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Perspective

Mediated Electrochemical Probing: A Systems-Level Tool for Redox Biology

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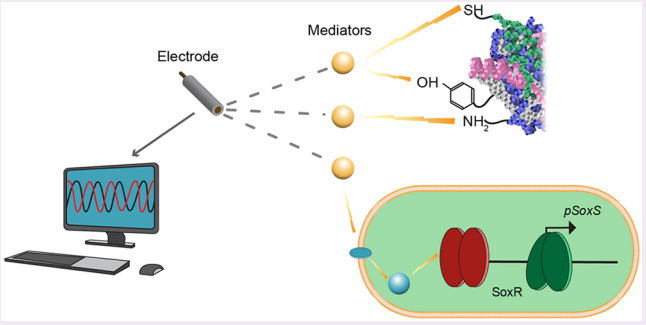
ABSTRACT: Biology uses well-known redox mechanisms for energy harvesting (e.g., respiration), biosynthesis, and immune defense (e.g., oxidative burst), and now we know biology uses redox for systems-level communication. Currently, we have limited abilities to "eavesdrop" on this redox modality, which can be contrasted with our abilities to observe and actuate biology through its more familiar ionic electrical modality. In this Perspective, we argue that the coupling of electrochemistry with diffusible mediators (electron shuttles) provides a unique opportunity to access the redox communication modality through its electrical features. We highlight previous studies showing that mediated electrochemical probing (MEP) can "communicate" with biology to acquire information and even to actuate specific biological responses (i.e., targeted gene expression). We suggest that MEP may reveal an extent of redox-based communication that has remained underappreciated in nature and that MEP could provide new technological approaches for redox biology, bioelectronics, clinical care, and environmental sciences.



INTRODUCTION

Reduction−oxidation (redox) reactions are integral to life and have well-defined roles in pathways for energy conversion and biosynthesis. Yet redox also appears to be a modality that acts at a global systems level.[1−4] For instance, oxidative stress has been one of the most important topics of redox biology because reactive chemical oxidants are common intermediates in biology's perception of and response to stress.[5−7] For instance, plants and animals respond to pathogen threats using an oxidative burst to generate reactive oxygen species (ROS).[8−10] Similarly, inflammation and associated ROS appear to be common consequences of psychological stresses,[11−14] as well as stresses associated with exercise.[15−17] While these reactive species are well-known for their detrimental effects associated with oxidative damage, aging,[18−20] and disease,[5] it has become clear that these same reactive species can perform beneficial functions. For instance, the ROS generated at a wound site appear to serve as critical cues to guide wound healing.[21,22] In fact, ROS are now known to perform various signaling functions, and these insights are revealing the broader richness of redox biology.[1]

One area in which a broader richness has been revealed involves the signals. As suggested in Table 1, reactive species do not just contain oxygen but may also contain nitrogen, chlorine, and sulfur, and there are significant differences among these reactive species.[23−25] Often, when a reactive species

Table 1. Partial List Illustrating the Diversity of Reactive Species and Typical Formation Reactions

reactive oxygen species	superoxide ($O_2^{\bullet-}$)	$O_2 + e^- \rightarrow O_2^{\bullet-}$
	hydrogen peroxide (H_2O_2)	$O_2^{\bullet-} + 2H^+ + e^- \rightarrow H_2O_2$
	hydroxyl radical ($HO^{\bullet}$)	$H_2O_2 + e^- \rightarrow HO^- + HO^{\bullet}$
reactive nitrogen species	nitric oxide ($NO^{\bullet}$)	
	peroxynitrite ($ONOO^-$)	$O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^-$
reactive chlorine species	hypochlorous acid (HOCl)	$H_2O_2 + H^+ + Cl^- \rightarrow H_2O + HOCl$
reactive sulfur species	thiyl radical ($RS^{\bullet}$)	$RSH \rightarrow RS^{\bullet} + H^+ + e^-$
	sulfenic acid (RSOH)	$RSH + H_2O_2 \rightarrow RSOH + H_2O$
reactive electrophiles	lipid hydroperoxide (LOOH)	
	quinones	

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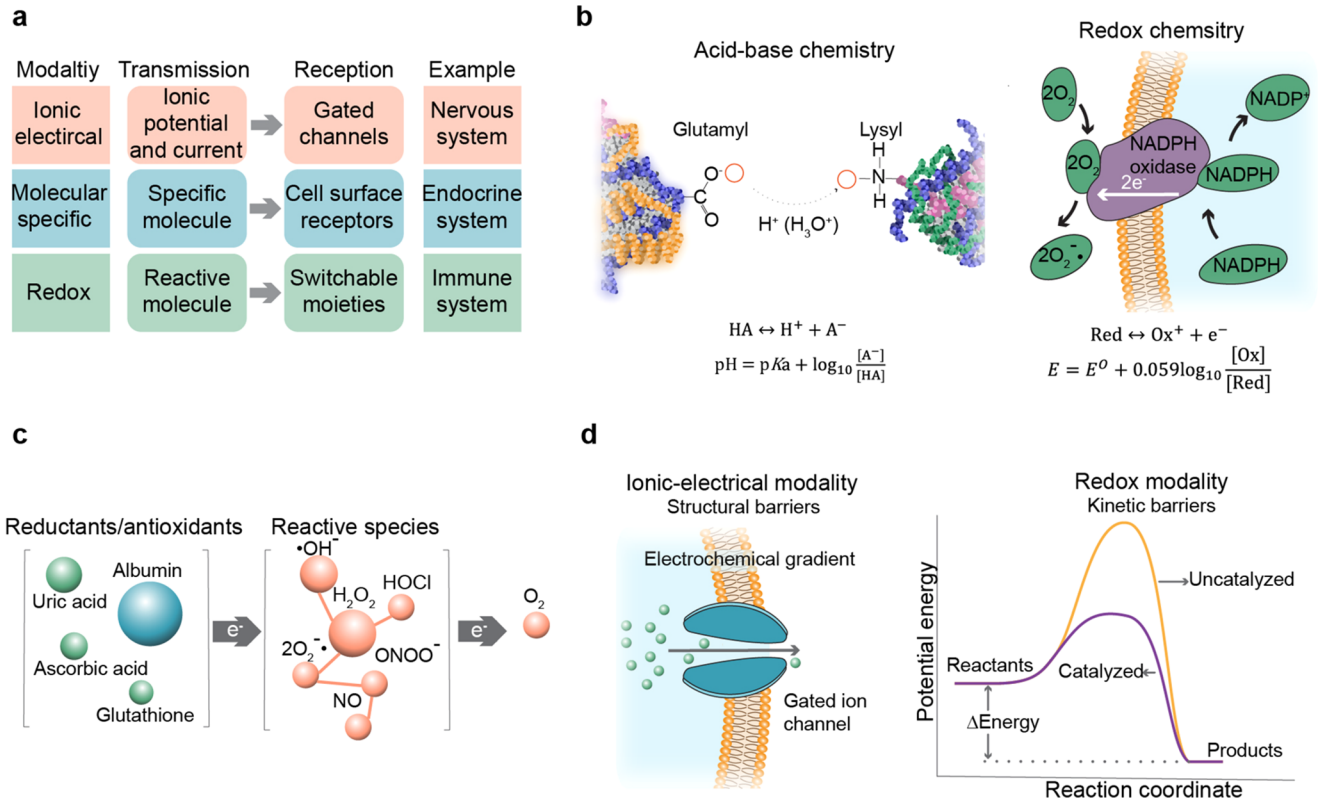

Figure 1. The unique features of the redox signaling modality. (a) Redox is distinct from other biological signaling modalities although it shares features with the ionic-electrical and molecularly specific modalities. (b) Redox chemistry is fundamentally different from acid−base chemistry because the charge carrier (the electron) is not soluble in the aqueous medium. (c) Redox reaction pathways are inherently networked because electrons cannot be transmitted through the medium but rather must "flow" through molecular intermediates. (d) Both the ion-based electrical modality and the electron-based redox modality couple electrical and molecular features: electrochemical gradients drive ionic currents, while redox potential differences drive the "flow" of electrons through redox reactions.

undergoes reaction, it consumes an antioxidant and generates another reactive product (e.g., a reactive electrophile as illustrated by quinones in Table 1).[26] From the perspective of signaling, these reactive products can serve as second messengers capable of propagating the original signal and possibly changing the "message" being conveyed.[1] Increasingly, the term "ROS-signaling" is being broadened to "redox-signaling", and the concept of oxidative stress is being broadened from a focus on the generation of reactive species to a balance (or imbalance) between pro- and antioxidant activities.[1]

A second area of increasing richness is the target of the reactive species.[1] For instance, the cysteine thiols of proteins are common molecular targets with their oxidation leading to disulfide bond formation.[1,27] Interestingly, this mechanism for signal reception is atomically (vs molecularly) specific, yet this sulfur-switching can still engage downstream intracellular signal transduction pathways that regulate gene expression (e.g., the Keap1−Nrf2−ARE pathway[28,29]). In addition, superoxide ($O_2^{\bullet-}$) based oxidation of Fe−S clusters provides another mechanism for the detection of a reactive oxidant and induction of a stress response (e.g., the SoxRS regulon in *E. coli*).[30] In addition to these well-defined targets, reactive oxidants can modify other amino acid residues (e.g., tyrosine and lysine) through a variety of mechanisms. A traditional view may consider such protein oxidation to be the collateral molecular damage of oxidative stress, while a different view considers these reactions as context-dependent post-translational modifications whose functional consequences are incompletely understood.[31]

A third area of increasing complexity involves context. For instance, the gut epithelium and microbiome contribute oxidative and reductive activities across an extracellular space that has steep gradients in O_2 and may contain a variety of redox-active metabolites and dietary antioxidants.[32,33] Thus, the lifetime and fate of a redox signal may vary in space and time and also may depend on diet and shifts in the microbiome. Importantly, such complex redox contexts are not unique. The plant's microbiome (the narrow rhizosphere surrounding its roots) is similarly complex with the roots supplying a local source of oxygen,[34−36] both the root "host" and its microbiome providing diffusible redox-active metabolites, and the extracellular matrix rich in redox-active humic substances.[37−39] Both these examples illustrate that redox signaling occurs within a complex redox context and conveys information across biological kingdoms (the host and its microbiome).

The above examples illustrate a complexity in redox signaling that has been difficult to fully appreciate. In this perspective, we suggest that electrochemistry may provide a valuable tool to reveal some of redox biology's complexity.

DISTINGUISHING FEATURES OF THE REDOX COMMUNICATION MODALITY

We consider four important features of the redox signaling modality. First, as illustrated in Figure 1a, redox signaling is

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different from the more-familiar biological communication modalities that transmit information using ionic electrical signals (e.g., a sequence of action potentials) or specific molecules (e.g., hormones, cytokines, or neurotransmitters).[40] In particular, the redox modality has its own code,[41] uses relatively simple reactive molecules (e.g., oxidants) as diffusible signals, and receives information through electron-transfer redox reactions (e.g., the oxidation of protein thiols to disulfides).

A second feature of the redox modality is the ubiquitous use of reduction−oxidation (redox) reactions for information transfer. From a chemistry perspective, it is tempting to think of redox reactions as being analogous to acid−base reactions. In fact, Figure 1b illustrates that acid−base and redox chemistries can be described by reactions and equilibrium equations that have similar forms. One crucial difference however involves the solubility of the "charge carrier". The proton (or hydronium ion H_3O^+) is soluble in an aqueous medium and thus proton transfer between an acid and base can occur indirectly through the intervening aqueous media. In contrast, the electron is not soluble, and electron transfer in aqueous solution generally requires chemical reactions in which one reactant directly transfers electrons to a second reactant. A second crucial difference is reaction kinetics. Protonation−deprotonation reactions are generally believed to rapidly equilibrate in the absence of catalysis while redox reactions can have strong kinetic barriers (e.g., the NADPH reductant can coexist with the O_2 oxidant in the absence of the enzyme NADPH oxidase).[42]

A third feature of the redox modality is its networked structure. Reactant nodes must directly interact to transfer electrons through redox reactions, and these interactions serve as network links (or edges). While some redox networks are well characterized (e.g., the electron transport chain in respiration), many redox interactomes[24,43] are incompletely understood. For instance, Figure 1c illustrates some features for the redox network in serum or tissue. A partial reduction of O_2 (e.g., by an inflammatory response or electron "leakage" from mitochondria) can generate ROS that can be interconverted through enzymatic and nonenzymatic mechanisms. Typically, these ROS are diffusible oxidants that can oxidize various reducing components that are present in the tissue or serum. These reductant "nodes" include small molecules (e.g., ascorbate and glutathione) and macromolecules. Particularly important is the protein albumin: it is quantitatively the most abundant reducing component in serum; it can transit between tissue and the vasculature; it has a comparatively long half-life (19 days); and it can undergo oxidation at various different amino acid residues.[44−47]

The fourth feature that makes the redox modality unique is the coupling of its electrical and molecular features. As illustrated in Figure 1d, the more familiar ionic electrical modality also couples electrical and molecular features: a membrane's electrochemical potential gradient provides a driving force for ion flow, while membrane-spanning protein channels serve as gates to control the flow of ionic charge across this membrane. In this case, biology uses structure (membranes and protein channels) to constrain the "flow" of the ionic charge carriers. In contrast, redox signaling often relies on kinetic barriers to constrain electron-flow through reactions that are otherwise thermodynamically favored.[48] For instance, H_2O_2 is a common redox signaling molecule, and although it is referred to as a reactive oxidant, it has considerable kinetic stability.[1,27] Thus, the coupling of the molecular and electrical features of the redox (vs ionic-electrical) modality is unique in that electrons (vs ions) are the charge carriers and the flow of charge involves a change in redox-state (vs ion-position). Table 2 summarizes key differences between biology's redox and ionic electrical modalities.

Table 2. Comparison of the Electrical Features of the Redox Modality and the Ion-Based Electrical Modality[a]

	redox modality	ion-based electrical modality
signal	reactive molecules (e.g., ROS)	ion-based activities (e.g., action potentials)
"charge carrier"	electron	ion
charge carrier solubility?	no (electrons "shuttled" by molecules)	yes (ions are soluble)
mechanism of current flow	electron-transfer reaction (change in redox state)	ion flow across membrane (change in ion position)
driving force for current flow	redox potential difference	electrochemical membrane gradient
constraints to current flow	kinetic barriers (activation energies)	physical barriers (membranes)
enabling mechanisms	enzymes	membrane-spanning channels
example of signal reception mechanism	sulfur switching (oxidation of protein cysteines to disulfides)	voltage-gated ion channels

[a]The statements are generalizations and may not be absolutely true in all cases.

MEDIATED ELECTROCHEMICAL PROBING (MEP) AS A TOOL FOR REDOX BIOLOGY

Unraveling the mechanistic details of biological function (e.g., communication and information processing) requires appropriate experimental tools. For instance, electrode measurements allowed early electrophysiologists to characterize important features of neuronal communication through biology's ionic electrical modality, even before the structure of DNA was resolved. However, electrode activity measurements are incapable of resolving the molecular level details of intracellular signal transduction pathways (e.g., G-protein signaling). Resolving such molecularly specific signal transduction requires advanced molecular methods. However, such molecular methods cannot replace electrodes for detecting ionic flows that occur over millisecond time scales and micrometer length scales. Thus, measurements of both molecules and electrical activities have been integral to characterizing biological signal transduction, and while these methods can be complementary, they are not interchangeable.

As noted above, redox signaling has both molecular and electrical features. While there has been exciting progress in applying molecular tools (e.g., omics) to characterize redox's molecular features (i.e., to detect the nodes),[49−55] we suggest that mediated electrochemistry is emerging as a unique tool to probe the electrical features of the redox modality. We suggest three analogies to illustrate the broad possibilities of MEP.

ANALOGY NO. 1: ELECTROCHEMISTRY AS A TOOL FOR REVERSE ENGINEERING

The use of mediated electrochemical probing (MEP) to characterize the electrical features of a redox network is

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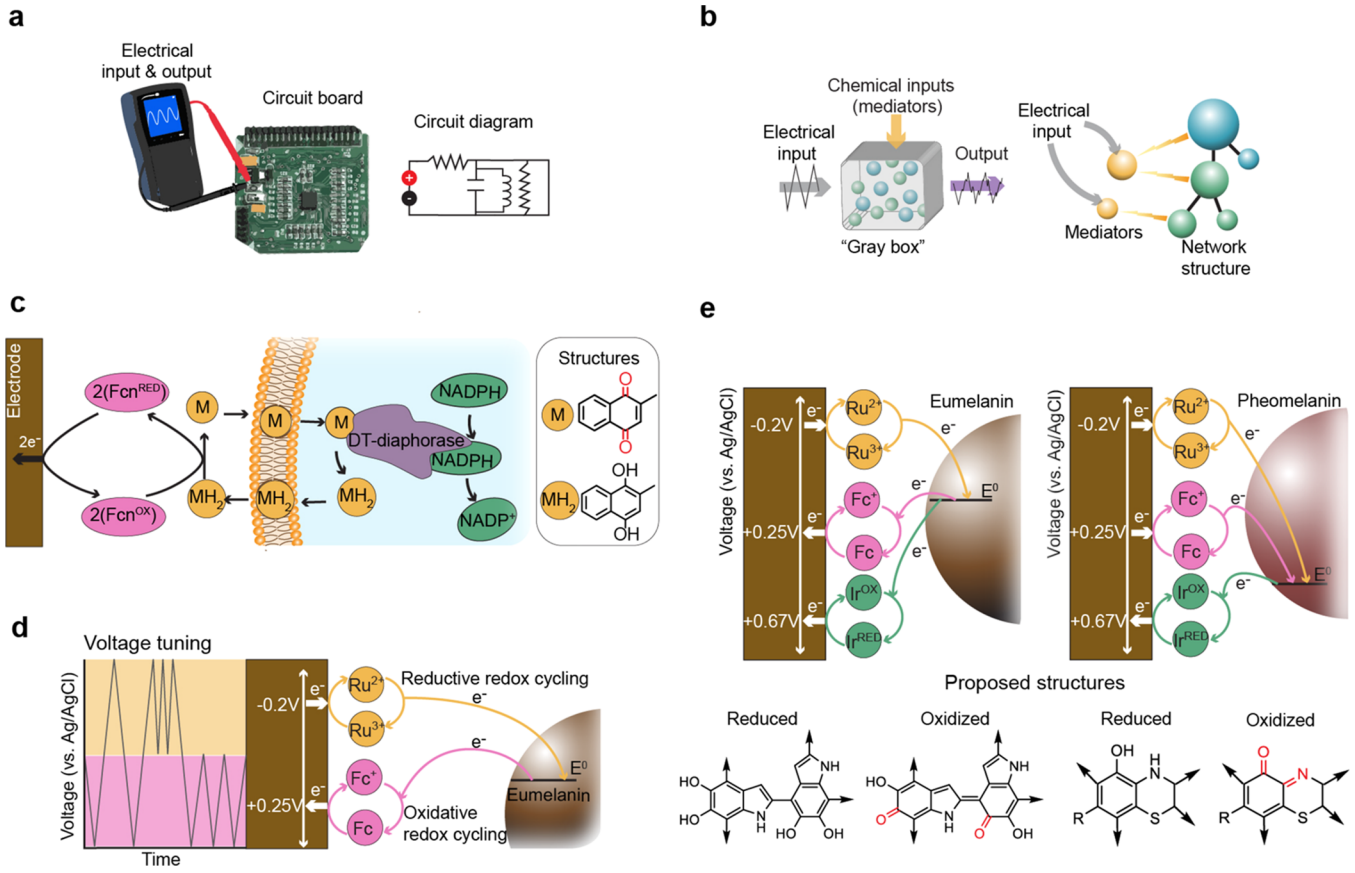

Figure 2. Mediated electrochemical probing (MEP) as a tool for reverse engineering. (a) Reverse engineering in electronics couples information of structure with electrical activity to characterize function. (b) MEP provides a method to probe the electrical features of a redox reaction network (i.e., a redox interactome). (c) Two mediators, ferrocyanide (Fcn^{RED}) and menadione (M), have been used to detect intracellular redox based activities. Multiple mediators and "tuned" voltage inputs have been used to (d) assess eumelanin's redox activities and (e) compare the redox activities of eumelanin and pheomelanin.

analogous to the reverse engineering of an electronic circuit as illustrated in Figure 2a. In electronic circuits, the flow of electrons depends on the characteristics of the individual circuit elements and how they are interconnected. Sometimes the function of an electrical circuit can be revealed by "simply" determining its structure (the elements and their connections), and this is possible because electrical circuits are generally built from a handful of circuit elements (e.g., resistors, capacitors, and inductors) that follow well-defined rules (i.e., current−voltage relationships). Other times, understanding a circuit's function requires the coupling of the available structural information with electrical measurements of the circuit's response characteristics (e.g., how the output current varies in response to input voltage).

By analogy, the flow of electrons through a redox network depends on the characteristics of the individual nodes and their interactions (i.e., reactions). Figure 2b suggests the reverse engineering of a redox network in which mediators are added and electrical inputs are imposed to induce electrons to flow through nodes. Independent molecular analysis (e.g., omics) may provide critical information on nodes; however the coexistence of two nodes is no guarantee that they are connected (i.e., that they can react with each other). At a minimum, two nodes can only be connected if there is a driving force and a mechanism: the redox reaction must be thermodynamically and kinetically favorable. Additionally, two nodes can only be connected if they have an appropriate spatial organization. Some redox networks have well-known spatial organizations (e.g., the respiratory electron transport chain). Other redox networks do not appear to have unique spatial architecture, but even in these cases, two nodes may remain disconnected if they are located beyond a relevant diffusional length scale (can be <1 μm).[56−58] Thus, chemical measurements alone may be unable to characterize how electrons (and information) flow through a redox network.

Two examples illustrate the use of electrical measurements to reverse engineer redox based activities. First, Figure 2c illustrates an early study in which two mediators were added to a cell culture.[59] One mediator (menadione, M) can cross the cell membrane and be reduced (to menadiol, MH_2) by accepting electrons from NAD(P)H through an enzyme-catalyzed electron transfer reaction (e.g., DT-diaphorase). The second mediator (ferricyanide, Fcn^{OX}) cannot cross the cell membrane but can be reduced (to ferrocyanide, Fcn^{RED}) by accepting electrons from menadiol. This dual mediator study illustrates the detection of an intracellular redox reaction by an external electrode.[59]

The use of mediated electrochemistry to access intracellular redox activities is being extended in many important ways: different mediators can be used to facilitate membrane transport or protein targeting,[60,61] voltage inputs can be "tuned" to probe defined activities,[62] nano- and micro-electrodes can be used to analyze single cells,[60,63,64] and additional measurement modalities (e.g., Raman) can be

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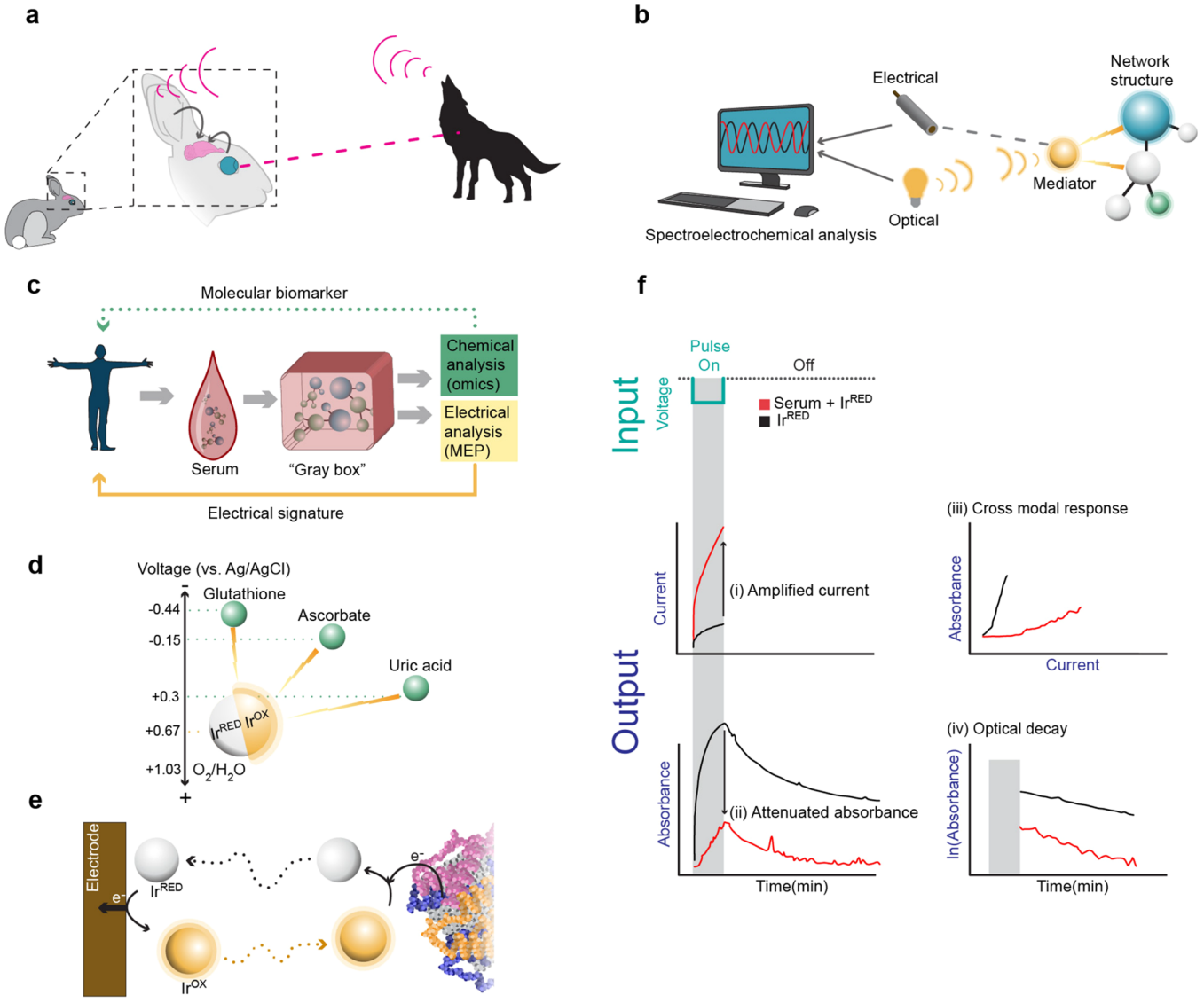

Figure 3. Electrochemistry as a tool for information processing. (a) Survival requires sensory systems (sensory organs plus brain) that efficiently acquire and process information. (b) Electrodes can sensitively detect redox signals and provide this information in a convenient electronic format that can be fused with information acquired from other sensor modalities (e.g., optical): mediator(s) facilitate interactions with redox nodes. (c) Illustrative example for the discovery of biomarkers for oxidative stress: molecular biomarkers can be discovered by reductionist measurements of individual chemicals (e.g., of network nodes), while electrical signatures can be discovered by systems-level MEP measurements (e.g., of network activity). Mediators (e.g., iridium, Ir) can extend an electrode's ability by (d) inducing interactions with redox nodes that (e) evoke readily measurable responses (analogous to echolocation's ability to extend hearing). (f) MEP uses mediator and voltage inputs to evoke dynamic responses that are characterized in terms of signal metrics (not molecular composition or concentration): four signal metrics are shown for a single oxidative voltage pulse. Adapted in part with permission from Kang et al.[82] Copyright 2018 Elsevier.

coupled with electrical measurements.[65] There are also challenges to using mediators to probe cellular redox activities. Mediated electrochemistry generally relies on the diffusion of a mediator between an electrode and the cells: while the electrode does not need to directly contact a cell, it does need to be relatively close (typically <100 μm). Further, the use of mediators to extract or donate electrons is not necessarily benign but could significantly perturb cellular metabolism or impose oxidative or reductive stresses. Nevertheless, *in vitro* MEP has been shown to be capable of detecting biologically relevant, redox-based differences (e.g., between metastatic and nonmetastatic cancer cells).[66]

A second example, illustrated in Figure 2d, is the natural pigment melanin, which is probably the biological material that has been most studied by MEP. Melanins are ubiquitous in biology and are believed to perform diverse functions. Surprisingly, basic questions of melanin's molecular structure (e.g., monomer, linkage, and size) and physicochemical properties (e.g., electrical conductivity) are not yet fully resolved and remain active areas of research.[67] MEP provides a means to reverse engineer melanin's redox properties without requiring knowledge of molecular structure.[68] As illustrated in Figure 2d, in initial MEP studies with eumelanin, we used two mediators: (i) $Ru(NH_3)_6Cl_3$ (Ru^{3+}) has a relatively reducing redox potential and can shuttle electrons from the electrode to the melanin through a reductive redox-cycling mechanism, while (ii) ferrocene dimethanol (Fc) can shuttle electrons from the melanin to the electrode through an oxidative redox-

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cycling mechanism. Reductive and oxidative redox cycling do not occur simultaneously, but these two mechanisms can be induced to alternate sequentially by imposing an oscillating electrode voltage. As illustrated in Figure 2d, if the voltage range is large, both reductive and oxidative redox cycling can be observed in a single cycle. However, the voltage input can be "tuned" to smaller voltage ranges that permit probing of a single redox-cycling mechanism. Initial MEP studies demonstrated that eumelanin is reversibly redox active and can be repeatedly oxidized and reduced: this suggests that melanin may be an important, but underappreciated, node in redox biology.[69,70]

In subsequent studies, we used three mediators to compare the redox properties of two melanin types: eumelanin, which is believed to be a protective antioxidant, and pheomelanin, which is believed to have detrimental pro-oxidant properties.[71,72] Figure 2e shows that probing with three mediators revealed that these two melanin types possess different redox potentials with the pheomelanin possessing a more-oxidative redox potential.[73] From independent chemical analysis putative molecular structures were proposed for the redox states of both melanins (Figure 2e).[73] These *in vitro* measurements suggest that pheomelanin may contribute to oxidative stress through a redox-buffering mechanism.

There have also been various extensions in which different redox-active molecules were tested as mediators that could engage melanins in redox cycling. For instance, our MEP studies have shown that the analgesic acetaminophen and the antipsychotic clozapine can engage melanin in oxidative redox cycling,[74] while the agricultural chemical paraquat can engage melanin in reductive redox cycling.[75] These *in vitro* studies suggest the possibility that melanins (e.g., in the skin or brain) may undergo previously underappreciated redox interactions that may contribute to the activities of drugs and environmental chemicals. MEP has also been extended from melanins to other natural materials with similar catecholic (or phenolic) structures. For instance, MEP has shown that dietary antioxidants (e.g., the spice clove),[76] plant lignins,[69] and the humics in soil[38,77] all possess redox activities. The facts that these materials are ubiquitous in nature, have redox potentials in the midphysiological range, and have low kinetic barriers for electron transfer suggest the possibility that phenolic and catecholic materials may serve as hubs in important extracellular redox networks.[78]

In summary, MEP can be viewed as a tool for reverse engineering that can provide functional measurements of redox-based electrical activities that complement molecular measurements. However, MEP measurements can be performed in the absence of structural knowledge (e.g., for melanins) to provide activity-based measurements of biologically relevant nodes and potentially to identify interactions (e.g., redox-based drug interactions) that warrant further study.

ANALOGY NO. 2: AN ELECTRODE AS A SENSORY ORGAN

We envision that electrochemistry can emerge as a systems-level tool for redox biology, but this may require a paradigm shift from a chemical analysis perspective to an information processing perspective. A chemical analysis perspective is intrinsically reductionist, tending to define information in terms of chemical composition and assuming a systems-level understanding will be revealed from the collective knowledge of the individual components. Information scientists use a definition for information that is broader, more abstract, and focuses on uncertainty (i.e., information decreases uncertainty). This information science perspective is increasingly being applied to biology[79−81] with prominent examples being predator−prey interactions (Figure 3a) that impose significant selective pressure on the evolution of systems for accessing and processing sensory information. These selective pressures tend to drive sensory *organs* to detect signals that are immediately available or easily evoked (e.g., echolocation), sensory *systems* to detect near the limit of signal-to-noise (resources cannot be wasted on unnecessarily high performance), and *brains* that integrate and weight inputs from multiple senses to inform decision-making (fight vs flight).

An electrode can be imagined as a technological version of a sensory *organ*, one with a high intrinsic "technological fitness" due to its low resource costs (electrochemical systems are cheap and portable), high sensitivity for signal detection, and electronic output format that is available for real time analysis and coupling to other sensor modalities.[83,84] As illustrated in Figure 3b, we envision the electrode as a "sensory" tool capable of detecting systems level information and providing these inputs for real time analysis (e.g., machine learning), fusion with inputs obtained from other sensors (e.g., optical), and adaptive learning to discover patterns and detect changes. Also illustrated in Figure 3b is the use of mediators that extend the electrode's capabilities for probing the nodes of an interactome much like echolocation extends hearing by using an imposed input to evoke outputs that are rich in information.

To illustrate the use of electrochemistry as a *systems* level tool for information processing, we consider the example of discovering a serum biomarker for oxidative stress as illustrated in Figure 3c. A chemical analysis approach (upper path in Figure 3c) would focus on the discovery of molecular biomarkers and sample analysis would often involve high throughput omics. The lower branch in Figure 3c illustrates a MEP-based information processing strategy, which aims to discover redox-based electrical signatures. These two approaches are complementary but not equivalent: the molecular analysis focuses on nodes, while the electrochemical analysis focuses on electrical activities of the network.

Iridium (K_3IrCl_6, Ir) was identified as a useful mediator for probing serum samples. As illustrated in Figure 3d, the oxidized form (designated Ir^{OX}) is a moderately strong oxidant (yet still in the physiological range) and can oxidize various biological reductants. In addition, Ir^{OX} can oxidize several protein amino acid residues and is especially sensitive to cysteine residues (presumably because it generates highly oxidized sulfur products).[85,86] Also illustrated in Figure 3d, the reduction of Ir can be detected optically because Ir^{OX} is yellow while Ir^{RED} is colorless. Initial studies used a single end-point measurement (either an electrochemical or optical measurement) to detect how much Ir^{OX} was reduced upon incubation with diluted serum:[86] serum from persons with greater levels of oxidative stress is expected to have less Ir-reducing capacity. These measurements demonstrated that persons diagnosed with schizophrenia had statistically greater levels of oxidative stress (as measured by Ir-reducing capacity) compared to healthy controls (N = 118; area under receiver operator characteristic curve = 0.89; $p = 9.3 \times 10^{-5}$).[87]

While this end-point measurement demonstrated that Ir could discern the extent of oxidative stress, the information being accessed is intrinsically limited by the format of measuring a single end-point value. Dynamic analysis of

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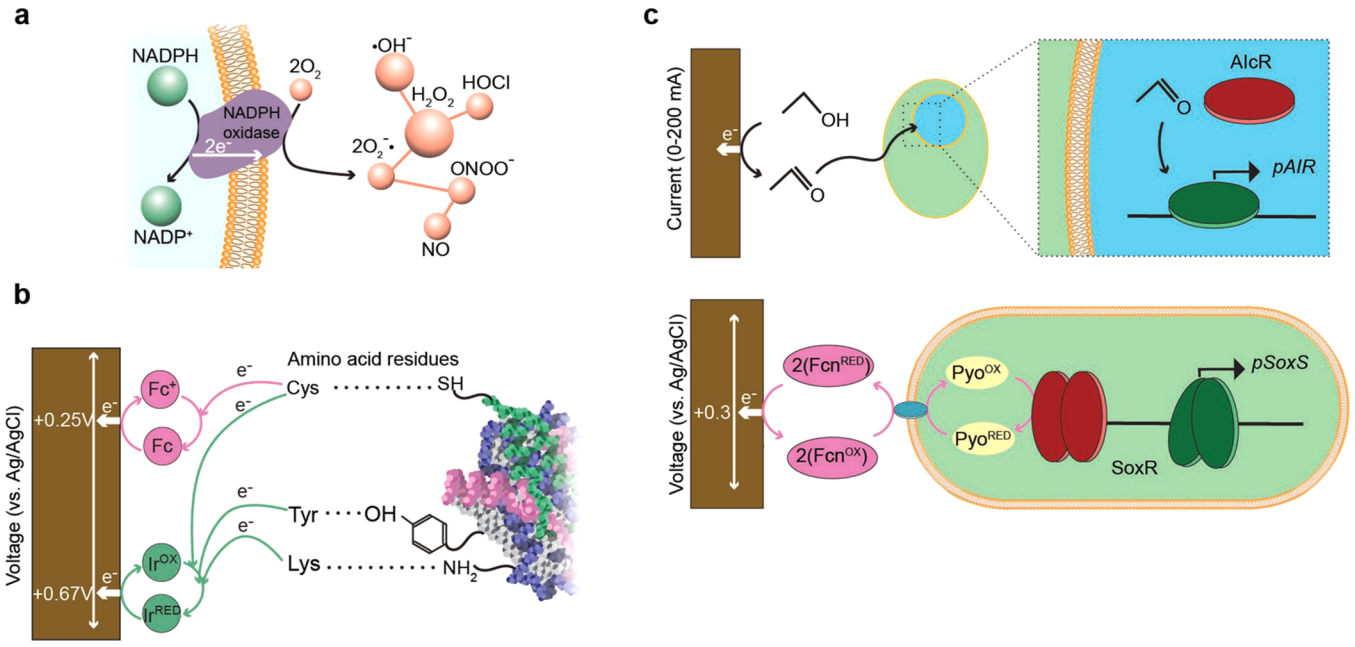

Figure 4. Electrochemistry as a redox-signal-generator to change molecular structure and actuate biological activity. (a) The immune system generates a range of reactive species that have differing chemical reactivities and targeting capabilities. (b) Mediators can selectively modify molecular structure based on their redox potential (e.g., thermodynamics can constrain reactivity). (c) Mediators can induce gene expression through redox-responsive regulons.

evoked responses, analogous to echolocation, offers the possibility of accessing more information as suggested in Figure 3e. For instance, the diluted serum can be mixed with the inert Ir^{RED} and oxidation can be initiated by a short oxidative pulse (e.g., 1 min) to generate Ir^{OX}, which can diffuse into the serum sample to extract electrons from (i.e., to oxidize) the various nodes. Samples with greater reducing capacity will tend to convert more of the electrochemically generated Ir^{OX} back to Ir^{RED}, which will lead to two measurable observations: the oxidative current drawn during this pulse will be amplified due to the oxidative redox cycling, and the optical absorbance will be attenuated due to consumption of the Ir^{OX}. Figure 3f illustrates such amplified current and attenuated absorbance and also shows a cross-modal correlation between these two outputs. After this oxidative pulse, the instrument voltage is turned off, there is no further generation of Ir^{OX} (no electrical output), and the optical absorbance decays due to consumption of the previously generated Ir^{OX}. The first-order rate constant for the decay of the optical signal provides a final observable measure related to the sample's reducing capacity. Thus, this single pulse generates four quantifiable signal metrics related to a sample's reducing capacity (current amplification, absorbance attenuation, electrical-optical cross-modal response, and absorbance decay rate).

The signal metrics generated by dynamic MEP are sensitive to the type and concentration of reductants present.[82] Further, these metrics depend on the pulse duration: short pulses should access information of only the most-reactive reductant nodes in a sample, while longer pulses should provide more time for less reactive reductants to be oxidized by Ir^{OX}. Also, MEP is not confined to probing with a single mediator or a single voltage pulse, but rather arbitrarily complex input voltages can be imposed (e.g., Figure 2d). Finally, additional signal metrics could be created and additional measurement modalities could be included to enhance the extraction of information from the sample. For the example of discerning signatures of oxidative stress from serum samples, a three-pulse input was used that generated 14 signal metrics. Mining of these metrics revealed that two metrics could distinguish persons diagnosed with schizophrenia from healthy controls ($N = 15$; $p = 0.003$ for one metric and $p = 0.013$ for the second metric), while a coupling of these independent metrics offered even greater discriminating capabilities (area under receiver operator curve = 0.98; $p < 0.001$).[82]

Thus, Figure 3c shows that the same sample can be analyzed by chemical and MEP-based information processing approaches but the information extracted is different. Molecular analysis provides chemical compositional information that has the potential benefit of being easier to interpret in terms of molecular mechanisms (e.g., of disease). MEP-based electrical measurements provide information of systems-level activities that offer the benefits of being easier to acquire and analyze by data analytics. While these two measurement approaches are complementary and the data should map onto each other, the real value of either measurement lies in whether they map onto the clinically driven question (e.g., which approach reduces the uncertainty in the diagnosis or management of disease).

In summary, dynamic MEP-based measurements use mediators and electrical inputs to evoke responses that appear as information-rich data streams that can be characterized in terms of signal metrics that can be mined for signature patterns and differences. This approach enlists the electrode (and spectrophotometer) as a simple and sensitive sensor that detects features of a sample, presents the data in real time for data analysis, and eventually will allow feedback and learning to be integrated into the data acquisition step.

ANALOGY NO. 3: AN ELECTRODE AS A REDOX SIGNAL GENERATOR

In Figure 3a, we illustrated sensory organs and the brain to emphasize that survival of a species depends on the efficient

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acquisition and processing of information about threats. Survival at the individual level requires more than just the information stored in genes but also depends on memory and learning. And survival depends not just on recognizing a threat but also on responding to the threat (fight vs flight), which often requires coordinated mechanical action. Thus, from an information science perspective, successful predator−prey outcomes depend on efficient detection, processing, and response to information, and this processing generally occurs in the central nervous system with much of the communication being transmitted through the ionic electrical modality. But organisms face another, different type of threat from pathogens, and in this case, the detection, processing, and response occurs through a distributed immune system that largely relies on molecular-based modalities for communication, learning, and actuation (e.g., cytokines and antibodies). Reactive species are major components at the front-line of host−pathogen interactions: the host generates these reactive species to perform effector actions (to kill the pathogen), while the pathogen responds to these stressors by upregulating defense responses (to induce protective antioxidant activities).

Figure 4a illustrates that immune cells generate a range of reactive species. Importantly, these reactive species are not all equivalent but can have chemical reactivities that vary by 13 orders-of-magnitude.[6] Because of these differences, the individual reactive species can act over different length and time scales, and they interact with different cellular and molecular targets. Thus, despite their simple molecular structures, reactive species offer differing capabilities for transmitting information. Several of these reactive oxidants and electrophiles can be generated electrochemically (e.g., H_2O_2, HOCl, and quinones), which suggests the possibility of using electrodes directly as redox signal generators capable of connecting biology to electronics.[88]

In addition to electrochemically generating the same redox signals as biology, Figure 4b shows that it is also possible to select mediators with tailored reactivities to engage biology's redox targets. For instance, the Fc mediator can be oxidized at the electrode to generate the Fc^+ oxidant that can diffuse from the electrode to target thiols to generate disulfide cross-links. However, as illustrated in Figure 4b, Fc^+ is a relatively weak oxidant and cannot oxidize lysine or tyrosine groups.[89] In contrast, the oxidized Ir mediator (Ir^{OX}) is a stronger oxidant that can target cysteine, lysine, and tyrosine residues. In addition to using mediators to target specific amino acids to modify protein structure, mediators have also been used to attenuate protein function (e.g., enzymatic activity).[90] These studies illustrate the extension of mediated electrochemistry from sensing redox-based information to transmitting redox-based signals that actuate molecular responses (i.e., changes in structure and function).

Mediated electrochemistry can also be used to provide redox cues that can actuate biology by altering gene expression through redox responsive transcription factors as illustrated in Figure 4c. To our knowledge, the first demonstration involved the electrochemical oxidation of ethanol to acetaldehyde to activate a fungal transcription system (AlcR transactivator) for heterologous expression in a mammalian host.[91] Later studies showed the purposeful use of two mediators (Fcn and the bacterial metabolite pyocyanin) to induce gene expression from the bacterial SoxR regulon.[92] Also, electrochemical oxidation of pyocyanin was used to create a chemical gradient for coordinating a differential response for multiplexed CRISPR activities.[93] In the latter case, the electronically generated signal was translated biologically and transmitted into biological networks. These three examples illustrate the breadth of possibilities both in terms of the imposed electrical input (currents vs voltage) and the biological targets (eukaryotes vs prokaryotes).

In summary, electrochemistry offers the opportunity to generate reactive oxidants (and reductants) that can engage biology through a native redox-based signaling modality. Thus, electrochemistry can be extended from observing and processing redox-based information to participating in redox-based communication and actuating biological responses.[94] From a technological perspective, these studies further suggest the potential for coupling the information processing capabilities of electronics with those of biology (e.g., through the use of synthetic biology)[95−98] to generate living electronics.[99]

PERSPECTIVE

One theme of this Perspective is that redox is a distinct modality for biological communication and actuation. Because redox signals are intrinsically molecular (e.g., reactive chemical species), the redox modality has similarities to more familiar molecularly specific signaling. In molecular signaling, information flows via molecular-level interactions (redox reactions vs ligand−receptor binding) that can be selective and can engage downstream signal transduction mechanisms that often lead to changes in gene expression. And because the redox modality has electrical features it also has similarities to ionic electrical modalities. However, the ionic electrical modality is rapid and centralized which is consistent with its role in interpreting inputs from sensory organs and coordinating outputs through neuromuscular activities. In contrast, the redox modality is slower and more distributed, consistent with its role in coordinating immune-related inputs and outputs.

The second theme is that redox communication is likely underappreciated in part because of the limited availability of experimental tools. We suggest that mediated electrochemistry provides a unique tool capable of engaging biology through a native redox communication modality. Also, electrochemistry can become a powerful experimental tool because the instrumentation is inexpensive and portable, measurements are highly sensitive and controllable in time and space (e.g., with nano- or microelectrodes), additional sensor modalities (e.g., optical) can be coupled with electrode measurements, and the data is generated in real-time in an electronic format accessible to instantaneous analysis. These instrumentation capabilities can be applied to redox biology by using diffusible redox mediators to "connect" the electrode into biological redox networks. Such mediator-based connections can be used to study relatively specific questions (Figure 2 illustrates probing of intracellular redox reactions and the redox activities of melanins) or to discover redox-based signatures of poorly understood phenomena (e.g., Figure 3 illustrates probing to discover signatures of oxidative stress). Mediated electrochemistry is also being used to access a native signaling modality for electrogenetic actuation (e.g., Figure 4 illustrates the potential to rewire redox-responsive regulons).[88,92,93,100]

We propose that redox-based communication, while ubiquitous in biology, has remained largely hidden from observation. It is well-known that redox-based communication and actuation is critical in determining outcomes of immune responses to pathogen threats. But redox-based communica-

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tion may also be integral to communicating information of immune system function across organs[1] (brain and gut) and also may be essential in sculpting communities that span Kingdoms (e.g., to maintain homeostasis in the gut microbiome or plant rhizosphere). We envision the emergence of a new type of bioelectronics that engages biology's redox-based modality (in addition to existing bioelectronics that engage biology's ion-based electrical modality). We suggest that such redox-linked bioelectronics would extend applications from the nervous and neuromuscular systems to the immune and gastrointestinal systems and could even provide new clinically relevant measurements (e.g., a vital sign for oxidative stress). Further, redox-linked bioelectronics can be extended to the biosphere for environmental and agricultural applications. From a methods perspective, there are many opportunities: How can scanning electrochemical methods be enlisted to resolve spatial gradients in redox activities (e.g., in a tissue section)? How can mediator-specificity be tailored (either to probe global redox context or a single enzyme's active-site)? How can information science be applied to maximize the information content of an evoked output response? In summary, we suggest that the "transition" of electrochemistry from the electrophysiology lab to the redox biology lab could open entirely new doors of opportunity at the interface of chemistry and biology.

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Notes

The authors declare no competing financial interest.

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KEYWORDS

redox: abbreviation for reduction−oxidation that is used to describe electron transfer chemical reactions
redox-active: describes a molecular species that can undergo a redox reaction by either donating or accepting electrons
bioelectronics: the use of electrodes often to acquire biological information (e.g., electrochemical biosensors and electrocardiograms) or actuate biological responses (e.g., neuroprosthetics and defibrillators)
mediators: chemical species that are often diffusible and redox-active allowing them to shuttle electrons (familiar biological examples are NAD(P)H and ascorbic acid)
oxidative stress: an imbalance between oxidative and antioxidant activities that has been linked to a diverse range of diseases through incompletely understood mechanisms
reactive species: reactive chemical molecules that usually contain oxygen but may also contain nitrogen, chlorine, and sulfur
electrogenetics: the use of electronic inputs to actuate biology often through alterations in gene expression
melanin: natural pigments that are ubiquitous in biology, have ill-defined structures and functions, but have recently been shown to possess redox-activity
reverse engineering: a method for studying the activities and functions of a system by imposing defined inputs and observing the response characteristics
sensor fusion: the combining of measurements from different sensors (e.g., electrical and optical sensors) to increase information and decrease uncertainty

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