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Excite the unexcitable: engineering cells and redox signaling for targeted bioelectronic control

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The ever-growing influence of technology in our lives has led to an increasing interest in the development of smart electronic devices to interrogate and control biological systems. Recently, redox-mediated electrogenetics introduced a novel avenue that enables direct bioelectronic control at the genetic level. In this review, we discuss recent advances in methodologies for bioelectronic control, ranging from electrical stimulation to engineering efforts that allow traditionally unexcitable cells to be electrically 'programmable.' Alongside ion-transport signaling, we suggest redox as a route for rational engineering because it is a native form of electronic communication in biology. Using redox as a common language allows the interfacing of electronics and biology. This newfound connection opens a gateway of possibilities for next-generation bioelectronic tools.

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Introduction

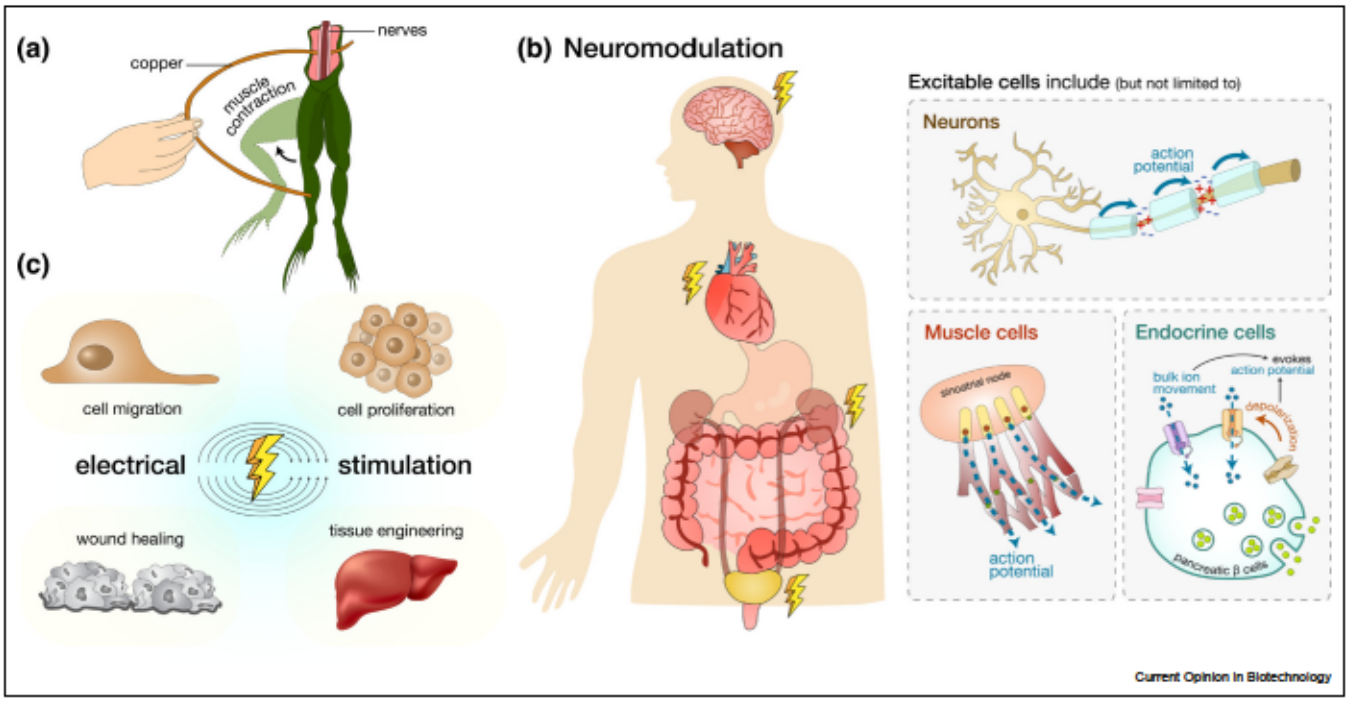
Electronic control of biological systems is an idea that dates back to the 18th century, a time when both Luigi Galvani and Alessandro Volta were doing pioneering work in the fields of electrophysiology and electrochemistry, respectively. It is through Galvani's work that we first caught a glimpse of the effects of electricity on biological systems: specifically, the ability to influence the movement of a frog's leg after exposure to an electric current, in which he concluded that electrical conduction was necessary for the muscle to contract (Figure 1a). Galvani's work later became the foundation for research on electrical control of cell physiology. From the start, electrophysiology was found to be inextricably linked to the study of electrically excitable cells such as neurons and cardiac cells. These cells are equipped with an excitable membrane with gated ion channels that enable controlled flow of ions that, in turn, generate action potentials. Through the propagation of action potentials, electrical signals are disseminated along cells or to neighboring cells.

While only certain cells can receive and spread electrical signals through ionic transport, recent studies suggest that in fact, virtually all cells are able to communicate electronically and this is made possible through redox-mediated electron transfer [1,2]. Redox reactions are ubiquitous in biology, enabling essential cell functions in central metabolism [3], energy harvesting [4], and cellular signaling [5]. Redox provides numerous opportunities for biological systems to be interrogated and even manipulated using electrochemical techniques; thus, we contend that redox can serve as the communication interface between biology and electronics to achieve targeted bioelectronic control. In this review, we summarize recent efforts that demonstrated electronic control of biological behaviors. These include electrical stimulation studies that manipulate or engineer ion-transfer communication, and additionally, intentional rewiring of biology's own redox mechanisms to allow for targeted, genetic control.

Electrical stimulation of excitable cells

Cells that natively use electrical communication via action potentials, such as neurons, muscle, and endocrine cells [6], are easily 'connected' to electronics through simple voltage stimulations (Figure 1b). The most well-known bioelectronic actuation devices could be the artificial cardiac pacemaker or the cardioverter-defibrillator, in which programmed pulses of voltage applied to heart chambers (i.e. cardiomyocytes) can stimulate the pacemaker cells to control the heart rate [7].

Figure 1



Electronic control in electrophysiology. **(a)** Galvani's frog leg experiment. The prepared leg of a frog twitches when a metal circuit connects a nerve to a muscle. **(b)** *Left*: Therapeutic targets for neuromodulation. These include organs that contain excitable cells, such as the brain, heart, gastrointestinal tract, and bladder. *Right*: Examples of excitable cells. Illustrated here are neurons (top), muscle cells (cardiomyocytes, bottom left), and endocrine cells (pancreatic β-cell, bottom right). **(c)** Electrical stimulation can modulate cellular behaviors of nonexcitable cells to aid in the process of wound healing and tissue engineering.

Bioelectronic neuronal stimulation has also been demonstrated as a tool for both studying electrophysiological behaviors and for therapeutic treatment (termed 'neuromodulation') to address clinical conditions. Neuromodulation is increasing in application, with trials in human diseases such as Parkinson's disease [8], schizophrenia [9], epilepsy [10], movement disorders [11], and chronic pain [12]. In addition to targeting the central nervous system, neuromodulation can be applied to peripheral and autonomic nervous systems to ameliorate or contain gastrointestinal disorders [13], bladder dysfunction [14], and obesity [15]. These treatments are promising and even offer the potential for neuroprosthetics [16]. Aside from neuronal activities, neuromodulation is shown to control the activity of excitable immunocytes [17,18], which in turn reduces the release of pro-inflammation factors. The coupling of neuronal circuitry with inflammatory responses offers new opportunities for using electronics for neuromodulation, however, a greater understanding will be required to determine how cellular-level activities (e.g. observable from patch-clamp recordings) lead to the change in emergent, systems-level function (e.g. cognition) [19]. It is important to note that despite its tremendous promise, neuromodulation is somewhat limited by the availability of electrically excitable cells (~2% of total cells in the human body [20]), leaving numerous cells with vital biological functions inaccessible to electrical control.

Electrical stimulation of nonexcitable cells

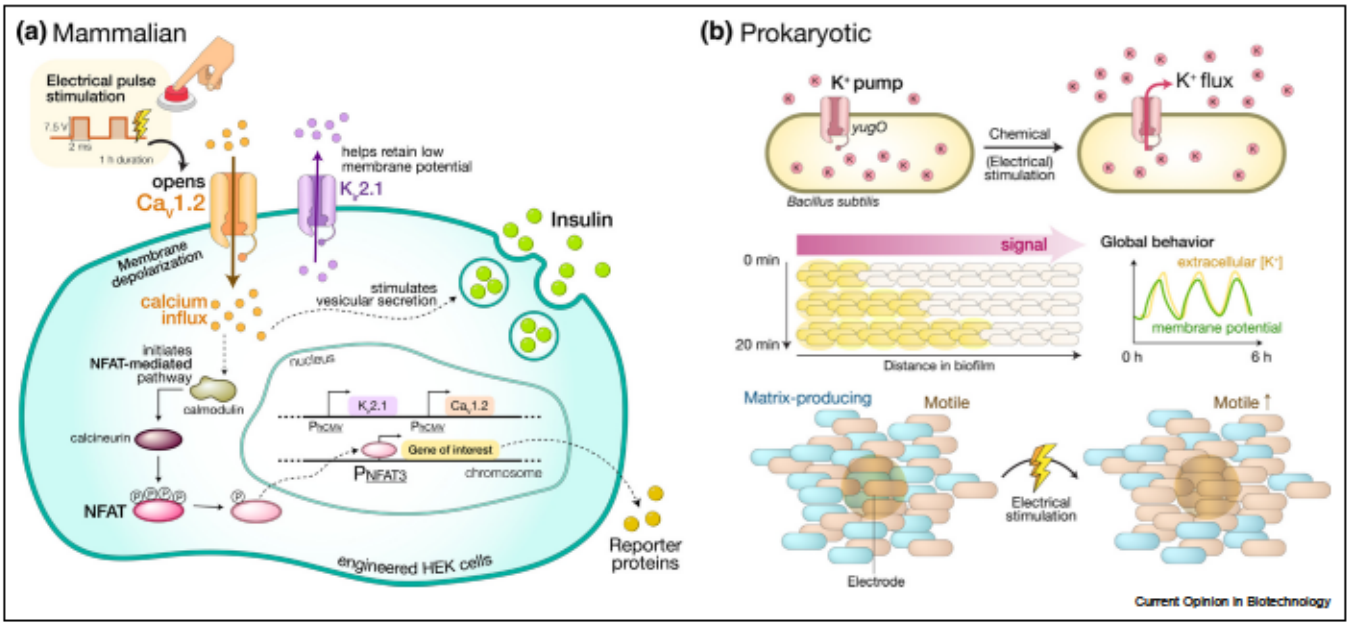
Many have recently argued that all cells are electrically active, that is, both prokaryotes and eukaryotes alike possess native bioelectric machinery such as ion channels to generate a resting membrane potential and endogenous electric fields that influence cell functions and communication [21]. While nonexcitable cells cannot propagate action potentials, exogenous electrical stimulation is still capable of polarizing cell membranes for activating intrinsic signaling pathways and influencing cellular microenvironments, which jointly lead to the modulation of essential cell functions such as cell proliferation [22], cell differentiation [23], and cell migration [24]. Therefore, electrical stimulation has emerged as a novel tool for guiding cell behavior. For example, in the field of regenerative medicine, electrical stimulation aids in the processes of tissue engineering [25] and wound healing [26] (Figure 1c). Novel microelectronic devices have been designed and fabricated as experimental platforms to instruct cell growth [27]. Additionally, exogenously applied electric fields have been shown to inhibit cell division by

globally disrupting a cell's native biophysical properties [28], or upregulating potassium ion channels [29] of malignant cancer cells to halt tumor growth [30]. Similar to neuromodulation of excitable cells, the underpinning mechanisms for these further types of bioelectronic control remain largely unelucidated, in turn, this somewhat hinders the implementation of targeted, intentional control of cell behavior.

Engineering the ionic electrical communication

Advances in synthetic biology have now given us many tools for the generation of 'designer cells' with programmable functions. In turn, this will enable more precise control. By expressing selected membrane ion channels (e.g. potassium and voltage-gated sodium channels) and gap junctions, researchers have demonstrated that nonexcitable mammalian cells can be engineered to generate and sustain action potentials for electrical communication [31]. Calcium channels ($Ca_v1.2$), too, are of particular interest due to their ability to further tune the electrical properties of the engineered excitable cells and couple intracellular signaling pathways to influence cell behavior (Figure 2a) [32]. For example, Xie et al. coupled glucose-sensing, adenosine triphosphate-sensitive potassium channels (K_{ATP}) [33] to a calcium-entry excitation–transcription response to produce insulin in human embryonic kidney (HEK) cells [34]. Specifically, K_{ATP}-mediated membrane depolarization, which results from glucose-initiated ATP generation, initiates calcium entry through ectopically expressed voltage-gated $Ca_v1.2$. The calcium influx subsquently activates the transcription factor nuclear factor of activated T cells (NFAT) and its translocation to the nucleus to activate gene expression. This synthetic process is later augmented to achieve direct electrical control of mammalian gene expression (Figure 2a): this time, ectopic expression of both the $Ca_v1.2$ and the potassium channel ($K_{ir}2.1$) allows the engineered HEK cells to become excitable and respond to electrical stimulation [35]. Before electrical stimulation, the inward-rectifying $K_{ir}1.2$ channel keeps the resting membrane potential low and the voltage-gated $Ca_v1.2$ closed, until pulses of applied voltage (7.5 V, 2-ms pulses) depolarize the membrane, open the $Ca_v1.2$, and trigger NFAT-mediated expression of insulin. In addition, Krawczyk et al. designed a custom bioelectronic device capable of performing wireless, real-time electronic

Figure 2



Engineering the ionic electrical communication. **(a)** Engineering the ion-channel signaling in mammalian systems. Ectopic ('out-of-place') expression of both the voltage-gated calcium ($Ca_v1.2$, orange) channel and the inward-rectifying $K_{ir}2.1$ (purple) allows direct electrical control of mammalian gene expression. Exogenous electrical stimulation causes the opening of $Ca_v1.2$, and the resulting calcium influx and membrane depolarization are shown to subsequently initiate NFAT-mediated gene expression and stimulate vesicle secretion of insulin in engineered HEK293 cells. **(b)** $K_{ir}2.1$-mediated signaling in prokaryotes is a potential target for engineering ionic electrical communication. *Top*: *B. subtilis* uses potassium ion channels to send signals within its biofilm for metabolism coordination. *Middle*: Propagation of extracellular potassium levels (yellow) in *B. subtilis* biofilm. Oscillations in membrane potential (green) and extracellular potassium level (yellow) are synchronized, suggesting that potassium release is involved in global membrane potential oscillations. *Bottom*: Electrical stimulation of a growing *B. subtilis* biofilm can actuate the change in consortia composition by promoting the proliferation of motile cells (cyan) but not extracellular-matrix-producing cells (brown).
Plots are partially adapted from [1].

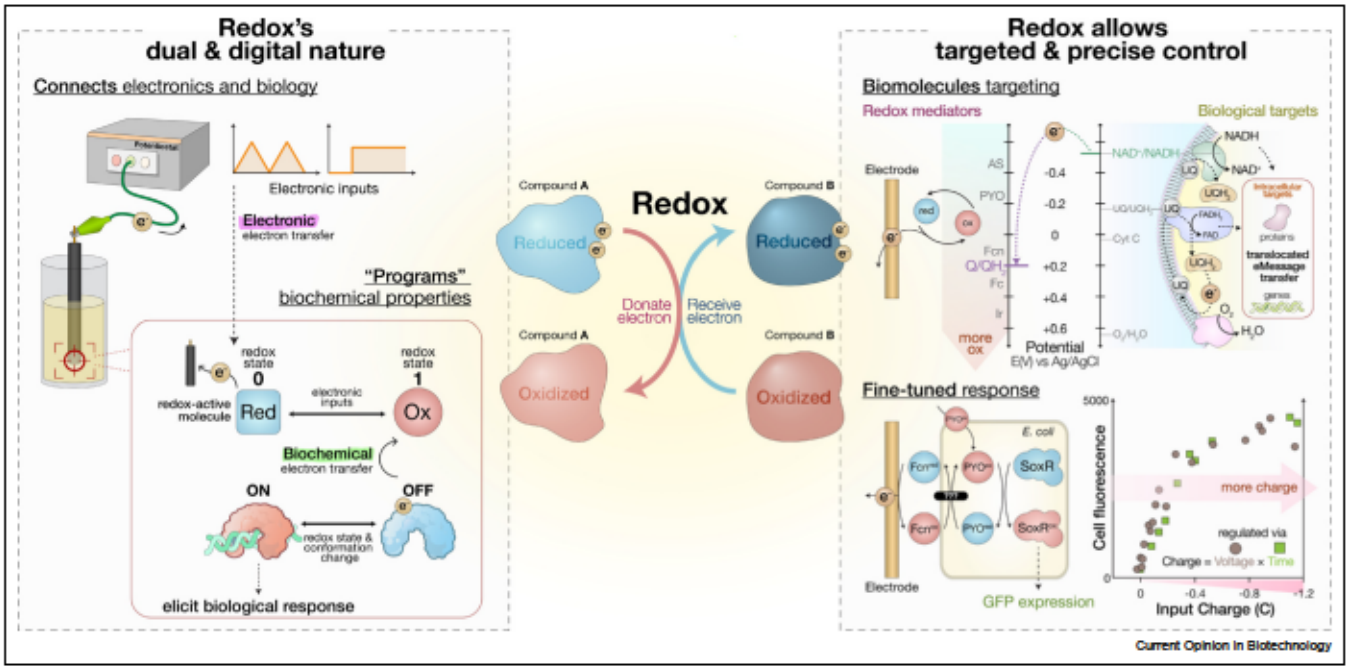
control on device-bound excitable HEK cells, and restored normoglycemia in type-1 diabetic mice [35]. This bioelectronic device has been recently updated as a self-sufficient, subcutaneous push button-controlled cellular implant, enabling electrically stimulated insulin release through simple button presses [36], clearly demonstrating general benefits from introducing electronics to the control of biological function.

Remarkably, ion-transport communication is not limited to eukaryotic systems. Surprisingly functional roles of ion-channel-mediated signaling in bacteria (e.g. *Bacillus subtilis* and *Escherichia coli*) [37,38] have been discovered, ranging from proliferation capacity [39], spore formation [40,41], and biofilm dynamics [42,43]. Electrical stimulation of a growing *B. subtilis* biofilm was recently reported to promote the proliferation of motile cells but not extracellular-matrix-producing cells, changing the local composition of the biofilm [44] (Figure 2b). These interesting findings on bacterial ionic transport can provide a future avenue for engineering electronic communication in prokaryotes and further enable ionic-based bioelectronic control.

Redox: an orthogonal modality for bioelectronic communication

Although there are growing avenues for ion-based electrical communication with biology, redox-mediated electron transfer offers an additional orthogonal communication modality that can be leveraged for bioelectronic control (Figure 3) (the differences between the ion-based and redox electrical modalities have been succinctly described by Zhao et al. [45]). In addition to cellular respiration/metabolism (e.g. oxidative phosphorylation), redox plays a vital role in several redox balancing systems for combating oxidative stress and maintaining a reducing intracellular environment [46]. Redox-active molecules such as Nicotinamide adenine dinucleotide phosphate, Nicotinamide adenine dinucleotide, glutathione, and others act as electron shuttles alongside metalloproteins (e.g. iron–sulfur [Fe–S] cluster containing proteins), facilitating intracellular redox-

Figure 3



Harnessing redox to enable bioelectronic control. *Center*: Redox, or reduction–oxidation reactions, allows electron transfer between two compounds, and the oxidation states of both compounds change accordingly. *Left*: Redox possesses a dual and digital nature that makes it a unique bridge between biology and electronics. Redox-active molecules can exchange electrons with both the electrode (electronics) and biological compounds, allowing direct 'programming' of the biochemical properties of a biological compound. The electronic-induced change in redox state and/or conformation of a biological can in turn elicit specific biological responses. *Right*: Redox allows targeted and precise control of biological behaviors. (Top) Respiratory components can be targeted by different redox mediators with varying redox potentials to probe or alter their redox state. Electronic messages conveyed by the electrode can thus be distributed to intracellular targets to elicit changes at the protein- or genetic level. Q/QH_2: quinone/quinol, Fc: ferrocene, Ir: iridium. UQ/UQH_2: ubiquinone/ubiquinol, Cyt C: cytochrome c. (Bottom) Cell fluorescence response (from SoxR-induced green fluorescent protein (GFP) expression) can be fine-tuned electronically. Here, GFP expression level in engineered *E. coli* is shown to be controlled by the electrode-oxidized redox mediators Fcn^{ox} and PYO^{ox}. PYO^{ox} oxidizes the transcriptional regulator SoxR to initiate transcription of its downstream gene-of-interest *gfp*. The data presented on the right are adapted from [2].

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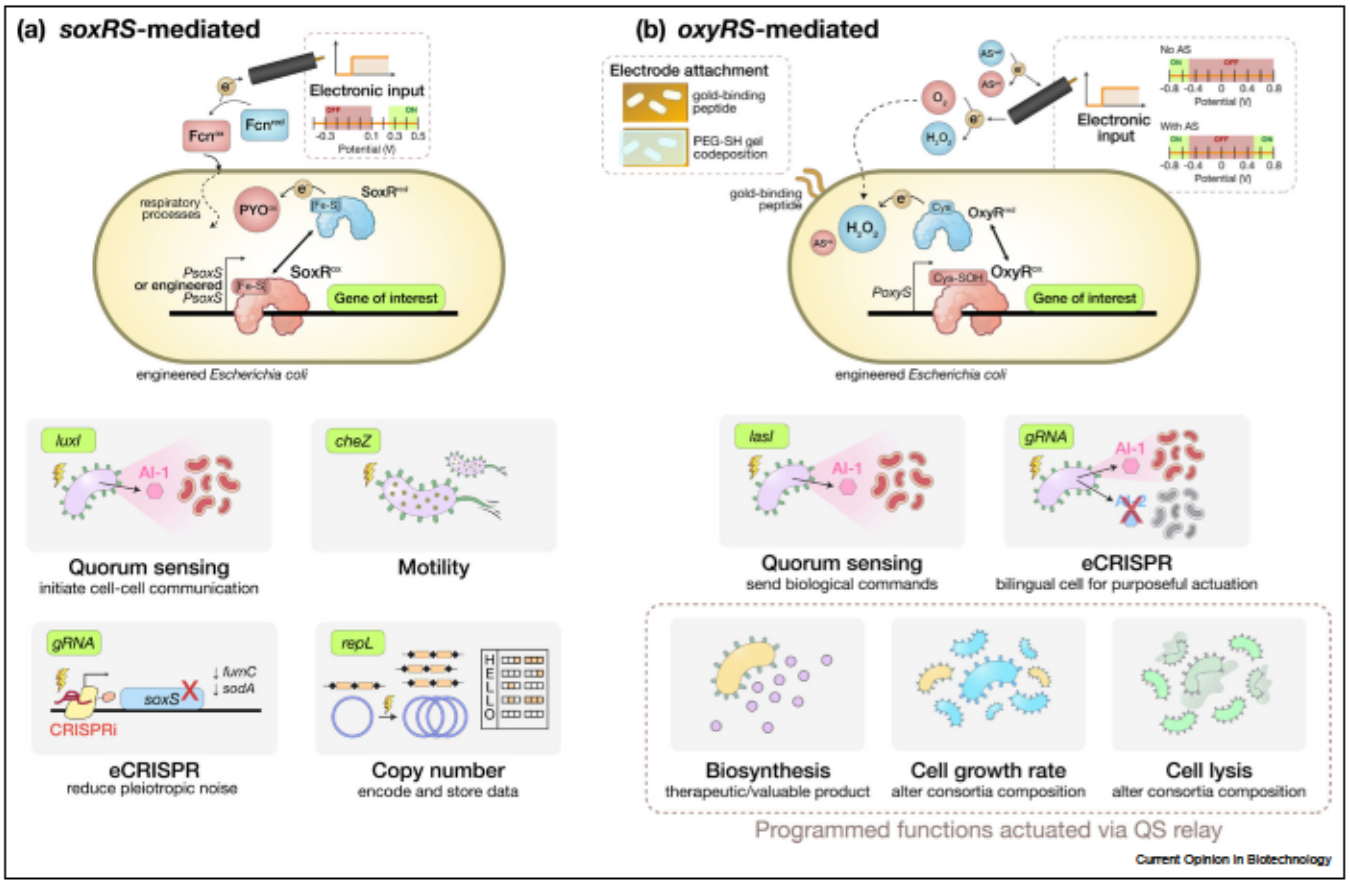
Figure 4



Targeted control of cell behavior through electrogenetics. **(a)** Mechanism of the *soxRS*-mediated electrogenetic system and its applications. Both ferrocyanide/Fcn and PYO are used as redox mediators to facilitate electron transfer between the electrode and transcriptional regulator SoxR. Electronic signals are shown to change the redox state of SoxR (through oxidation of its [Fe–S] clusters) to initiate transcription of the gene of interest (shown in green rounded rectangles) in engineered *E. coli*. **(b)** Mechanism of the *oxyRS*-mediated electrogenetic system and its applications. Electrode-generated H_2O_2, via the reduction of oxygen (O_2), oxidizes the transcriptional regulator OxyR (through oxidation of its cysteine residue) to initiate transcription of the gene of interest (shown in green rounded rectangles). Oxidized plant phenolic AS is reported to also activate OxyR-induced expression. Surface display of gold-binding peptide or electro-codeposition with thiolated PEG (PEG-SH) hydrogel can both enable engineered *E. coli* to attach onto electrodes and ensure efficient electronic signal transfer. Programmed functions can be electronically actuated either directly through the rewired *oxyRS* or indirectly through *oxyRS*-initiated QS communication.

effort foreshadowed the burgeoning field that exists today that directly employs electronic signals.

Recently, through the rewiring of native redox mechanisms (i.e. *soxRS* regulon, Table 1), an orthogonal methodology has been developed in which the engineered construct responds through redox-mediated electron transfer from the electrode-oxidized signal molecule directly to biological signaling molecules, linking genetic circuitries to electronics [52]. As an example, pyocyanin (PYO) was employed to shuttle electrons from an electrode into engineered *E. coli* (Figure 4a). Naturally secreted by *Pseudomonas aeruginosa*, PYO is a toxin that can disrupt the oxidative states of competing microbes or mammalian cells [58]. In its oxidized form, PYO readily activates *E. coli*'s native oxidative stress sensor SoxR, and in turn, initiates transcription from the *soxS* promoter [59]. The purported mechanism underpinning this electrogenetic switch was the oxidation of SoxR's [Fe–S] cluster either directly by PYO or by superoxide (O_2^-) that was, in turn, generated by PYO. Interestingly, expression levels of *soxRS*-mediated electrogenetics were found to be regulated not only by the magnitude of total charge input (e.g. 0 ~ −1.2 Coulombs) but by the direction of electron flow (i.e. applying a reducing (−0.3 ~ +0.1 V) or oxidizing (+0.25 ~ +0.5 V) potential) since both factors influence the ratio of oxidized to reduced form of the signal molecules. Importantly, the addition of terminal electron acceptor, potassium ferricyanide (Fcn), enabled dramatic amplification of the *soxS*-mediated transcription, presumably owing to its continued reoxidation at the electrode and re-reduction of intracellular SoxR.

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