Bio-electrical engineering: a promising frontier for synthetic biology

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What do the following have in common? The production of methane gas from farm waste; toilets at a music festival, lit with LED lights; a bacterial biofilm that is on the brink of starvation. All of these involve microbes that are making use of bio-electrical processes. Though it is difficult to define the limits of what can be called bio-electrical, these processes are typically responding to or creating a current or voltage, with the electrical effects extending beyond the limit of an individual cell. In the examples above, current is flowing between organisms of different species or between an organism and an abiotic material, or voltage changes are being sensed and propagated across a colony of cells. Our appreciation of the extent of electrical phenomena in microbial biology has seen a recent revival, with studies revealing not just the variety of bio-electrical processes that exist but also defining the molecular mechanisms responsible. Now, we can begin to apply the approaches and techniques of synthetic biology. By re-engineering natural systems, we can hope to improve our understanding of how their components function and repurpose them for exciting biotechnological applications.

The best-known electron transfer processes within a bacterial cell are arguably those involved in energy production at the cell membrane. A typical view of respiration is that electrons that have been harvested from the breakdown of sugars are passed down a chain of electron carriers, with the energy released at each transfer step being used for ATP production. Oxygen is often thought of as the final electron acceptor in respiration, but other high-potential molecules—ones that readily take up electrons—can also be used to pull current through respiratory chains. But what if the cell could benefit from using an external electron acceptor that it cannot import? A number of microbes have become adapted for this lifestyle, and have developed different mechanisms for transporting electrons from their intracellular metabolism to their outer surface and beyond.

In the absence of oxygen, metal-containing compounds such as iron oxides make attractive final electron acceptors for respiration, but these are often in a granular, insoluble form. The metal-reducing pathway from *Shewanella oneidensis* MR-1 is one of the best understood electron export mechanisms in Gram negative bacteria. Electrons use a chain of cytochromes to move from the quinol pool in the cytoplasmic membrane, across the periplasm and through a pore in the outer membrane to the extracellular surface of the cell. Large cytochromes on the outside of the cell are then able to directly transfer electrons to the metals (Figure 1). Other direct electron transfer systems exist, often utilizing the same functional motif of an outer

![Figure 1. The *Shewanella oneidensis* MR-1 metal-reducing pathway.](image-url)
membrane porin with associated cytochromes. A special case involves the *Geobacter* species that also use conductive pili (protein filaments) to transfer electrons across distances equivalent to many times the length of the cell, literally wiring themselves to the metal oxides they are using. A simpler method for EET is the indirect or mediated route, which uses soluble molecules to shuttle electrons away from the cell. Some mediators, such as flavins, collect electrons from components on the outside of the cytoplasmic membrane, whilst others such as phenazines can pass through the membrane to access the intracellular metabolism. Electron transfer using mediators typically occurs at a slower rate compared with direct transfer due to the requirement for diffusion.

Electron-exporting bacteria can be put to work in a microbial fuel cell (MFC), where they transfer electrons to the anode electrode. A high potential reaction at the cathode drives electrical current through an external circuit between the electrodes. One strength of biological systems is that they are adaptive and regenerative, so compared with typical abiotic fuel cells, MFCs can process a much broader range of substrates and are more robust to the presence of impurities. MFCs fed with wastewater generate useful electricity, and by oxidizing the organic compounds present in the liquid they also decontaminate it. An MFC fed by raw urine was demonstrated at Glastonbury Festival, generating enough power to run its own lighting (Figure 2), and in the lab this treatment was shown to effectively suppress the growth of pathogenic bacterial species. Electron-exporting bacteria can also be used for the bioremediation of toxic metals in waste streams by reducing metal ions to a less soluble form for collection and removal.

**Engineering electron export**

So where does synthetic biology come in? Perhaps we would like to endow the ability for electron export on an organism with an interesting metabolism: a photosynthetic bacterium for example, which generates electrons from light and water. Reconstructing these multi-component systems in a new host requires extensive optimization of gene expression, as well as modifying the host metabolism to interface effectively with the EET pathway. In addition to being a pure engineering goal, building many variants of the EET pathway verifies we understand the contribution each component makes to the whole system. Good progress has been made in this regard with the *S. oneidensis* metal-reducing pathway, but there is still a way to go before electron export rates reach those seen in the native system, let alone surpass them. Energy production is not the only useful consequence of electron export: the flow of current is also a flow of information. EET pathways will enable biosensors that integrate directly with computers, and researchers are already creating synthetic electron transfer pathways that are post-translationally activated by binding target molecules.

Focusing down to the molecular level of EET components highlights further engineering opportunities. Conductive pili from different species of *Geobacter* possess different levels of electrical resistance; using the comparison to understand what makes for highly conductive filaments will hopefully inform the design of synthetic proteins with even faster electron transfer rates—perhaps through the incorporation of unnatural amino acids. The conditions at the microbe/electrode interface are vital for ensuring effective current transfer and an important target for engineering. Proteins that have evolved to interact with metal oxides might be redesigned for more effective electron transfer to the carbon electrodes typically used in MFCs, perhaps through chemical linkage to doped electrode surfaces. This controlled adhesion could also enable patterning of cells on surfaces to create electronic circuits many orders of magnitude larger than the cells themselves.

**Reversing the flow**

Electron export is only half of the picture! Electrons can flow into the cell too, if the organism is able to make use of a higher-potential electron acceptor than the metal (or electrode) it is oxidizing—for example, oxygen in the case of the iron(II)-oxidizing bacterium *Acidithiobacillus ferrooxidans*. Lithotrophic bacteria (those that take up electrons from inorganic sources) use the incoming electrons for energy conservation, but can also regenerate low-potential electrons needed for anabolic reactions such as carbon fixation.

![Figure 2. The ‘Pee Power’ unit feeds urine into microbial fuel cells which generate electricity to light the unit. Image courtesy of Ioannis Ieropoulos via www.susana.org](https://portlandpress.com/biochemist/article-pdf/41/3/10/851400/bio041030010.pdf)
Uptake of current from an electrode for use in the biosynthesis of organic compounds is termed electrosynthesis. Adjacent to this is electrofermentation, where an external electrical influence is used to change the balance of the cell's metabolism, biasing it towards the production of a desirable product. In the context of transitioning to a sustainable global economy there is growing interest in using (renewable) electrical energy to drive the synthesis of useful molecules such as bio-plastics or fuels. Many current synthetic biology efforts are concerned with the construction and optimization of biosynthesis pathways, and efforts have already been made to link these to electron uptake pathways. An advanced form of this goal might be the combination of electron uptake and export abilities with electrosynthesis of energy-storage compounds such as glycogen, to create an organism capable of taking up electrical current, storing it as chemical energy and releasing it again as electrical current when required: a living biological battery.

**Electricity for the community and the importance of direct interspecies electron transfer (DIET)**

In nature, microbes exist in communities; in this context it is possible to think of microbial metabolism as being spread across multiple species that are each adapted to perform particular types of reactions and excrete the products for another organism to use. Bio-electricity can play an important role here too, through DIET. *Geobacter* species in particular have been identified as being adept at DIET, transferring electrons to other organisms via their conductive pili. Anaerobic digesters make use of bacterial consortia to break down organic matter and produce biogas. Here, *Geobacter* species have been shown to transfer electrons directly to methanogens, facilitating the conversion of more complex organic matter into methane (Figure 3). The full mechanism for electron uptake is not understood, but is clearly adaptable, as the function of the conductive pili can be replaced by other conductive material such as activated carbon granules. Synthetic biologists are starting to explore the potential benefits of splitting biosynthetic pathways between different strains in order to reduce their metabolic burden. DIET could be a useful tool in this context; whilst reconstituting the necessary molecular machinery for DIET is not yet a matter of ‘plug and play’, metabolic engineering of species already capable of mutual electron exchange is more accessible.

Bio-electrical activity in microbial consortia has implications for human health too: many Gram-positive bacteria that colonize or act as pathogens in the human gut possess a flavin-based EET mechanism that is required for competitive growth. EET may prove to be important as synthetic biologists consider how our own microbiomes might be engineered to combat disease.

**Electrical feedback**

Organisms use EET mechanisms in order to alter their own redox state, so it is natural that these processes are subject to regulation based on sensing of the intracellular redox balance. This regulation has been repurposed to enable electronic control of transcription, using mediated electron transfer to control the oxidation state of the regulator protein SoxR: when oxidized SoxR activates transcription from the *psoxS* promoter. Electronic control of gene expression is easily reversible and likely cheaper than using chemical inducers in large-scale cultures.

**Voltage in action**

Other bio-electrical processes are at work in bacterial communities. In addition to being the gateway for electron exchange between cellular metabolism and the extracellular environment, the cytoplasmic membrane is a barrier for electrical charges. Regulating voltage across the membrane is vital for an individual cell’s transport processes and energy production, but can also play a role at the community level. *Bacillus subtilis* cells grown in a biofilm were shown to coordinate their metabolism through the propagation of a wave of potassium ion efflux across the colony (Figure 4). This electrical signalling, analogous to a neuronal action potential, allows starving individuals in the centre of the colony to communicate to their brethren at the edges that they should slow their growth and let more nutrients diffuse in. Communication has also been demonstrated to occur between separate colonies, and influences the attraction of nearby motile cells of different species. The ion channel responsible has been identified, and so taking control over this process could

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**Figure 3.** *Geobacter* species (red) form a close association with methane-producing *Methanosaeta* species (green) in aggregates from an anaerobic digester, as they perform direct interspecies electron transfer. Adapted from Energy Environ. Sci. (2014) 7(1), 408–415; reproduced with permission from The Royal Society of Chemistry.
be useful for a number of applications, for example, patterning of microbial communities or enabling signal amplification and ultrasensitivity in biosensors.

**Completing the circuit**

Of course, bio-electricity is not restricted to bacteria. Optogenetics—using light to stimulate action potentials via photo-responsive ion channels—is a good example of how synthetic biology has been applied to control eukaryotic neuronal cells and interrogate how they function in their networks. Bio-electric effects in nature are continually being revealed, from their role in cancer to plant immunity and physiology, and each discovery generates a new opportunity for synthetic biologists to redesign and regulate.

By viewing biological phenomena through the conceptual framework of bio-electricity we allow ourselves to bring in tools from multiple scientific disciplines to deepen our understanding, at scales ranging from the atomic to entire ecosystems. Synthetic biology will be an integral part of this effort, not just because of the many useful techniques developed by the field, but because the interdisciplinary and applications-focused nature of the community is well matched to the challenges of bio-electrical engineering. Many design principles used by synthetic biologists are taken from electrical engineering; with the rise of bio-electrical engineering we are on the threshold of bringing those ideas full circle.

**Figure 4.** A time-course of a biofilm of *Bacillus subtilis* shows waves of membrane polarization spreading from the centre to the edges. Thioflavin T (ThT) fluorescence increases when the inside of the cell becomes more negative. Adapted from Nature (2015) 527, 59–63; reproduced with permission.

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**Further reading**

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Robert Bradley is a Postdoctoral Research Associate at Imperial College London. He researches how extracellular electron transfer by bacteria can be controlled and exploited for applications including bio-energy production, bio-sensing and the biosynthesis of useful molecules. His interests in this area began with investigations into EET from cyanobacteria, which has led to efforts to reconstitute and add synthetic control over EET systems in a variety of contexts. Robert is currently working with a number of talented students to create genetic tools to engineer iron-oxidizing bacteria for the efficient electrosynthesis of chemicals.

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