Electronic control of gene expression and cell behaviour in *Escherichia coli* through redox signalling

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The ability to interconvert information between electronic and ionic modalities has transformed our ability to record and actuate biological function. Synthetic biology offers the potential to expand communication ‘bandwidth’ by using biomolecules and providing electrochemical access to redox-based cell signals and behaviours. While engineered cells have transmitted molecular information to electronic devices, the potential for bidirectional communication stands largely untapped. Here we present a simple electrogenetic device that uses redox biomolecules to carry electronic information to engineered bacterial cells in order to control transcription from a simple synthetic gene circuit. Electronic actuation of the native transcriptional regulator SoxR and transcription from the PsoxS promoter allows cell response that is quick, reversible and dependent on the amplitude and frequency of the imposed electronic signals. Further, induction of bacterial motility and population based cell-to-cell communication demonstrates the versatility of our approach and potential to drive intricate biological behaviours.
The exchange of information between electrons and ions has been a mainstay in a variety of biochemical applications for decades. Small molecules, however, represent a much wider ‘repertoire’ for biological information transfer, or ‘molecular communication’. Gaining the ability to measure, disrupt or enhance these biomolecular signals would allow for development of advanced technologies to study and manipulate the biological environment. Specifically, molecular connectivity with electronics can benefit from the fact that electrochemical detection is sensitive, selective, cost-efficient and label-free in small volumes. Such connectivity presents a unique opportunity to apply our knowledge of and control over electronic-device form and function to study biological systems, improve biosensors and create wearable and implantable bio-hybrid devices.

Redox biomolecules have significant roles in a wide array of cellular functions, and present a means for electronically interceding with both native cell pathways and redox-sensitive engineered constructs. Bioelectrochemical technologies, such as microbial fuel cells (MFCs) and bioelectrosynthesis systems (BESs), use electrochemical techniques to interact with cellular electronic processes and transport electron mechanisms to change or measure cellular behaviours. A plethora of literature exists on MFCs, where microbial communities metabolize organic compounds, resulting in production of electricity. Conversely, BESs aim to electrochemically intercede with microbial metabolism for the production of various compounds of interest. Electronic interrogation of biological systems with redox molecules has allowed for detection of changes in cell metabolic activity, redox state, toxicity and other parameters. Cells have been engineered for enhanced electron flow and to allow for electronic detection of engineered cell activity. Electronic signals translated through redox molecules also show controlled glucose consumption and regulation of enzymatic activity. The use of the above-mentioned and other bioelectrochemical methods will no doubt continue to have impactful applications in fields such as bioenergy, biotechnology, biosensing and biocomputing.

However, while the accomplishments above are impressive, they are limited in their cellular effects to those that are naturally responsive to changes in electron transfer or redox status. Linking electronic signals, through redox molecules, to engineered gene expression, opens the doors for electronically studying and controlling a much wider array of behaviours and thus the possibility of many additional applications. Such an electrogenic device was previously explored in mammalian cells. We advance this idea by working with Escherichia coli, a widely used synthetic biology chassis, and show circuit versatility and quick response times.

We use pyocyanin (Pyo) for gene induction and ferricyanide (Fc) for response-amplification and electronic control to guide production of proteins that act as reporters or that otherwise direct cell function. Pyocyanin is secreted by Pseudomonas aeruginosa and is implicated in community organization, pathogenicity and interspecies behaviour. To use pyocyanin as an inducer, we employed one of the best-characterized redox-responsive regulons in E. coli, the SoxRS regulon, which functions to sense and respond to oxidative stress. In E. coli, the SoxR protein contains an iron-sulfur cluster (2Fe-2S) that is maintained in a reduced form by NADPH-dependent enzymes. When oxidized (for example, by redox-cycling drugs), SoxR activates transcription of the SoxS protein from the PsoxS promoter. The SoxS protein, in turn, regulates dozens of other genes, mainly with the aim of detoxifying the cell.

Studies of the mechanisms of redox-drug activation of SoxR show that conditions that promote cellular respiration increase expression from the PsoxS promoter. They suggest that this is due to increased electron flow through the respiratory machinery, which could allow increased re-oxidation of the redox drugs and SoxR activation. We worked from this hypothesis, and propose that using a redox molecule that acts as an electron acceptor and whose form we could electronically regulate would allow us to amplify the intracellular Pyo redox cycling that leads to SoxR-mediated transcription. We chose ferricyanide as our alternative electron acceptor. Ferricyanide (oxidized, Fcn(O)) and ferrocyanide (reduced, Fcn(R)) (with a standard potential, E\text{0}, of +0.2 V versus Ag/AgCl—silver/silver chloride) have been used for decades in studies of electron transport processes, where Fcn(O) reduction rates correlate with microbial respiratory activities.

Our method demonstrates electronic control of a native redox process to actuate gene expression. This bacterial electrogenic device is simple, specific and versatile. We take advantage of the well-characterized native redox-response of the SoxRS regulon and proposed electron transport mechanisms so that minimal genetic ‘rewiring’ is required. Induction levels are controlled by varying either the applied electronic potential or its duration, and correlate to the measured charge through Fcn(O/R) redox form interconversion. We show that gene expression is functionally reversible on relatively short time scales (30–45 min) and that this allows for response ‘ON’/‘OFF’ cycling. Additionally, we expand on this genetic circuit by demonstrating electronic induction of cell motility and by connecting electronically actuated cells to non-actuated cells via generation of the native signalling molecules associated with bacterial quorum sensing. Thus, electrons are converted to biological signalling molecules that, in turn, influence phenotype in otherwise unaffected cell populations. Importantly, the ‘controlled’ behaviours that our electrogenic device controls are typically not responsive to such redox changes.

Results

Redox mediator effects on cells and gene expression. Figure 1a provides a schematic representation of our approach. To test the effect of pyocyanin, Fcn(O), and Fcn(R), on gene expression and their interactions with cells we first carried out studies using chemical systems (for example, without electrodes; chemical structures are presented in Supplementary Fig. 1). These results, which for brevity are presented in the Supplementary Information, set the stage for electrode-based studies. We constructed plasmid pTT01, from the pBR322 vector, that includes the soxR gene and the overlapping divergent PsoxR and PsoxS promoters. The gene coding for the fluorescent reporter protein phiLOV, which can fluoresce in anaerobic conditions, was placed downstream of PsoxS (Fig. 1b). We incorporated an ssRA degradation tag—AANDENYADAS (DAS) on the C terminus of phiLOV in plasmid pTT01 (forming plasmid pTT03), which significantly increases protein degradation and thus results in an overall lower steady-state protein level, but also a more rapid return to baseline levels upon cessation of induction (denoted ‘OFF’). All constructs, unless otherwise stated, were tested in the strain DJ901 (soxRS). This strain allowed for higher reporter levels, but cells with intact soxRS were still responsive (see ‘Electronic actuation of bacterial motility’ section and Supplementary Fig. 2). See Methods, Supplementary Fig. 3, and Supplementary Tables 1–3 for all plasmid and cell engineering information and sequences.

The addition of pyocyanin alone (0–10 μM) resulted in modest phiLOV expression (fluorescence increase from 200 to 500 au).
The addition of 5 mM of Fcn(O) amplified this pyocyanin-induced fluorescence ~17-fold. Control cultures showed no increase in fluorescence (for example, Fcn (R) + PyO or Fcn(O) only). See Supplementary Note 1 and Supplementary Fig. 4.

Additionally, phiLOV fluorescence increased with ferricyanide (0–25 mM) while pyocyanin was kept at 5 μM, in an apparent dose-dependent response (Supplementary Fig. 4). The results indicated the importance of the redox status of Fcn(O/R) since Fcn(O) but not Fcn(R) amplified pyocyanin-induced gene expression. Since this is the first study of this electrogenetic device, we performed the above and all following experiments anaerobically to exclude oxygen’s interference with pyocyanin redox state and for better control of redox conditions. However, the system could be adapted for conditions that span a variety of oxygen gradients through further optimization. We discuss this and show preliminary data in Supplementary Note 2 and Supplementary Fig. 5.

Based on the above, we worked with 5 μM pyocyanin and 5 mM Fcn (O/R) for the remaining studies. At these levels, neither mediator significantly altered cell viability, though the combination did alter acetate production per glucose consumed (Supplementary Fig. 6 and Supplementary Note 3). We found that Fcn(O) reduction by cells depended on both the amount of cells and starting Fcn(O) concentration (Supplementary Fig. 7), consistent with above-mentioned literature regarding Fcn(O) use for respiratory activity measurement. As mentioned previously, others have proposed that redox-cycling drugs which oxidize SoxR and drive expression from the PsoxS promoter interact with the electron transport machinery. We propose that in our system, after oxidation of SoxR, the now-reduced drugs are re-oxidized intracellularly when an electron acceptor is present. We provide corroborating evidence in Supplementary Fig. 8 and Supplementary Note 4, though we cannot rule out alternative mechanisms.

In sum, chemical studies demonstrated that Pyo induces phiLOV expression from the PsoxS promoter and Fcn (O) but not Fcn (R) amplifies this expression in a dose-dependent manner.

Electronic control of gene expression and dose-response. The above results suggested the possibility for genetic induction in situ by applying electronic signals that provide negative charge (oxidation) to ensure both that PYO is oxidized and to increase Fcn(O) from Fcn(R), and positive charges (reduction) for subsequently halting gene induction through Fcn(O) reduction to Fcn(R) (Fig. 2a). We interconverted bulk Fcn (O/R) redox state electrochemically in a three-electrode set-up (Supplementary Fig. 9, Methods). In our system the E° of the Fcn (O/R) couple was about +0.2V (grey cyclic voltammogram in Fig. 2b, Supplementary Fig. 10a). For complete and quick bulk oxidation and reduction (<20 min, Supplementary Fig. 10b and c), we biased electrodes significantly more positively than the oxidation peak (+0.5 V for ~+0.25 V peak) or more negatively than the reduction peak (~0.3 V for ~+0.1 V peak). We could use potentials closer to the peak potentials, but conversion...
efficiency would suffer; conversely, higher voltages can generate unwanted reactive species. The measured charge (integrated current over time) correlated well with Fcn(O) absorbance (Fcn (O) is yellow) (Supplementary Fig. 10d) and repeated oxidation and reduction of the same solution did not degrade Fcn (O/R) (Supplementary Fig. 10e).

Correspondingly, in Fig. 2b, we show that varied electrode potential modulates Fcn(R) oxidation (charge) and cell (phiLOV) fluorescence. In these experiments, we applied different voltages to Pyo + Fcn(R) solutions with cells for 15 min, followed by incubation to allow for phiLOV accumulation, and flow cytometry measurements. Figure 2b shows the resulting total charge and average cell fluorescence levels at specific potentials. Three response ranges were observed based on the potential used. When we applied potentials more negative than the reduction peak, which did not promote significant Fcn(R) -> Fcn(O) conversion, charge and fluorescence outputs were negligible. Applied potentials between the reduction and oxidation peaks (∼0.1 and +0.25 V) resulted in proportionally more negative charge (partial Fcn(R) to Fcn(O) conversion) and increasing fluorescence. Potentials more positive than that of the oxidation peak resulted in a leveling off of charge and increasing fluorescence. Potentials more positive than that of the oxidation peak resulted in a leveling off of charge and increasing fluorescence.

We tested whether Fcn(R) oxidized in situ by a constant oxidizing potential could amplify gene expression and whether increasingly negative charge, mediated by increasing duration (10–900 s), could elicit a dose-dependent response. A heat map depicts the cell fluorescence due to varied durations of applied +0.5 V (Fig. 2d). Low charge (closer to zero) resulted in low cell fluorescence. For charges between zero and ∼−0.27 C, initial increases in fluorescence were followed by decreases. In these situations, the Fcn(R) amount converted to Fcn(O) was not sufficient to enable continued expression over the timeframe tested and ssRA-mediated phiLOV degradation brought the reporter quantity down. More negative charges than ∼−0.27 C resulted in higher Fcn(O) levels and continued increase in fluorescence for the length of the experiment (despite the ssRA-mediated degradation). A heat map with the corresponding cell fluorescence of induction with varied potentials is shown in Supplementary Fig. 11, and results are comparable.

In Fig. 2e, we show that the average fluorescence (via moving-window time average) increased with applied charge whether +0.5 V was applied for varied lengths of time or potentials were varied but applied for 15 min. We found no significant differences between the two methods—highlighting that it was the applied electronic charge, not the voltage or its duration, which correlated with fluorescence. The response appeared linear until ∼−0.5 C. These experiments demonstrate a direct relationship between applied potential (electronic signal), resulting charge (Fc(R) to Fcn(O) interconversion at the electrode), and average cell fluorescence, confirming electronic control of gene expression and defining the redox-based communication pathway.
Dynamic control of gene expression. We wanted to take advantage of the dynamic electrochemical control of the redox state of Fcn(O/R) to drive overall reporter response ‘ON’ or ‘OFF’, characterized by increased protein production (‘ON’) or decreased protein production and quantity via the ssRA tag (‘OFF’). We thus first tested the effects chemically by centrifuging and re-suspending cells in fresh media with different mediators to evaluate the genetic response from an ‘ON’ (Pyo + Fcn(O)) to ‘OFF’ (Pyo + Fcn(R)) transition (Supplementary Fig. 12a). In this situation phiLOV induction is reduced, the remaining protein (with ssRA tag, see Supplementary Fig. 12b) degrades, and the total fluorescence decreases. Repeated cycling of ‘ON’ to ‘OFF’ induction conditions at 1h intervals showed corresponding fluorescence increases and decreases, with cells fluorescing similarly after each ‘ON’ cycle (Supplementary Fig. 12c). Cells showed significant fluorescence degradation upon switching of Fcn(O) to Fcn(R) in <45 min of exposure (Supplementary Fig. 12d).

Thus, Fcn (O) amount defines whether protein production increases (high Pyo- and SoxR-mediated induction from PssoxS promoter, and total protein increase despite ssRA-mediated protein degradation) or decreases (low induction from PssoxS promoter, and total protein decrease due to degradation). We predicted that by electronically controlling the Fcn (O/R) redox form we could similarly specify increases or decreases in protein levels.

We thus introduce the ‘OFF’ component of the electronic control scheme, where we stop amplifying gene expression and rely on the biological system (ssRA tag) to drop the output signal in a similar manner as in the chemical experiments. Cells are turned ‘ON’ with electronic oxidation of Fcn(R) to Fcn(O) (+0.5 V) and off with electronic reduction of Fcn(O) to Fcn(R) (−0.3 V) (Fig. 3a). In Fig. 3b, we show dynamic control of cell fluorescence with repeated ‘ON’/‘OFF’ electronic signals in a continuous culture. We show that the cells remain responsive and the cycling process is reproducible.

In Fig. 3c, we evaluated the ‘ON’/‘OFF’ profile by varying the cycle time. We found that the fluorescence measured at the half cycle time (after an ‘ON’ signal) increased monotonically with cycle time. After the half cycle measurement, an ‘OFF’ signal was passed to the cells. The fluorescence then decreased significantly by the termination of the cycle for half-cycle times above ~45 min. One aspect to keep in mind is that the full electrochemical interconversion between Fcn (O) and Fcn(R) takes about 15 min using our current set-up. Therefore, for the shorter cycle time (for example, 15 min), cells remain exposed to Fcn(O) for the majority of the time. This results in continual gene expression. For a 30 min cycle time, the cells are not in the presence of Fcn(O) for at least half the duration, and we see more pronounced effects of degradation. Longer ‘ON’ states result in greater fluorescence and longer ‘OFF’ states result in greater degradation. Fully developed ‘ON’/‘OFF’ switching occurs for cycle times near 30–45 min.

We constructed a simple mathematical model (see Methods and Supplementary Software) to delineate phiLOV synthesis from its degradation (Supplementary Note 5, Supplementary Fig. 13). In Fig. 3d, we show the calculated accumulation of phiLOV in the absence of degradation over time, and found a positive linear relationship between charge and synthesis of phiLOV protein, consistent with data in Fig. 2. This is a
Electronic actuation of bacterial motility. To show that the redox-driven control scheme can actuate behaviour more complex than fluorescence reporter production, we first electrically induced bacterial swimming. We placed the E. coli motility effector gene, cheZ, under the PsosxS promoter, creating pHW01 (details in Methods). CheZ stimulates dephosphorylation of CheY, which drives flagellar motor function and swimming versus tumbling behaviour (Fig. 4a)\(^5\). CheZ null mutants were transformed with pHW01 and first chemically induced with pyocyanin (+/− Fcn(O/R)) (Supplementary Note 6). CheZ expression (via western blot) showed similar trends to previously shown fluorescence induction results (Supplementary Fig. 14a). Further, cells stimulated with +0.5 V with Pyo and Fcn(R) showed that higher charges correlated with increased CheZ production, almost to the level of wild-type cells (Fig. 4b, Supplementary Fig. 14c shows expanded western blot).

To characterize swimming, we developed a video-analysis algorithm that calculates per-cell swimming velocities (see Methods and Data and Code Availability). CheZ amplification from its background level in the null mutant should correspond to higher velocities as its presence induces more straight swimming and less tumbling. Cell trajectories, showing individual cell paths starting at the origin and spanning 3 s, are smoother and longer with Pyo + Fcn (R) at higher charges (Fig. 4c). Figure 4d shows that velocity of tracked cells significantly increased with charge. Importantly, we observed no interference on cell motility or CheZ production from non-inducing controls (Supplementary Fig. 15). These results indicate that our redox-mediated approach can electronically stimulate a complex cell behaviour—bacterial swimming—through gene induction, and do so without apparent interference with motility mechanisms.

Electronic actuation of bacterial communication. We aimed to create a bio-electronic cellular information relay: electronically induced cells produce a signalling molecule that is interpreted by a second set of cells that, in turn, responds with altered behaviour specifically encoded by the molecular signal. In this way, we can separate the redox-based electronic-actuation components (relay cell) from the resultant behavioural changes (receiver cell). This could be useful in cases where interactions between Pyo, Fcn(O/R) and the engineered electrogenic circuit are of background importance. As seen in Fig. 5a, in our relay cell, SoxR induces Vibrio fischerii LuxI (instead of phiLOV) expression from the plasmid pTT05. LuxI produces an acylated homoserine lactone (AHL), a bacterial signalling molecule that can diffuse through the membrane to guide quorum sensing (QS) behaviour. The V. fischerii LuxI QS system has been widely used to engineer communication networks between non-communicating bacteria\(^6\). The AHL receiver cell interprets the AHL cue by binding the LuxR protein and expressing phiLOV from the PluxI promoter in the plasmid pTT06. As before, adding various Fcn (O) concentrations with Pyo in solution resulted in amplified gene expression in co-cultures of the relay and receiver cells (Supplementary Fig. 16a). Supplementary Fig. 16b shows electronic induction of cell fluorescence of co-cultures over time, the average of which correlates with the charge (Fig. 5b).

In electronically induced co-cultures, the AHL receiver cells exhibited an increase in fluorescence and emerged as a distinct fluorescent population, as can be seen from the flow cytometry histograms in Fig. 5c. Supplementary Fig. 16c shows results of electronic induction between non-co-cultured cells, also with a charge-dependent response (Supplementary Note 7). In addition, quantitative PCR analysis corroborates gene
expression results for all electronically induced proteins presented (Supplementary Fig. 17), demonstrating messenger RNA decay following the ‘OFF’ transition in dynamic studies.

These results demonstrate successful biomolecular information transfer through redox-mediated electronic signals to native quorum sensing signalling molecules.

**Discussion**

Our work shows, for the first time, the utility of using biologically relevant redox molecules in translating electronic signals to changes in engineered bacterial gene expression. Our system is based on coupling Pyo-driven SoxR activation\(^{31,35}\) with electronic control of Fcn(O/R) redox form\(^{18,41,42}\). This integration allows us to open a new communication pathway and develop a novel framework to connect electronic signals to gene expression. We present in this paper robust evidence and thorough characterization of a functioning bacterial electrogenetic device. To our knowledge, our work is first in demonstrating and characterizing an electrode-based system for reversible and specific redox-driven genetic control in bacteria.

Our applications of this system to genetically induce bacterial motility and cell-to-cell communication highlight its versatility in that it builds upon advances in using electronic control of behaviours that are naturally redox-dependent. Additionally, although we highlight the dynamic gene-actuation capabilities of our system, our aims differ from those of other synthetic biology efforts that enlist non-native components to recognize alternative input signals for precise genetic control using light\(^{47–50}\) or magnetic and radio waves\(^{51–53}\). Instead, we minimally rewired the cells to take advantage of native redox interactions, and provide insights into their developing role as mediators for bio-electronic communication.

We foresee that our system can be tailored to produce a variety of responses, guide various behaviours, and further the use of other electronic\(^{28,31}\) and redox-based systems to access and affect biomolecular information transfer, perhaps as part of MFC or BES systems for gene expression based on potential, current or electron acceptor availability. We show preliminary results that expand on the redox mediators, cell genetic background and oxygen levels that are used. Our approach may prove useful for spatio-temporal control of cells immobilized at or near electrode surfaces, for metabolic engineering applications, gut-on-a-chip systems, and other bio-hybrid devices where precise cellular spatio-temporal control is desirable. Additionally, our system offers an additional mode (in addition to light, magnetic, and radio) of relaying electronic signals to cells. Such cells can be programmed to respond to an unprecedentedly wide array of biological and non-biological information and make ‘smarter’ decisions than previously possible. Our electrogenetic device additionally offers electronic interrogation of SoxRS-specific targets and electron-flow-dependent processes. In sum, our work to translate electronic signals to bacterial gene expression represents a new way of using redox molecules and electron flow for guiding biological function.

**Methods**

**Cell strains and plasmids.** The majority of the experiments used *E. coli* D1901 (lacU169 rpsL ΔsoxRS901) (ref. 36). For experiments with CheZ induction W3110 E.coli with CheZ genomic deletion were constructed (CheZ KO). Plasmid vectors include pBR322 (for phiLOV and LuxI expression) and pFZY1 (for CheZ expression). Briefly, the complete DNA region encompassing the

**Figure 5 | Electronic control of cell-to-cell communication.** (a) Schematic of electronic control of cell-to-cell communication. Electronic signals modulating Pyo and Fcn (R) to Fcn (O) result in LuxI-laa and AHL production from relay cells. The receiver cells produce LuxR. When LuxR detects AHL, phiLOV is induced from the luxI promoter. (b) Average fluorescence of biosensor cells within co-cultures in which relay cells are electronically induced with the indicated charges. (c) Flow cytometry histograms showing the emergence of a fluorescent receiver-cell population at the indicated time points after induction.
soxR (Gene ID: 948566), and the PsoxR and PsoxS promoters (entire region between soxR and soxS) was PCR-amplified from the genome of E.coli MG1655. The gene coding for the proteins phiLOV (fluorescence), LuxI (autoinducer production), or CheZ (motility), with and without soxR degradation tags, were placed downstream of the PsoxS promoter. Standard restriction cloning techniques and Gibson assembly were used. NEB5α (New England Biolabs, Ipswich, MA) and Top10 Chemically Competent (ThermoFisher Scientific) cells were used for construct assembly. Details of plasmid construction and all sequences are in the Methods and Supplementary Tables 1-3.

Cell culture. Cells were grown overnight in lysogeny broth (LB) at 30 °C aerobically with 250 r.p.m. shaking, were inoculated from the overnight cultures at 1.5% in LB, and grown in 37 °C with 250 r.p.m. shaking, until OD_{600} 0.2–0.5. The cells were re-suspended in M9 media (1 x M9 salts, 0.4% glucose, 0.2% casamino acids, 20 mM MgCl2, and 100 mM MOPS) and then grown at 37 °C in a mini-incubator inside the Coy chamber for anaerobic experiments or in a shaking incubator (250 r.p.m.) aerobically.

Establishment of anaerobic conditions. A Coy Laboratory Products (Grass Lake, MI) anaerobic chamber maintained anaerobic conditions—set up as per the manufacturer’s instructions, with nitrogen and CO_{2}/H_{2}/N_{2} mix.

Spectrophotometric readings. A SpectraMax M2 plate reader ( Molecular Devices, Sunnyvale, CA) was used to read absorbance of ferriycentane (420 nm) and cell amounts (600 nm).

Cell fixing. Typically, 100 μl of cells were taken per sample for fluorescence measurements. Cells were washed in PBS, re-suspended in 2% paraformaldehyde in PBS, and incubated for at least 30 min at room temperature before flow cytometry measurements.

Flow cytometry. Flow cytometry was performed using a BD Biosciences (Franklin Lakes, NJ) FACS Canto with the BD FACS Diva software. 50,000 cells were collected for each sample and consistently gated by forward scatter and side scatter. The mean green fluorescence levels of phiLOV (488 nm laser and 530/30 green filter) are based on the means of 40,000–50,000 cells from the number of indicated samples. Analysis was done in FACS Diva, FlowJo and Excel.

Electrochemical set-up. For bulk electrolysis, 50 cm-long gold electrodes (Franklin Lakes, NJ) were placed downstream of the PsoxS promoter. An electrochemical set-up as described above was used—except the working electrode was placed on a microscope slide, and a video was recorded using a Bioptechs software and DXM microscope equipped with a DP72 camera (Olympus, Waltham, MA). Approximately 100 frames are recorded for each video, using a 20 x objective lens with a green fluorescent protein (GFP) filter. Motility video analysis was done using Matlab based on methods in literature.

Using Otso’s method, each frame of the motility video was segmented into a binary image. The built-in function regionprops provided the location and shape of each cell. The tracking algorithm uses a nearest-neighbour approach that links cells in subsequent frames based on closeness, size similarity and pixel intensity. The velocity was determined from centroid data. The program accounts for cells that are stuck for part or the entire duration of the video and cells that are under the influence of background flow. To create the trajectory diagrams in Fig. 4c, the first 3 x of each cell trajectory in the video are shown, translated and plotted at the origin (0,0).

Motility video analysis. Cells in chemotaxis buffer were removed from the anaerobic chamber, placed on a microscope slide, and a video was recorded using a CellSens software and Olympus microscope equipped with a DP72 camera. A Coy laboratory Products (Grass Lake, MI) anaerobic chamber, placed on a microscope slide, and a video was recorded using a Bioptechs software and DXM microscope equipped with a DP72 camera (Olympus, Waltham, MA). Approximately 100 frames are recorded for each video, using a 20 x objective lens with a green fluorescent protein (GFP) filter. Motility video analysis was done using Matlab based on methods in literature.

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Induction of motility. Overnight cultures were grown as above. Following re-inoculation in LB, cells were grown to an OD_{600} of 0.45 at 37 °C shaking at 250 r.p.m. aerobically. Cells were spun at 400 g for 5 min and re-suspended in an equal volume of M9 media. Cells were placed in the anaerobic chamber, where mediators were added as indicated. Non-electrically stimulated samples were induced in the anaerobic chamber at 37 °C for 90 min. Electrically stimulated cells were induced with various charges (constant potential, varying time, as above), after which cells were placed into Eppendorf tubes and cultured for a total of 30 min before analysis. Western Blot analysis is described in Methods and was done using standard techniques. For video analysis, cells were spun down at 400 g for 5 min and re-suspended in chemotaxis buffer (CB: 1 x PBS, 0.1 mM EDTA, 0.01 mM L-methionine, 10 mM L-1-lactate) while still in the anaerobic chamber.

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Construction of pTT01-pTT04 plasmids. The DNA region containing the soxR gene and the region between soxR and soxS was amplified from the E.coli MG1655 genome and ligated into the PCR-Blunt II-TOPO plasmid. The fragment was then digested out with the BamHI and HindIII enzymes and ligated into a similarly digested pBR322 vector. The gene coding for the phiLOV protein was produced as a gBock by IDT, with E.coli codons optimized using GenScript framing of acidic sequence from Christie et al. The pTT01 (phiLOV) and pTT02 (phiLOV-LAA) plasmids were assembled using the Gibson Assembly method (NEB Gibson Assembly Master Mix) by PCR amplifying both the phiLOV sequence (with or without the AADDENYADAS (LAA) degradation tag) and the pBR322-oxsR-PsoxS constructs with overlaps. The AADDENYADAS (DAS) tag was added to phiLOV by PCR amplifying pTT02, treating the PCR with T4 polynucleotide kinase and ligating with T4 ligase to create plasmid pTT03. The plasmid pTT04 was created by PCR-amplifying pTT03 without the soxR coding sequence, treating the PCR with T4 polynucleotide kinase and ligating with T4 ligase. The relevant primers can be found in Supplementary Table 2. The relevant genetic element sequences, including the tags, can be found in Supplementary Table 3.

Construction of pTT05 and pTT06. The plasmid pTT05 was created from pTT01 by PCR amplification without phiLOV. The lux gene with the LAA tag was amplified from the plasmid pLuxR12 (ref. 57). Gibson Assembly Master Mix

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From NEB was used to assemble the final construct. Plasmid pTT06 was created by amplifying pTT01 plasmid without the soxR through PsoXI restriction and ligated by ligating the chz and soxPoxX fragments via PCR. The Gibson assembly method was used as above. The relevant primers can be found in Supplementary Table 2. The relevant genetic element sequences, including the tags, can be found in Supplementary Table 3.

Construction of motility plasmid pHW01. To create the plasmid pHW01 with the chz gene under control of the PsoXI promoter, E. coli W3110 cells were used as the host. Amplification of the chz and soxPoxX fragments via PCR, the primer set BamHI-SoxX-F and SoxX-ChzR was used for the amplification of the soxR-PoxX fragment, while the primer set SoxX-ChzF and ChzR-HindIII-R was utilized for the chz fragment. In both cases, the primer SoxX-chz-F and, therefore, both resulting PCR products shared an overlapping fragment. After selection of PCR products, both were mixed together at equimolar ratio and an extra PCR was performed with the primers BamHI-SoxX-F and ChzR-HindIII-R for ligating soxR-PoxX-chzR. The resulting product was inserted into pFZY1 (ref. 58) vector through BamHI and HindIII restriction enzyme cloning.

Construction of constitutive fluorescent plasmid pTSG. The plasmid pTSG was derived from a plasmid previously used for constitutive expression of DsRedExpress2 (refs 59,60). First, a redundant HindIII restriction endonuclease site (AAGCTT) was deleted from plasmid pTSR7G through plasmid PCR using primers HindIIIdel-F and HindIIIdel-R. The product was phosphorylated with T4 PNK and re-ligated with T4 ligase. Next, the reporter gene egFP was amplified using the pEGFPR2.2 primers. The gfp2luxExpR was excised from the pTSR7G derivative via EcoRI and HindIII digestion and egFP was inserted. Transformation and recovery of the ligation product yielded Top10+pTSG cells that constitutively expressed EGFP and thereby fluoresced green. The plasmid was transformed into cheZ KO cells (below) with the motility plasmid pHW01 to allow for fluorescent video recording.

Construction of plasmid pTG1. The E. coli K-12 genomic region that constitutes the soxR protein and the divergent overlapping PoxX and PsoXI promoters was inserted into a pCRII-BluntII-TOPO plasmid (Thermo Fisher Scientific). This construct and the plasmid pFZY1 were digested with BamHI and HindIII and ligated such that the lacZ gene in pFZY1 was downstream of the PsoXI promoter. pTG1 allowed for SoxR-mediated expression of β-galactosidase.

Genomic cheZ deletion. Chromosomal deletion of cheZ in E. coli W3110 was carried out using the one-step inactivation method described by Datsenko and Wanner. In this method, a phase λ. Red recombination system was introduced to facilitate the homologous replacement of W3110 cheZ gene with kanamycin resistance gene cassette followed by the excision of the resistance cassette for creating cheZ knockout of W3110 (W3110 chez −). Specifically, the Red helper plasmid pKD46 (GenBank Accession: AY048746.1) was first transformed into W3110 electro-competent cells by electroporation. The transformed cells were grown and selected on LB agar plates which contained 50 μg ml⁻¹ ampicillin at 30 °C. A positive colony was picked and inoculated into a 50 ml LB medium which contained 50 μg ml⁻¹ ampicillin and 1 mM l-araabinose. The cells were cultivated at 37 °C overnight. The transformation step was repeated and the cells were freshly prepared and kept on ice until the next transformation of a kan resistance cassette. To synthesize the kanamycin resistance cassette, we conducted a PCR using the primer set (cheZ-KO-P1F and cheZ-KO-P2R) and the plasmid pKD4 (GenBank Accession: AY048743.1) as the template.

The resulting PCR product of the kanamycin cassette flanked by FLP recognition target sites was produced and gel-purified for subsequent transformation into pKDe46 carrying W3110 electro-competent cells above-mentioned. In all, 300–500 ng of the kanamycin cassette product was introduced into 50 μl of competent cells by electroporation followed by the incubation with 50 μl SOC broth and 1 mM l-araabinose at 37 °C for 250 p.s. shaking for 2 h. Cells were grown overnight on an LB-agar plate containing 30 μg ml⁻¹ Kanamycin for screening the recombinants. We further isolated colonies from the kanamycin plate and conducted PCR verification for cheZ deletion (cheZ_seq-P1F and chez,seq-P2R) and kanamycin cassette insertion (primer set 1: chez- upstream and Kc primer set 2: k2 and cheZ- downstream). Isolated cells were also inoculated in LB medium supplemented with 50 μg ml⁻¹ ampicillin for checking the curing of pKDe46 plasmid. Subsequently, the removal of the kanamycin resistance cassette from the isolated clones was also implemented by the electro-transformation and temperature upshift induction of the 707-FLPe plasmid. Upon temperature shifting, 30 °C to 37 °C, chez mutations carrying 707-FLPe plasmid expressed FLPe recombinase and then triggered FLP-mediated excision of the FRT-flanked kanamycin resistance cassette. After incubating at 37 °C for 3–5 h, the cells were plated and grown on LB-agar plates. We then picked single colonies from the plates and inoculated into LB only, LB with 30 μg ml⁻¹ kanamycin, and LB with 3 μg ml⁻¹ tetracycline for screening of kanamycin removal and 707-FLPe plasmid curing. PCR verification of kanamycin cassette removal was further performed with the primer set k1 and k2.

General cloning procedures. DNA was extracted from cells using either a Qiagen (Hilden, Germany) or a Zymo Research (Irvine, CA) Miniprep kit according to the manufacturer’s instructions. Polymerase chain reaction (PCR) was used to amplify genes or DNA of interest using Q5 DNA Polymerase (NEB). Primers were ordered from Integrated DNA Technologies (IDT, Coralville, IA). NEB restriction enzymes such as BamHI and HindIII were used to generate restriction digests of desired PCR products or plasmids. Agarose gel electrophoresis was used to separate DNA fragments based on size and the gel bands (as visualized with SYBR Safe, Invitrogen) as well as DNA sequencing by Genewiz was used to verify the constructs. Digested fragments were ligated using either NEB Quick Ligase or NEB T4 Ligase. Gibson Assembly was performed with NEB’s Gibson Master Mix chemistry in accordance with the manufacturer’s instructions. Each of the electro- or chemically competent cells (either from NEB, Invitrogen (Carlsbad, CA), electrocompetent, or made with Zymo Research’s Z- Competent E. coli Transformation Kit) were used for transformation.

β-galactosidase (Miller) assay. The Miller assay was performed on ZK126 cells with the pTG1 plasmid expressing β-galactosidase according to standard protocols. Miller assay was performed according to standard protocols. Briefly, cells were lysed with chloroform and sodium dodecyl sulfate (SDS) to release β-gal. The substrate ortho-nitrophenyl-β-galactoside was added and cleaved by β-gal into a yellow molecule, o-nitrophenol. Absorbance at 600, 550 and 420 nm was quantified by a SpectraMax M2 plate reader. The OD at 600 nm was measured from 250 μl of cells and the ODs at 420 and 550 nm were measured from 200 μl of cells.

Electrochemical ferricyanide reduction measurement. To measure ferricyanide reduction, cells, a three-electrode set-up was used: an Au working electrode (2 mm diameter, CH Instruments, Inc., Austin, TX), a 4-cm-long platinum wire counter electrode (Alfa Aesar, Haverhill, MA) and Ag/AgCl reference electrode (BASI, West Lafayette, IN). We used about 1.5 ml of cells at OD600 of 1.5, with a mini magnetic stir bar (see bulk electrolysis set-up in Methods). The cells were grown at 37 °C above and incubated inside the anaerobic chamber at 37 °C during measurements. An oxidation potential of +0.5 V was applied over time to measure ferricyanide.

Propidium iodide staining. Propidium iodide was used to stain dead bacteria. Cells were washed in 10 mM MgSO4 (pH 6.5), then PBS, and finally re-suspended in PBS with 5 μg ml⁻¹ of propidium iodide added. The cells were incubated at room temperature while covered with foil for 50 min. Afterwards cells were spun down and re-suspended in PBS. Fluorescence of cells was measured with flow cytometry as described in Methods, with the excitation and emission set for DiRed detection.

Glucose and acetate measurement. Glucose was determined by the YSI 2700 SELECT Biochemistry Analyser (YSI Life Sciences, Yellow Springs, Ohio). Acetate was determined by high-performance liquid chromatography, Hewlett Packard 1100 Series using an Aminex resin-based HPX-87H column (Bio-Rad, Hercules, CA). The analysis conditions were as follows: temperature 210 nmol, mobile phase 0.008 N H2SO4, flow rate 0.6 ml per min, temp 35 °C, calibration was done using organic acid analysis standard (Bio-Rad, Hercules, CA).

qPCR analysis. To study the messenger RNA levels in response to mediator treatments qPCR was performed. Cells were grown as stated in the Methods, taken to the anaerobic chamber, and let sit for 15 min before treatments. Cells were induced with the indicated mediators for 30 min (if in solution). Electrochemical induction was performed as in the Methods—in all cases +0.5 V was applied for 15 min, resulting in the indicated changes, after which the cells were cultured a further 15 min before addition of RNA later. Cells were treated as indicated and ~2 × 10⁸ total cells were washed in equal volume of PBS and then re-suspended and stored in RNA later (Ambion, Austin, TX) at 4 °C overnight. Before RNA isolation cells were pelleted by centrifugation at 9500 g for 10 min. The supernatant was discarded, and the remaining pellets were frozen at ~ −80 °C. Upon thawing, samples were lysed in 250 μl BugBuster HT (Novagen, Madison, WI) according to the
manufacturer’s protocol. Lysozyme concentrations were assessed via BCA assay (Pierce, Rockford, IL) according to the manufacturer’s protocol. Lysates were normalized to 500 ng µL⁻¹ with water and boiled with SDS loading dye.

**SDS-polyacrylamide gel electrophoresis and western blotting.** Purified CheZ was shipped to New England Peptide (Gardner, MA) for antibody generation in rabbit using the Customer Supplied Antigen package. The antiserum was used in Western blotting analysis of living cells and microfluidics: a convergence of fields. Biochemistry. (Mosc) 7, 706–716 (1990).

17. Morris, K., Catterall, K., Zhao, H., Pasco, N. & John, R. Ferricyanide mediated biochemical oxygen demand—a novel rapid alternative for measuring biochemical oxygen demand assay. Anal. Chir. Acta 442, 129–139 (2001).

18. Pasco, N., Baronian, K., Jeffries, C. & Hay, J. Biochemical mediator demand—a novel rapid alternative for measuring biochemical oxygen demand. Appl. Microbiol. Biotechnol. 53, 613–618 (2000).

19. Harrington, T. D. et al. Neutral red-mediated microbial electrosynthesis by Escherichia coli, Klebsiella pneumoniae, and Zymomonas mobilis. Biosens. Bioelectron. 19, 57–65 (2015).

20. Rawson, F. J., Downward, A. J. & Baronian, K. H. Electrochemical detection of intracellular and cell membrane redox systems in Saccharomyces cerevisiae. Sci. Rep. 4, 5216 (2014).

21. Rabinowitz, J. D., Vaccino, J. F., Beeson, C. & McConnell, H. M. Potentiometric measurement of intracellular redox activity. J. Am. Chem. Soc. 120, 2464–2473 (1998).

22. Kim, E. et al. Redox-capacitor to connect electrochemistry to redox-biology. Analyst 139, 32–43 (2014).

23. Liu, C., Yong, D., Yu, D. & Dong, S. Cell-based biosensor for measurement of phenol and nitrophenols toxicity. Talanta 84, 766–779 (2011).

24. Jensen, H. M. et al. Engineering of a synthetic electron conduit in living cells. Proc. Natl Acad. Sci. USA 107, 19213–19218 (2010).

25. Allerov, S. et al. Electrical communication of cytochrome enriched Escherichia coli JM109 cells with graphite electrodes. Electrochem. Acta. 54, 4979–4984 (2009).

26. Tschirhart, T. et al. Electrochemical measurement of the β-galactosidase reporter from live cells: a comparison to the Miller assay. ACS Synth. Biol. 5, 28–35 (2016).

27. Collier, J. H. & Mrksich, M. Engineering a biospecific communication pathway between cells and electrodes. Proc. Natl Acad. Sci. USA 103, 2022–2025 (2006).

28. Song, Y., Wang, J. & Yau, S.-T. Controlled glucose consumption in yeast using a transistor-like device. Sci. Rep. 4, 5429 (2014).

29. Gordonov, T. et al. Electronic modulation of biochemical signal generation. Nat. Nanotechnol. 9, 605–610 (2014).

30. TerAvest, M. A. & Ajo-Franklin, C. M. Transforming eukaryocytes for biotechnology using synthetic biology. Biotechnol. Bioeng. 113, 687–697 (2016).

31. Weber, W. et al. A synthetic mammalian electro-genetic transcription circuit. Nucleic Acids Res. 37, e33 (2009).

32. Gu, M. & Inlay, J. A. The SoxRS response of Escherichia coli is directly activated by redox-cycling drugs rather than by superoxide. Mol. Microbiol. 79, 1150–1159 (2011).

33. Price-Whelan, A., Dietrich, L. E. & Newman, D. K. Pyocyanin alters redox homeostasis and carbon flux through central metabolic pathways in Pseudomonas aeruginosa PA14. J. Bacteriol. 189, 6372–6381 (2007).

34. Gamby, S. et al. Altering the communication networks of multispecies microbial systems using a diverse toolbox of AI-2 analogues. ACS Chem. Biol. 7, 1023–1030 (2012).

35. Demple, B., Ding, H. & Jorgensen, M. Escherichia coli SoxR protein sensor/transducer of oxidative stress and nitric oxide. Methods Enzymol. 348, 355–364 (2002).

36. Greenberg, J. T., Monach, P., Chou, J. H., Josephy, P. D. & Demple, B. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in Escherichia coli. Proc. Natl Acad. Sci. USA 87, 6181–6185 (1990).

37. Tsaneva, I. R. & Weiss, B. soxR, a locus governing a superoxide response regulon in Escherichia coli K-12. J. Bacteriol. 172, 4197–4205 (1990).

38. Koo, M. S. et al. A reducing system of the superoxide sensor SoxR in Escherichia coli. EMBO J. 22, 2614–2622 (2003).

39. Shoji, A. K., Shin, J. H., Lee, K. L., Imlay, J. A. & Roe, J. H. Comparative study of SoxR activation by redox-active compounds. Mol. Microbiol. 90, 983–996 (2013).

40. Wu, J. & Weiss, B. Two divergently transcribed genes, soxS and soxR, control a superoxide response regulon of Escherichia coli. J. Bacteriol. 173, 2864–2871 (1991).

41. Morris, K., Zhao, H. & John, R. Ferricyanide-mediated microbial reactions for environmental monitoring. Aust. J. Chem. 58, 237–245 (2005).

42. Catterall, K., Robertson, D., Teasdale, P. R., Welsh, D. T. & John, R. Evaluating use of ferricyanide-mediated respiration biosaas to quantify stimulatory and inhibitory effects on Escherichia coli populations. Talanta 80, 1980–1985 (2010).

43. Christie, J. M. et al. Structural tuning of the fluorescent protein iLOV for improved photostability. J. Biol. Chem. 287, 22295–22304 (2012).

44. McGinness, K. E., Baker, T. A. & Sauer, R. T. Engineering controllable protein degradation. Mol. Cell 22, 701–706 (2006).
45. Zhao, R., Collins, E. J., Bourret, R. B. & Silversmith, R. E. Structure and catalytic mechanism of the E. coli chemotaxis phosphatase CheZ. *Nat. Struct. Mol. Biol.*, **9**, 570–575 (2002).

46. Danino, T., Mondragon-Palomino, O., Tsimring, L. & Hasty, J. A synchronized quorum of genetic clocks. *Nature* **463**, 326–330 (2010).

47. Olson, E. J., Hartsough, L. A., Landry, B. P., Shroff, R. & Tabor, J. J. Characterizing bacterial gene circuit dynamics with optically programmed gene expression signals. *Nat. Meth.* **11**, 449–455 (2014).

48. Wang, X., Chen, X. & Yang, Y. Spatiotemporal control of gene expression by a light-switchable transgene system. *Nat. Meth.* **9**, 266–269 (2012).

49. Levskaya, A. et al. Synthetic biology: engineering *Escherichia coli* to see light. *Nature* **438**, 441–442 (2005).

50. Schmidt, S. R., Sheth, R. U., Wu, A. & Tabor, J. J. Refactoring and optimization of light-switchable *Escherichia coli* two-component systems. *ACS Synth. Biol.* **3**, 820–831 (2014).

51. Ortner, V. et al. Magnetic field-controlled gene expression in encapsulated cells. *J. Control. Release* **158**, 424–432 (2012).

52. Stanley, S. A. et al. Radio-wave heating of iron oxide nanoparticles can regulate plasma glucose in mice. *Science* **336**, 604–608 (2012).

53. Stanley, S. A., Sauer, J., Kane, R. S., Dordick, J. S. & Friedman, J. M. Remote regulation of glucose homeostasis in mice using genetically encoded nanoparticles. *Nat. Med.* **21**, 92–98 (2015).

54. Meijering, E., Dzyubachyk, O. & Smal, I. Methods for cell and particle tracking. *Methods Enzymol.* **504**, 183–200 (2012).

55. Otusu, N. A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man and Cybernetics, B*, **9**, 62–66 (1979).

56. Gibson, D. G. et al. Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* **6**, 343–345 (2009).

57. You, L., Cox, 3rd R. S., Weiss, R. & Arnold, F. H. Programmed population control by cell-cell communication and regulated killing. *Nature* **428**, 868–871 (2004).

58. Koop, A. H., Hartley, M. E. & Bourgeois, S. A low-copy-number vector utilizing beta-galactosidase for the analysis of gene control elements. *Gene* **52**, 245–256 (1987).

59. Servinsky, M. D. et al. Directed assembly of a bacterial quorum. *ISME J.* **10**, 158–169 (2016).

60. Wu, H. C. et al. Autonomous bacterial localization and gene expression based on nearby cell receptor density. *Mol. Syst. Biol.* **9**, 636 (2013).

61. Datserko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97**, 6640–6645 (2000).

62. Miller, J. H. *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, 1972).

63. Pottash, A. E., McKay, R., Virgile, C. R., Ueda, H. & Bentley, W. E. TumbleScore: run and tumble analysis for low frame-rate motility videos. *BioTechniques*. (in the press).

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**Author contributions**

T.T., E.K., G.F.P. and W.E.B. developed the concepts. T.T., H.-C.W. and A.Z. genetically engineered the constructs. H.U. and A.E.P developed and ran Matlab tools and analyses. T.T., R.M. and A.N. designed and ran the experiments. T.T., E.K., R.M., H.U., G.F.P. and W.E.B. analysed the data. T.T., G.F.P. and W.E.B. wrote and edited the manuscript and figures. J.S., G.F.P. and W.E.B. supervised the work.

**Additional information**

**Supplementary Information** accompanies this paper at http://www.nature.com/naturecommunications

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