial fuel cell that often relies on diffusible redox mediators to enable electron transfer between the microbes and electrode (e.g., neutral red,[11] thionin,[12] methyl viologen,[13] and phenazine[14]).

Initial Examples of Biorelevant Redox-Based Detection and Actuation. The first obvious question is what useful redox-based communication is possible through a sonar-like mediated electrochemical probing? We cite conclusions from three initial examples to suggest the possibilities (the reader is referred to the original papers for detailed discussions of these examples). In our first example, we performed *in vitro* measurements with the natural pigment melanin, which is believed to offer protective functions (e.g., photoprotection by eumelanin) but in some cases can have deleterious activities (e.g., pro-oxidant activities of pheomelanin).[15,16] As illustrated in Figure 3a, our approach resembles reverse engineering as we use mediators to interact with (i.e., probe) the melanin samples in different ways (e.g., at different redox potentials E^0) and observe redox responses that suggest biologically relevant differences in redox activities.[17−21] Second, we used such a reverse engineering approach to probe serum samples to discover signatures of oxidative stress[22,23] (oxidative stress is ill-defined but believed important in various diseases).[5] As illustrated in Figure 3b, we probed serum samples using a single mediator and a complex pulse sequence to discover output response signatures that could distinguish healthy controls from persons diagnosed with schizophrenia based on differences in their oxidative stress.[23] Finally, we engineered a synthetic biology (synbio) construct of *E. coli* through the SoxRS regulon of Figure 1d to enable a redox-imposed input to actuate biology.[24] As illustrated in Figure 3c, the diffusible mediators (Med) were used to impose an oxidative stress, while the stress-response regulon of this synbio construct was

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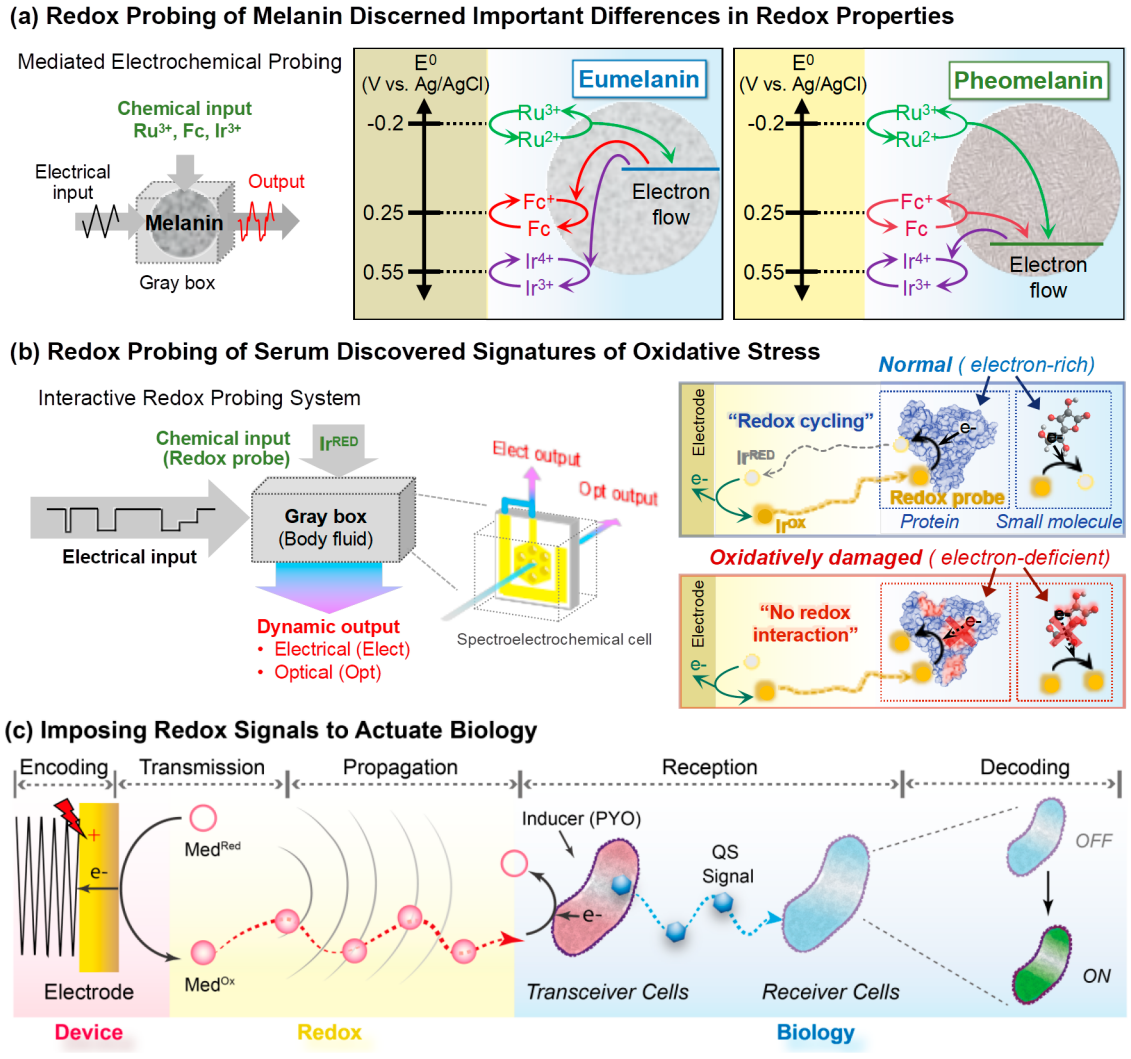

Figure 3. Initial examples illustrating that mediated electrochemical probing can access useful redox-based information and actuate biology. (a) Redox probing with multiple mediators (Ru^{3+}, Fc, and Ir^{3+}) allowed differences between eumelanin and pheomelanin to be discerned, which may provide insights of pheomelanin's pro-oxidant activities. Adapted with permission from ref 21. Copyright (2018) American Chemical Society. (b) Redox probing of serum samples using a single mediator (iridium, Ir) and a voltage pulse sequence could discern signature patterns of oxidative stress. Adapted with permission from ref 23. Copyright (2018) Elsevier. (c) Redox inputs could impose oxidative stresses to a synbio construct whose stress response regulon was rewired to enable stress-induced gene expression. Adapted with permission from ref 2. Copyright (2019) IEEE.

"rewired" to synthesize a quorum sensing (QS) signaling molecule capable of communicating with a broader bacterial population.[24] These initial examples encouraged us that redox is an interesting bioelectronics modality both to access biologically relevant information and to communicate with biology.

■ CATECHOL–CHITOSAN AS A REDOX CAPACITOR

The next question is how to enhance our information processing capabilities for such redox-based bioelectronics? Interestingly, this same question is being asked by researchers focused on ion-based bioelectronics. In both cases, the electrode interconverts device-compatible electrical signals and biologically compatible signals (either a redox or ionic signal). To enhance ionic–electronic (i.e., "iontronic") communication, electrodes are commonly modified with charged and/or conducting polymers to create organic electronic circuit elements (e.g., field-effect transistors) that can control the flow of ions to perform specific functions.[25,26] In our work, we modify the electrodes using redox-active biopolymer-based films.

Electrofabrication of Catechol–Chitosan Film. Figure 4a shows that we created a redox-active film in two electrofabrication steps involving chitosan and catechol.[27,28] Chitosan is a pH-responsive film-forming aminopolysaccharide that can be easily electroassembled at the electrode surface by a reversible cathodic electrodeposition step.[29,30] Specifically, chitosan is a water-soluble cationic polyelectrolyte at pHs below the pK_a (~6.3) but undergoes gel formation (i.e., self-assembly) when the pH is increased. Cathodic reactions result in the high localized pH adjacent to the electrode surface that induces chitosan's deprotonation and self-assembly into crystalline regions. The resulting electrodeposited chitosan hydrogel is physically cross-linked and stable at neutral and basic pHs but can be redissolved under mildly acid conditions (e.g., with acetic acid). Catechols are among nature's most abundant redox-active compounds[31] and can be readily grafted to the deposited chitosan by an anodic oxidation that generates

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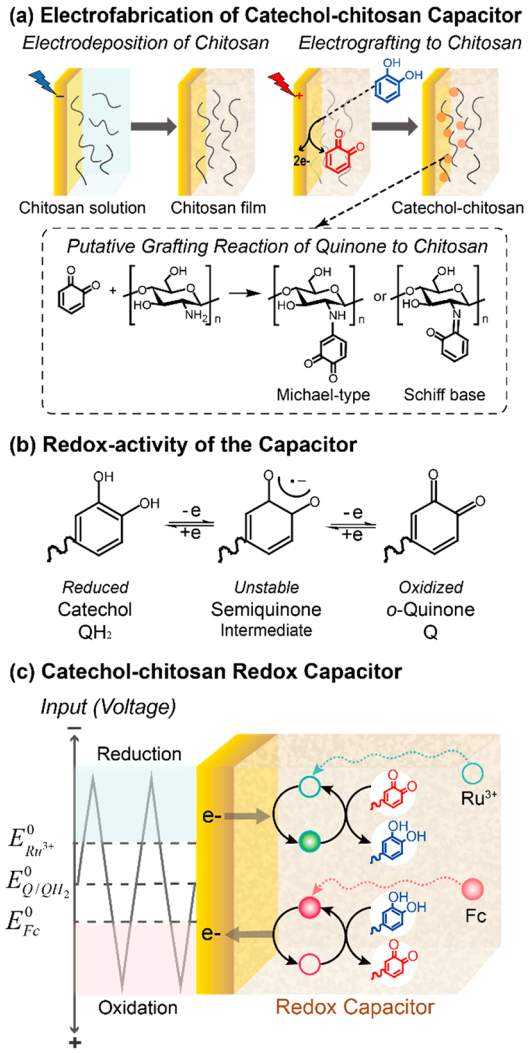

Figure 4. Catechol–chitosan redox capacitor film. (a) Electrofabrication of the redox capacitor in two steps: cathodic chitosan electrodeposition and anodic catechol grafting. (b) The proposed redox activity of the capacitor relies on the reversible interconversion of two stable redox states. (c) Redox-cycling reactions mediate the transfer of electrons between the electrode and the film: reductive redox cycling transfers electrons from the electrode to charge the film with electrons, and oxidative redox cycling transfers electrons to the electrode to discharge electrons from the film.

a reactive *o*-quinone that can diffuse into and react with the chitosan hydrogel film. The putative grafting reactions of quinone to chitosan include Schiff-base linkages or Michael-type adducts.[32,33] Operationally, the grafting is simple, although the underlying chemistries are complex, likely resulting in diverse chemical moieties. There are several important features of this film electrofabrication method: it is simple, aqueous-based, and does not require reactive reagents; it is rapid with each step requiring only minutes to complete; it is spatiotemporally selective in the sense that deposition occurs at an electrode surface in response to imposed electrical signals (i.e., films can be electroaddressed on patterned electrodes in parallel or in sequential steps); and electroassembly is convenient for functionalizing lab-on-a-chip devices because it can be performed in covered fluidic channels without the need for line-of-sight (required for photolithography) or direct contact (required for printing).[34]

Catechol–Chitosan Film as a Redox Capacitor. Initial studies demonstrated the catechol–chitosan films do not conduct electrons: they cannot exchange electrons directly with the underlying electrode surface, and electrons do not appear to flow in response to an applied electrical field.[35] This lack of electronic conductivity suggests that the grafted aromatic moieties do not form an extended aromatic network and/or do not form intimate contacts with the underlying electrode (the thickness of the wet hydrogel films can be varied but are typically on the order of several micrometers). Although nonconducting, these initial studies demonstrated that the catechol–chitosan films are redox-active: they can accept, store, and donate electrons.[35] As suggested in Figure 4b, we hypothesize that such redox capacitor properties result because the grafted moieties can be reversibly switched between two stable states: the reduced catechol (designated QH_2) and the oxidized *o*-quinone (designated Q).

Figure 4c illustrates that switching the redox state requires diffusible mediators to shuttle electrons between the electrode and film. For instance, when reducing voltages are imposed at the electrode surface, the mediator $Ru(NH_3)_6Cl_3$ (Ru^{3+}) can undergo reductive redox cycling in which Ru^{3+} is reduced at the electrode, diffuses into the film, is oxidized by donating its electrons to the film, and then diffuses back to the electrode where it can be rereduced. This reductive redox cycling serves to switch the grafted moieties to their reduced (e.g., catecholic) state. Similarly, when oxidizing voltages are imposed, a different mediator (e.g., ferrocene dimethanol, Fc) can undergo oxidative redox cycling which serves to switch the grafted moieties in the film to their oxidized (e.g., *o*-quinone) state. Importantly, mediator–film electron transfer is controlled by thermodynamics: electrons tend to flow from species with more-negative redox potentials to species with more-positive redox potentials. Also important is that both (or multiple) mediators can be present in the same solution, but each individual mediator is only excited to redox cycle when the imposed electrode voltage approaches its E^0.

Molecular Electronic Properties of Catechol–Chitosan Redox Capacitor Film. The catechol–chitosan films offer unique molecular electronic properties because they possess redox activity in the absence of conductivity.[36] The uniqueness is illustrated by the cyclic voltammograms (CVs) shown in Figure 5a and the input–output curves shown in Figure 5b. Both representations of the data compare the response of an electrode coated with the catechol–chitosan redox capacitor film against a control chitosan film (neither conducting nor redox-active) and a conducting graphene–chitosan film.[37] These three film-coated electrodes were tested in solutions containing two diffusible mediators, Ru^{3+} and Fc (50 μM each). The control chitosan film shows small peaks associated with the reversible oxidation and reduction of these two mediators, while the catechol–chitosan and graphene–chitosan films show markedly different responses, as summarized in Table 1 and further illustrated in Figure 5c.

The first difference illustrated in Figures 5a and 5b and Table 1 is amplification: both the catechol–chitosan and graphene–chitosan films show considerably amplified output currents compared to the control chitosan film. However, the mechanisms responsible for current amplification based on redox cycling and conductivity are different.[38−40] Amplification for the catechol–chitosan films occurs because electrons are

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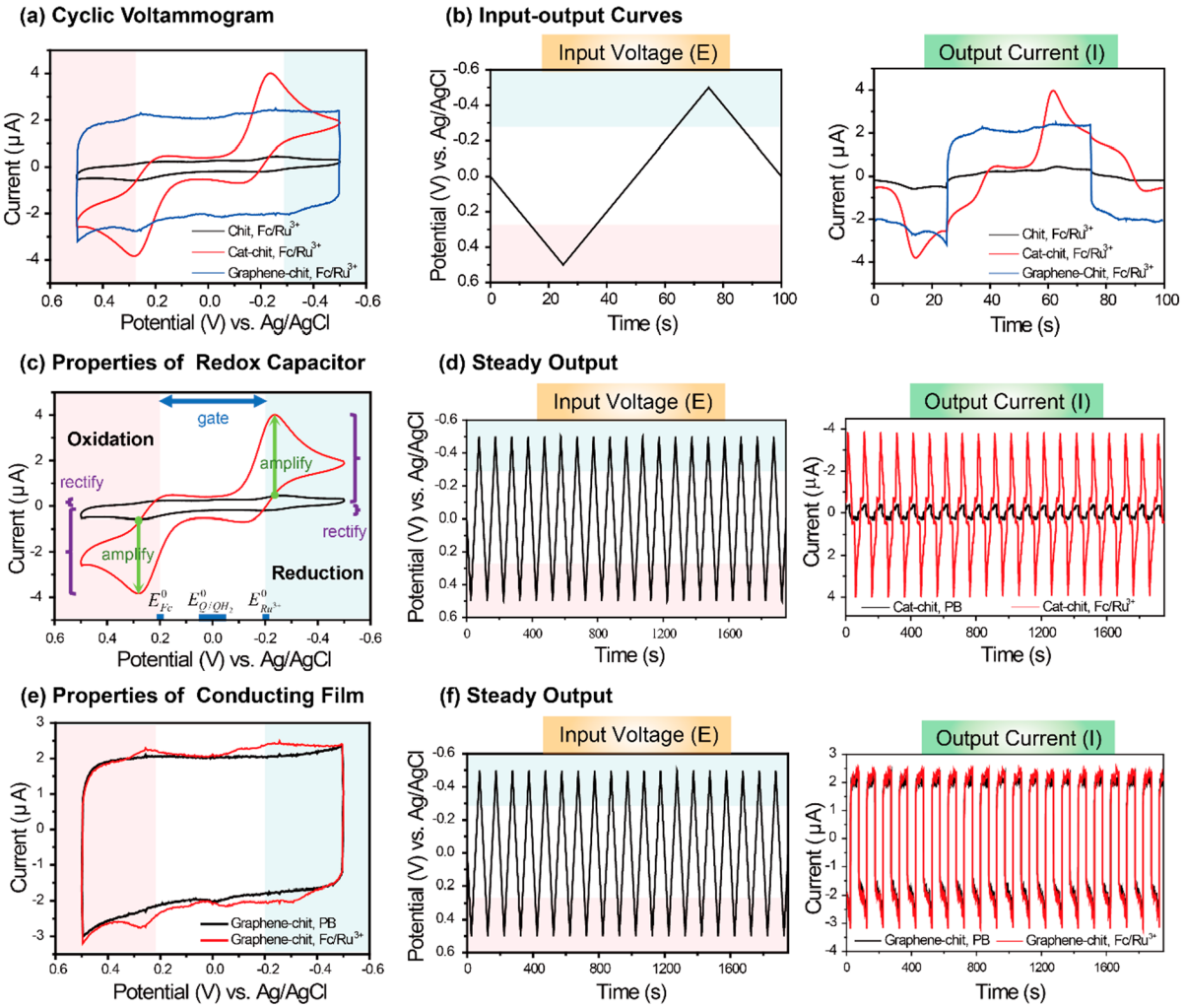

Figure 5. Molecular electronic properties of catechol−chitosan redox capacitor when probed in the presence of both reductive and oxidative redox-cycling mediators (50 μM Ru^{3+} and 50 μM Fc). (a) CV and (b) input−output curves comparing responses of three film-coated electrodes: control chitosan film (nonconducting, nonredox-active), graphene−chitosan film (conducting), and catechol−chitosan film (nonconducting, redox active). (c) CV highlighting the amplification, rectification, and gating properties of the catechol−chitosan redox capacitor. (d) Long-term, multicycle input−output curves shows steady (time-invarying) output response of capacitor film to oscillating voltage inputs. (e) CV illustrating that the electrical signal obtained from the graphene−chitosan conducting film is largely insensitive to the mediators. (f) Long-term, multicycle input−output curves shows steady (time-invarying) output response of the conducting film to oscillating voltage inputs. (Note: PB refers to phosphate buffer without mediators.)

Table 1. Comparison of Properties between Redox-Active and Conducting Films[a]

	redox capacitor (catechol−chitosan film)	conducting film (graphene−chitosan film)
amplification	electron storage allows the accumulation of signal for subsequent measurement	an increase in conducting surface area and electrocatalysis both enable signal amplification
rectification	thermodynamic constraints control the direction of electron flow	
gating	mediators control electron flow based on their redox properties (i.e., the mediators' E^0)	

[a]See Figure 5.

accumulated in the film during reductive redox cycling (i.e., the film is being charged with electrons) and electrons are depleted from this film during oxidative redox cycling (i.e., the film is being discharged). In contrast, current amplification for the graphene−chitosan film occurs because of the large charging currents associated with the large conducting surface area and also because of graphene's electrocatalytic properties.[41−43] Graphene's electrocatalytic activity is illustrated by the small difference in voltages between the peak currents for Fc oxidation and Fc reduction ($\Delta E_{p,Fc} = E_{p,Fc,ox} - E_{p,Fc,red} = 0.02$ V) compared with the control chitosan film ($\Delta E_{p,Fc} = 0.11$ V).

The second difference illustrated in Figure 5c and Table 1 is rectification: because of thermodynamic constraints, Ru^{3+} can engage the catechol−chitosan film through reductive (but not oxidative) redox cycling, and similarly, Fc can engage the catechol−chitosan film through oxidative (but not reductive) redox cycling. As a result, the currents associated with Ru^{3+} reduction (but not Ru^{3+} oxidation) are amplified, and the

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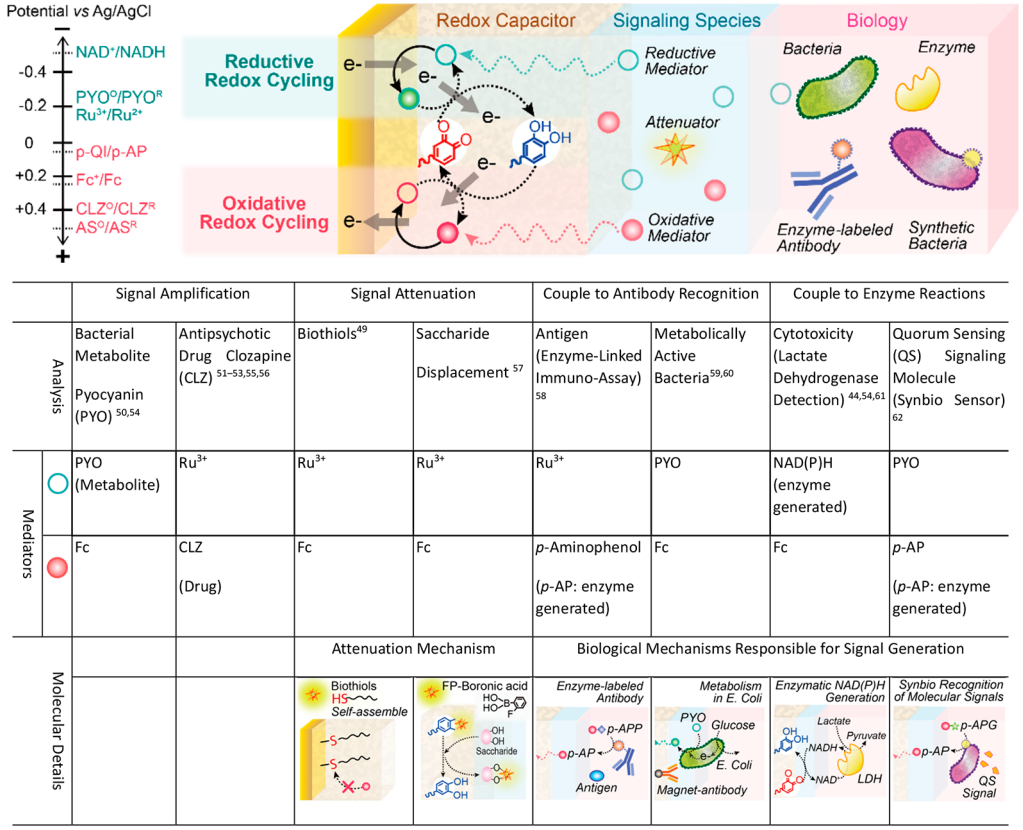
Table 2. Applications of the Catechol−Chitosan Redox Capacitor for Sensing



		Signal Amplification		Signal Attenuation		Couple to Antibody Recognition		Couple to Enzyme Reactions	
Analysis		Bacterial Metabolite Pyocyanin (PYO) [50,54]	Antipsychotic Drug Clozapine (CLZ) [51−53,55,56]	Biothiols[49]	Saccharide Displacement [57]	Antigen (Enzyme-Linked Immuno-Assay) [58]	Metabolically Active Bacteria[59,60]	Cytotoxicity (Lactate Dehydrogenase Detection) [44,54,61]	Quorum Sensing (QS) Signaling Molecule (Synbio Sensor) [62]
Mediators	○	PYO (Metabolite)	Ru^{3+}	Ru^{3+}	Ru^{3+}	Ru^{3+}	PYO	NAD(P)H (enzyme generated)	PYO
	●	Fc	CLZ (Drug)	Fc	Fc	*p*-Aminophenol (*p*-AP: enzyme generated)	Fc	Fc	*p*-AP (*p*-AP: enzyme generated)
Molecular Details				Attenuation Mechanism		Biological Mechanisms Responsible for Signal Generation			
				Biothiols Self-assemble	FP-Boronic acid Saccharide	Enzyme-labeled Antibody *p*-APP *p*-AP Antigen	Metabolism in E. Coli PYO Glucose E. Coli Magnet-antibody	Enzymatic NAD(P)H Generation Lactate Pyruvate NADH NAD+ LDH	Synbio Recognition of Molecular Signals *p*-APG *p*-AP QS Signal

currents associated with Fc oxidation (but not Fc reduction) are amplified. This rectification of mediator currents provides the distinctive waveforms in Figure 5b for electrodes coated with catechol−chitosan films.

The third difference illustrated in Figure 5c and Table 1 is gating: because the mediators are essential for transferring electrons to/from the catechol−chitosan film, the mediator's redox potential (E^0) controls the conditions in which electron transfer can occur. It is important to note that this electron transfer-based gating mechanism is intrinsically different from electric field-based gating mechanisms responsible for the voltage gating of protein-based ion channels or field effect transistors.

In addition to amplification, rectification, and gating, the catechol−chitosan redox capacitor offers steady (i.e., time-invarying) behavior, as illustrated in Figure 5d. Steady output currents are observed when the redox-cycling reactions are reversible, and an oscillating voltage is imposed to sequentially engage oxidative and reductive redox cycling to repeatedly switch the catechol moieties between their oxidized and reduced states. This steady output response facilitates the use of information processing methods to extract information from our redox signals.

To further illustrate the differences between the catechol−chitosan redox capacitor film and graphene−chitosan conducting film, we subjected the latter to repeated cyclic input voltages either in the presence or absence of mediators. Figure 5e shows large currents are observed for the electrode coated with the conducting film, but these currents were largely insensitive to the presence/absence of mediators. Figure 5f shows that, like the capacitor film, the conducting film generates steady, time-invarying output current responses to oscillating input voltages.

Relevance to Biological Redox-Based Communication. To consider the biological relevance, it is helpful to consider the catechol−chitosan as a redox catalyst facilitating the transfer of electrons from reductants to oxidants (e.g., in Figure 4c, Ru^{2+} is the reductant and Fc^+ is the oxidant). Importantly, this catechol-based redox catalyst has a somewhat broad substrate-specificity in that it has been observed to accept electrons from various reductants (including the biological reductants NADPH[44] and ascorbic acid[45]) and to donate electrons to biologically relevant oxidants such as O_2.[46] This ability of catechol−chitosan to exchange electrons with biologically relevant molecular species provides a mechanism for the exchange of redox-based information between biology and electronics.

In summary, biology routinely uses diffusible redox species (e.g., NADPH) to transport electrons to perform various functions. We suggest that electrochemical redox probing provides a means to observe and participate in such biological redox activities (e.g., for sensing and actuation). Further, we suggest the catechol−chitosan redox capacitor is a useful tool because it can exchange electrons with various mediators (both electrochemical and biological) in ways that dramatically alter the measured electrochemical outputs and because analysis of these changes can be guided by (bio)chemical intuition to enable meaningful interpretation of this redox-based information.[47−49]

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INITIAL APPLICATIONS OF THE REDOX CAPACITOR

To date, the catechol−chitosan redox capacitor has primarily been used for sensing, and Table 2 lists various applications reported. In the initial examples, this capacitor was used to directly detect molecules that could engage in redox cycling for signal amplification. The first example was the detection of the virulence factor pyocyanin from bacterial cultures of *Pseudomonas aeruginosa*. Pyocyanin was observed to undergo reductive redox cycling with the redox capacitor, and amplified currents were observed when an oxidative redox cycler (e.g., Fc) was included in the solutions.[50] Next, the antipsychotic drug clozapine was reported to undergo oxidative redox cycling with the redox capacitor, and this offered the possibility for simple detection for point-of-care applications.[51−53] The advantage of a detection scheme that enlists redox cycling is that it is comparatively simple, rapid, and sensitive, while the advantage of the catechol−chitosan redox capacitor is that can be conveniently electroassembled for on-chip applications.[52,54−56,61] The disadvantage of a detection scheme based on redox cycling is that while it detects an activity (e.g., redox activity),[47] it may not offer sufficient selectivity to establish the molecular species responsible for this redox activity.

Later studies used molecular interactions that suppressed redox cycling for selective signal attenuation. In one example illustrated in Table 2, the self-assembly of biothiols onto a gold electrode served to attenuate mediator currents. By using the capacitor to amplify mediator currents through redox cycling, it was possible to quantify this thiol-based signal attenuation.[49] One interesting feature of this approach is that standard curves for several biothiols were linear over multiple orders-of-magnitude of concentration.[49] Another interesting feature of this approach is that thiol self-assembly and signal attenuation could be reversed by imposing a reducing voltage that disassembled the thiols.[63−65] Thus, while the redox capacitor may not offer intrinsic molecular selectivity, this work demonstrates that the electrical inputs could be tuned to test hypotheses of expected molecular-level behaviors (e.g., voltage-dependent thiol disassembly) to provide greater confidence that the activities being measured result from a specific class of molecules (e.g., biothiols).

In a separate study, a related dopamine-chitosan redox capacitor was used to detect saccharides based on a displacement mechanism.[57] As illustrated in Table 2, this mechanism uses the binding of 2-fluorophenylboronic acid (FP-boronic acid) to the catecholic diols to block redox activity, prevent redox cycling, and thereby attenuate the signal. In the presence of competing saccharides, FP-boronic acid was displaced from the catecholic moiety; the redox activity was recovered, and the film could engage in mediator-based redox cycling. This redox cycling led to an amplification of mediator currents that could be correlated to the saccharide concentration.[57]

Additional studies have attempted to further enhance selectivity by coupling antibody-based molecular recognition with the redox capacitor's capabilities to enhance sensitivity through signal amplification.[58] For instance, Table 2 shows a strategy to enhance the electrochemical detection from an enzyme-linked immunoassay. In this example the alkaline phosphatase-linked secondary antibody converts a redox-inactive substrate (*p*-aminophenyl phosphate; *p*-APP) to a redox-active product (*p*-aminophenol; *p*-AP) that can undergo redox cycling with the capacitor. The authors reported an eightfold signal amplification and that this redox cycling-based amplification could be coupled to additional amplification strategies (e.g., enzymatic amplification and magnetic concentration).[58]

A similar antibody recognition approach was used for microbe detection (e.g., for detecting food pathogens).[60] In this example, an antibody capable of selectively binding bacteria was coupled to magnetic particles that could be magnetically collected at the surface of a multifunctional film[66] that included the redox capacitor. Localization of such a viable bacterial population at the film surface altered the local redox context and this perturbation of redox context could be detected by redox cycling (e.g., by the changes in the amplification and rectification of mediator currents).[59] As in the previous example, the antibody provided the molecular recognition to confer selectivity, while the electrochemical reactions of the mediators provided the signal transduction mechanism: again, the redox capacitor enhanced sensitivity through signal amplification. The authors reported that this approach provides a simple, rapid, and portable approach to pathogen detection.[60]

A more recent study coupled the redox capacitor to an enzyme reaction that is commonly used to report cytotoxicity. This cytotoxicity assay is based on the observation that when cells are damaged, they release the lactate dehydrogenase (LDH) enzyme into the extracellular space, and thus, measurement of LDH has become a standard measure of cytotoxicity. Previous studies have shown that the catechol−chitosan redox capacitor can accept electrons from the biological reducing agent NADPH[44] that is generated from the LDH reaction. In this example, a redox capacitor was electroassembled onto an electrode address within a microfluidic device, and this capacitor-coated electrode was used to detect LDH. As illustrated in Table 2, LDH transfers electrons from the added lactate substrate to NADPH, which undergoes redox-cycling reactions that donate electrons to the grafted-moieties to charge the capacitor with electrons (i.e., to convert the quinones to catechols). Intermittent discharging of the capacitor by Fc-mediated redox cycling generated an amplified current that could be correlated to the LDH activity (and therefore cytotoxicity). In this example, the capacitor served to store electrons liberated by the LDH reaction. It is important to note that the reduced NADPH is not readily detected by direct electrochemical oxidation due to kinetic limitations (i.e., high overvoltages must be imposed to electrochemically oxidize NADPH). Thus, this example illustrates that the catalytic properties of the catechol−chitosan redox capacitor facilitate redox communication with biology.[67−69]

The final example in Table 2 illustrates a dual film system for coupling the redox capacitor with a synthetic biology-based cell biosensor.[48,62] Specifically, *E. coli* reporter cells were rewired to recognize the bacterial QS molecular signal autoinducer-2 (AI-2) and transduce this recognition into the expression of an enzyme (*β*-galactosidase) that can convert a redox-inactive substrate (4-aminophenyl *β*-D-galactopyranoside; *p*-APG) into a redox-active intermediate (*p*-aminophenol; *p*-AP)[70] that can undergo redox cycling in the capacitor film. Essentially, this dual film system enlists the capabilities of synthetic biology to recognize the QS molecular signal (the AI-2 signaling molecule) and enzymatically generate an intermediate chemical output (i.e., a redox-active intermediate) that can

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ACS Appl. Electron. Mater. 2019, 1, 1337−1347

engage the redox capacitor in redox cycling to generate an amplified electrical output.

CONCLUSIONS

Redox-linked bioelectronics offers exciting possibilities for connecting biology and electronics. Redox is different from the conventional ion-based electrical modality of biology as it involves the flow of electrons and is integral to communication within the immune system, across biological systems (e.g., the gut−brain axis), and even across biological kingdoms (e.g., the microbiome and epithelium). But, redox is similar to the ion-based electrical modality in that both modalities are accessible to electrode measurements that are simple, rapid, and portable. Recently, there have been advances in redox biology that are demonstrating how biology perceives, processes, and responds to redox-based information, while molecular and synthetic biology provide the means to apply this knowledge to design biological information processors for redox connectivity. Recent advances in electrochemistry (e.g., mediated probing) enable electrical signals to be used for biodevice communication (both to acquire information and to actuate responses), while the application of information theories to this redox modality offers the promise of further extending the vast capabilities of information science to biology. We envision the electrical features of this redox modality will enable entirely new bioelectronics applications for point-of-care diagnostics and wearable electronics but also imagine the molecular features of this modality will offer the potential to couple the computational power of electronics to the molecular logic of life.

Here, we highlight several important features of the catechol−chitosan redox capacitor for accessing and processing redox-based information. First, this capacitor is electrofabricated at an electrode address using steps that are simple, rapid, and spatiotemporally selective: thus, fabrication is appropriate for a broad range of applications (e.g., lab-on-a-chip devices). Second, this capacitor offers important molecular electronic properties: mediator-based redox cycling with the capacitor amplifies, rectifies, and gates currents, and the reversibility of these redox-cycling reactions enables the capacitor to yield long-term, time-invarying steady outputs that are integral to information and signal processing methodologies. Third, the capacitor can exchange electrons with a range of biological and electrochemical oxidants, reductants, and mediators, which facilitates a bridging of biodevice communication. Finally, the capabilities of the redox capacitor can be integrated with additional components from biology: several examples in Table 2 illustrate the coupling of the redox capacitor with antibody-based molecular recognition elements and synthetic biology constructs. These initial reports suggest a broad potential for integrating more (and more complex) components into the circuitry of redox-linked bioelectronic circuitry.

There are limitations to our redox-based bioelectronics approach. First, while electrode measurements provide rapid and sensitive measurements, redox measurements require direct electron transfer at the electrode surface, and thus, measurements will be both invasive and operate over a relatively small length scale (e.g., millimeters). Second, the redox-based measurements access redox information at a global level, and while these measurements reflect the chemical context, it is not always possible to "map" the electrochemical measurements directly to chemical compositions and concentrations. Third, redox is not always the most relevant modality for biological communication. For instance, the neuromuscular system can be readily observed through the ionic electrical modality, while the endocrine system will likely require molecularly specific modalities for biodevice communication. We envision that redox will be the most appropriate modality for probing the immune system and also for observing interactions across biotic−abiotic interfaces (e.g., the microbiome associated with the gut and plant rhizosphere). Finally, the current understanding of redox biology is rather limited, and thus, we imagine that in the immediate term, our redox-based measurements may be more valuable as a tool for study rather than a means to solve clinical or environmental problems.

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Notes

The authors declare no competing financial interest.

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