INTRODUCTION

The fields of bioelectronics, synthetic biology and electromicrobiology are converging through the development of new living electronics where biological entities (e.g., biomolecules, cells or cellular communities) are directly integrated as electrical components into electronic circuits (Dunn, 2020). Biohybrid devices have been built that integrate a diversity of living electronic components spanning many length scales. Research in the field of bioelectronics is yielding materials innovations that improve the abiotic:biotic interface between biological entities and electrodes (Tseng et al., 2020). Synthetic biologists are leveraging concepts from electrical engineering as analogies when designing DNA-, RNA- and protein-based logic circuits to program predictable and dynamic phenotypes in cells and biomolecular networks (Brophy & Voigt, 2014; Gao et al., 2018; Green et al., 2017). Meanwhile, advances in electromicrobiology are providing a mechanistic understanding of molecular components underlying the ability of microorganisms to electrically interact with insoluble materials and other cells in their environment (Kracke et al., 2015).

The cross-disciplinary integration of the recently gained knowledge in each of these fields is uniquely poised to produce novel living electronics that leverage the unique capabilities of biological and solid-state systems. Herein, we summarize recent advances in the characterization of biological entities as electrical components, the integration of biological entities into electrical devices, and provide a prospective for new types of electrical devices that could be constructed using living components. Living electronics offer unique capabilities including the ability to self-assemble, self-power, repair, biodegrade, process molecular information and...
store information in low-energy structures. Living electronics can be produced with renewable feedstocks at ambient temperature and pressures, thereby offering a path towards sustainable production of electronics. To leverage these unique capabilities the field needs a framework to integrate these components into conventional electrical circuits.

**CATALOGUING LIVING ELECTRONIC COMPONENTS**

Circuit engineering has benefited from having a catalogue of electronic components with well-characterized input–output relations, allowing them to be combined in a hierarchical manner. To integrate living components into electronic circuits it is useful to categorize these components according to their functions and behaviours so that they can be integrated into more complex devices.

**Wires**

One of the simplest electronic components is a charge-carrying wire or a conductive path. These components allow for the flow of current and have characteristic conductivities/resistances. Biological systems also take advantage of wires that exist at a range of length scales and conductivity to facilitate energy conservation and cellular communication (Figure 1).

**Redox-active molecules as nanometre scale wires**

All living cells form intracellular electron transfer (ET) networks using diffusible redox-active molecules that function as charge carriers coupling oxidative and reductive metabolic pathways. These ET networks enable energy extracted from substrates to be conserved in chemical bonds or membrane potentials, thereby allowing cells to perform work including active transport, biosynthesis, motility and cell division. Redox-active molecules include both organic small-molecules (e.g. nicotinamides, flavins, quinones and phenazines) and proteins (e.g. ferredoxins, flavodoxins, cytochromes, thioredoxins and azurins) (Atkinson et al., 2016). Redox-active molecules have a range of redox potentials at which they undergo ET and are compartmentalized in different regions of microbial cells including the cytosol, inner-membrane, periplasm and extracellular space. These properties constrain the connectivity of these ET networks and tailor their activity towards select chemical reactions.

In addition to forming intracellular ET networks, redox-active molecules can be excreted from the cell and used as mediators to form ET connections between cells and insoluble materials or other cells in their environment. This has been observed in electroactive organisms such as *Shewanella oneidensis* (Marsili et al., 2008; Xu et al., 2016), *Shewanella algaee* (Turick et al., 2002) and *Pseudomonas aeruginosa* (Hernandez et al., 2004) that excrete flavins, melanin and phenazines respectively. The diffusible nature of these mediators enables them to function as charge-carrying shuttles that connect cells to distant materials in their environment. To increase electron flux, multicellular communities have also evolved strategies for concentrating these diffusible molecules into extracellular networks, which enables them to generate a gradient, or ‘electrocline’, of reduced and oxidized redox-active molecules (Bellin et al., 2016; Koley et al., 2011). For example, during biofilm formation, *P. aeruginosa* populations release extracellular DNA (eDNA) that binds and retains the phenazine pyocyanin while also functioning as a nanometre scale charge transporter to enable ET rates that are not limited by long-distance diffusion.
of the phenazine (Saunders et al., 2020). However, using biofilms containing diffusible redox-active molecules to construct living electronic devices would be challenging as their conductive properties can change if there is a decrease in the concentration of the redox-active molecule within the biofilm environment (Saunders et al., 2020).

However, redox-active molecules have been used as nanoscale wires for the construction of molecular electronic devices where these molecules are covalently tethered to electrodes. While not the focus of this review, as they have been extensively reviewed elsewhere (Bostick et al., 2018; Cahen et al., 2021; Xiang et al., 2016), such devices have been constructed using carotene (Visoly-Fisher et al., 2006), Cu-azurin (Artés et al., 2012; Fereiro et al., 2018), c-type cytochromes (Amdursky et al., 2013, 2014; Futera et al., 2020) and ferredoxins (Zhang et al., 2004). Recently, proteins that do not undergo redox reactions in cellular contexts have been shown to carry charge likely via redox-active aromatic amino acids tyrosine and tryptophan in the peptide backbone rather than redox-active co-factors (Zhang et al., 2019, 2020). Such a non-traditional redox-active molecule has been employed as a molecular wire within scalable semiconductor chips with an ~25-nm-long alpha helical peptide containing metal binding domains enabling it to bridge pairs of ruthenium nano-electrodes (Fuller et al., 2022). Using this device, fluctuations in picoamp currents with an applied voltage of 0.7–1 VDC could be used to estimate the occupancy of biomolecules that bind to modified wires (Fuller et al., 2022). This exemplifies how nanoscale wires made of redox-active molecules can function as modular interfaces for biomolecular sensing (e.g. nucleic acids, peptides) in molecular electronics.

**Supramolecular assemblies of proteins as nano-to-micrometre scale wires**

Controlled assembly of charge carriers into supramolecular structures can extend the nanoscale electron transfer (ET) properties to enable electron transport (ETp) at micrometre scales (Ing, El-Naggar, & Hochbaum, 2018). This is best exemplified by microbial nanowires, conductive appendages that project out of microbial cells allowing them to reach insoluble substrates or other cells in their environment (Lovley & Yao, 2021). The term, microbial nanowires, has been used to describe a variety of disparate biological structures including conductive pili (Reguera et al., 2005; Walker et al., 2018), polymeric cytochromes (Filman et al., 2019; Wang, Gu, et al., 2019; Yalcin et al., 2020) and outer-membrane protrusions laden with cytochromes (Pirbadian et al., 2014). The charge transfer capabilities of these diverse supramolecular assemblies have been extensively studied using both dry, solid-state techniques as well as solution-based techniques including electrochemistry and spectroscopy. These measurements have revealed a range of charge transfer properties and suggested mechanisms including thermally activated hopping, diffusion-assisted hopping, delocalized band formation, flickering resonance and superfusion as extensively reviewed by Ing, El-Naggar, et al. (2018) (Table 1). When comparing these properties careful attention must be paid to both how these measurements are made (solid-state vs. solution based), the proposed mechanism of conductivity, the sample purity, the spatial geometry, the charge carrier concentration and what property is being reported (bulk conductance vs. intrinsic conductivity or electron diffusion coefficients).

Conductive microbial nanowires were initially discovered in *Geobacter sulfurreducens* (Reguera et al., 2005). These microbial nanowires extend micrometre distances away from the cell surface and based on early genetic studies were long thought to be electrically conductive pili (e-pili) consisting of polymers of a truncated pilin, PilA-N (Reguera et al., 2005), that contains a relatively high number of aromatic amino acids relative to other Type IV pilin proteins (Vargas et al., 2013). Many studies characterized the conductive properties of these wires without direct evidence of their molecular identity and suggested a mechanism of delocalized band formation through π-stacking of aromatic amino acids within supramolecular assemblies of PilA-N. Individual e-pili were found to form ~3 nm diameter wires with conductivity on the order of ~10−2 S cm−1 as measured in hydrated conditions at pH 7 in a two-electrode configuration (Adhikari et al., 2016). In one of the most compelling studies linking a conductive function to PilA-N identity, using synthetic biology techniques Ueki et al. were able to determine the conductance of e-pili heterologously expressed in *Escherichia coli* using a four-electrode conductance measurement (Ueki et al., 2020). The conductance of films of these *E. coli* produced e-pili was found to be ~10−6 S under dry conditions, which is similar to the conductance of films of *G. sulfurreducens* produced nanowires measured under similar conditions (Walker et al., 2018). This synthetic biology approach helped to overcome the challenge of confounding alternative nanowires structures encoded in the *G. sulfurreducens* genome. However, in both studies, the purity and identity of the nanowires were not subsequently confirmed beyond targeted Western blots and scanning probe measurements of nanowire diameter (~3 nm) that do not preclude the presence of potential confounding conductive macromolecules such as B-DNA which has been observed in recent cryo-electron microscopy (cryo-EM) studies of *G. sulfurreducens* filament extractions (Wang, Mustafa, et al., 2022). To date, PilA-N filaments have not been structurally identified using cryo-EM approaches. However, heterodimeric PilA-N-C filaments have been observed (Gu et al., 2021; Wang, Mustafa, et al., 2022).
**Table 1** Measurements of natural supramolecular wires. A description of the electronic measurements of natural supramolecular nanowires.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Charge carrier</th>
<th>Cofactor</th>
<th>Scaffold</th>
<th>Diameter (nm)</th>
<th>Conductivity (S cm⁻¹)</th>
<th>Conductance (S)</th>
<th>Measurement</th>
<th>Measurement orientation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. sulfurreducens</em></td>
<td>PilA</td>
<td>–</td>
<td>Self-polymerized</td>
<td>3</td>
<td>10⁻²</td>
<td>–</td>
<td>Hydrated solid-state</td>
<td>Transverse Individual</td>
<td>Adhikari et al. (2016)</td>
</tr>
<tr>
<td><em>G. sulfurreducens</em></td>
<td>PilA</td>
<td>–</td>
<td>Self-polymerized</td>
<td>3</td>
<td>10⁻⁹</td>
<td>–</td>
<td>Dry solid-state</td>
<td>Cross-sectional Individual</td>
<td>Walker et al. (2019)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>gsPilA</td>
<td>–</td>
<td>Self-polymerized</td>
<td>3</td>
<td>10⁻⁶</td>
<td>–</td>
<td>Dry solid-state 4-electrode</td>
<td>Transverse Film</td>
<td>Ueki et al. (2020)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>gsPilA</td>
<td>–</td>
<td>Self-polymerized</td>
<td>3</td>
<td>10⁻⁹</td>
<td>–</td>
<td>Dry solid-state</td>
<td>Cross-sectional Individual</td>
<td>Ueki et al. (2020)</td>
</tr>
<tr>
<td><em>G. sulfurreducens</em></td>
<td>OmcZ</td>
<td>Haem</td>
<td>Self-polymerized</td>
<td>2.5</td>
<td>10²</td>
<td>–</td>
<td>Hydrated solid-state</td>
<td>Transverse Individual</td>
<td>Yalcin et al. (2020)</td>
</tr>
<tr>
<td><em>S. oneidensis</em></td>
<td>MtrCAB OmcA MtrDEF</td>
<td>Haem</td>
<td>Outer-membrane extension</td>
<td>10</td>
<td>10⁻²</td>
<td>–</td>
<td>Hydrated solid-state 2-electrode</td>
<td>Transverse Individual</td>
<td>Grebenko et al. (2018)</td>
</tr>
<tr>
<td><em>M. huntgatei</em></td>
<td>Archaealum</td>
<td>–</td>
<td>Self-polymerized</td>
<td>10</td>
<td>–</td>
<td>10⁻⁸</td>
<td>Dry solid-state 2-electrode</td>
<td>Cross-sectional Individual</td>
<td>Walker et al. (2019)</td>
</tr>
<tr>
<td>Cable bacteria (marine)</td>
<td>unknown</td>
<td>Ni-S</td>
<td>unknown</td>
<td>50 (26)</td>
<td>10⁻²–10²</td>
<td>–</td>
<td>Dry solid-state 2-electrode</td>
<td>Transverse Fibre-sheath</td>
<td>Meyesman et al. (2019); Boschker et al. (2021)</td>
</tr>
</tbody>
</table>

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*Unspecified genus assignment.*  
*Hypotheses from spectral studies of proposed metal centres (Boschker et al., 2021).*
In 2019, a paradigm-shifting set of cryo-EM studies (Filman et al., 2019; Wang, Gu, et al., 2019) revealed the identity of certain nanowires isolated from G. *sulfurreducens* to be a polymeric assembly of the multihaem cytochrome Omcs (Yalcin & Malvankar, 2020). Omcs wires were found to form 4 nm diameter wires with closely spaced haems that have conductivities on the order of $10^{-2}$ S cm$^{-1}$ as measured in hydrated conditions at pH 7 in a two-electrode configuration (Wang, Gu, et al., 2019). Subsequently, a different multiheme cytochrome, OmcZ, was found, using scanning probe techniques, to polymerize into 2.5 nm diameter wires with 1000-fold higher conductivity ($10^{-1}$ S cm$^{-1}$) than what was reported for Omcs using a two-electrode configuration (Yalcin et al., 2020). Filaments of a third multiheme cytochrome, Omce, were structurally resolved recently and found to share a conserved haem arrangement with Omcs, but have little sequence or structural similarity (Wang, Mustafa, et al., 2022). Recently, a thorough mechanistic study of Omcs revealed interesting temperature dependence in nanowire conductivity, resulting in a 300-fold increase in conductivity when cooled due to changes in hydrogen-bonding network that makes neighbouring haems more planar (Dahl et al., 2022). While questions remain about the specific roles of the multiple nanowires produced by *G. sulfurreducens*, they likely play different roles as the biofilm develops with PilA being important for the maturation and export of the polymeric cytochrome wires (Gu et al., 2021), Omcs being important for initial colonization and growth of the biofilm (Dahl et al., 2022), and OmcZ playing an important role as the biofilm thickens, enabling cells far from the surface to still maintain electrical contact as the local biofilm redox potential decreases (Yalcin et al., 2020).

*S. oneidensis* also produces nanowires that extend micrometres away from the cell (El-Naggar et al., 2008; Gorby et al., 2006), but in contrast to *G. sulfurreducens*, are not solely proteinaceous. Rather, nanowires from *S. oneidensis* consist of outer-membrane and periplasmic extensions that are packed with membrane-associated cytochromes (Pirbadian et al., 2014; Subramanian et al., 2018). When dried and chemically fixed, these membrane extensions form 8 to 10 nm diameter nanowires with conductivities of $-1$ Scm$^{-1}$ under dry, solid-state conditions in a two-electrode configuration (El-Naggar et al., 2010). However, such dry conditions do not reflect physiologically relevant conditions and individual nanowires measured under hydrated conditions yielded conductivity of $10^{-2}$ Scm$^{-1}$ (Grebenko et al., 2018). This is especially important because the cytochrome charge carriers in these nanowires are mobile and diffuse along the cell surface and the nanowires (Chong et al., 2022). This diffusivity is thought to contribute to the overall conductivity of these nanowires in a collision-exchange mechanism whereby the lateral diffusion of cytochromes leads to collision and interprotein electron transfer via redox-hopping (Chong et al., 2022; Subramanian et al., 2018). Additionally, the individual cytochromes that span the outer membrane of *S. oneidensis* can be thought of as nanoscale wires spanning ~15 nm and have electron fluxes that are about 30-fold lower than *G. sulfurreducens* Omcs over similar length scales (Jiang et al., 2019).

Other microbes have recently been discovered to produce diverse conductive structures that function as supramolecular wires. In the archaean *Methanospirillum hungatei*, conductive atomic force microscopy was used to show cross-sectional electron transport in the archaeallum that is thought to arise from a core of tightly packed aromatic phenylalanine residues (Walker et al., 2019). Anaerobic methanotrophic archaea (ANME) express multihaem cytochrome/surface-layer fusion proteins that have been implicated in direct interspecies electron transfer with sulfate-reducing bacteria (McGlynn et al., 2015). While the structure and electron transport properties of ANME cytochrome/s-layer fusions have not yet been investigated, they hold exciting promise for engineering self-assembling 2D conductive materials (Charrrier et al., 2019; Schuster & Sleytr, 2013). Recently, extracellular flavinated proteins have been found in a wide array of Gram-positive bacteria including multi-flavinated proteins that may serve similar functions as multihaem cytochromes (Light et al., 2018; Méheust et al., 2021). While it remains to be seen if similar micron-scale flavinated proteins exist, there are exciting opportunities for developing conductive nanowires that do not require metallocofactors for conductivity but instead harbour redox-active organic molecules such as flavins.

In addition to these natural examples, there are examples of synthetic proteinaceous nanowires that have been engineered to facilitate micrometre-scale electron transport (Table 2). Synthetic nanowires use scaffold proteins to self-assemble into nano- or micro-metre length fibres that are often modified with metal binding moieties or metalloproteins that endow these fibres with conductive properties (Altamura et al., 2017; Nguyen et al., 2014; Scheibel et al., 2003; Seker et al., 2017). Frequently, amyloid fibril-forming proteins are used for the scaffold, such as fungal prions (Altamura et al., 2017; Scheibel et al., 2003) and bacterial curlins (Nguyen et al., 2014; Seker et al., 2017). These scaffold proteins have relatively low intrinsic conductivities (Del Mercato et al., 2007; Dorval Courchesne et al., 2018; Kalyoncu et al., 2017). To confer metallic conductivity to these nanowire scaffolds, the monomer can be functionalized to bind exogenously supplied metal nanoparticles by fusing them to metal binding moieties such as surface-exposed cysteines (Scheibel et al., 2003), poly-histidine (Chen et al., 2014; Seker et al., 2017), or metal-binding peptides (Nguyen et al., 2014; Seker et al., 2017). Redox-hopping conductivity has been engineered into nanofibres by fusing amyloid-forming
monomers to rubredoxin, a metalloprotein containing an Fe^{2+/3+} centre enabling electron hopping with a conductivity of $\sim 10^{-6}$ S cm$^{-1}$ in both hydrated electrochemical measurements and in solid-state two-electrode measurements (Altamura et al., 2017). Similarly, the bacterial curli CsgA has been engineered to display a minimal cytochrome domain, but charge transport of these engineered curli was not measured (Chen, Yang, et al., 2022). To generate nanofibres with intrinsic conductivity based on band conduction with $\pi$-stacking, the amyloid-forming scaffold CsgA has been engineered to increase the number of amino acids with aromatic side chains through peptide fusion (Kalyoncu et al., 2017) and site-directed mutagenesis (Dorval Courchesne et al., 2018). The CsgA modified to have more aromatic residues resulted in nanowire films with enhanced conductivity relative to WT CsgA that, when characterized as dried fibre mats, displayed conductivities of $\sim 10^{-9}$ S cm$^{-1}$ and $\sim 10^{-8}$ S cm$^{-1}$ for the WT and mutant CsgA respectively (Dorval Courchesne et al., 2018). These studies illustrate the flexibility of amyloids as a platform for generating engineered conductive biomaterials.

Other fibre-forming scaffolds have been leveraged to generate conductive nanowires. For example, alpha-helical bundles with internal aromatic amino acids form micrometre length nanofibres with an exceptionally high conductivity of $\sim 1$ S cm$^{-1}$ as measured in solid-state conditions using a two-electrode configuration (Ing, Spencer, et al., 2018). Mechanistic electron transport measurements revealed that these nanowires had properties of ohmic charge conductors rather than those of redox-hopping or $\pi$-stacked charge conductors (Ing, Spencer, et al., 2018). The archaeal filament-forming protein gamma-prefoldin (γPFD) from Methanocaldococcus jannaschii has been engineered to assemble the tetra-haem cytochrome c3 (Cytc3) from Desulfovibrio vulgaris (Chen et al., 2020) into filaments in a modular way using the SpyTag/SpyCatcher system (Zakeri et al., 2012). γPFD-Cytc3 fibres formed 3.6 nm diameter nanowires that display redox-hopping-based conductivity in electrochemical measurements and displayed conductances of $10^{-7}$ S when characterized using a solid-state, two-electrode configuration (Chen et al., 2020). This modular bioconjugation strategy could be adapted to assemble other redox-proteins into nanowires.

Synthetic wires have also been constructed that are not based on linear scaffolds but rather three-dimensional networks of charge carriers. Crystals of a tetra-haem cytochrome from S. oneidensis, CctA, have been generated and studied for their charge-carrying properties in solution using photoreduction and spectroscopy (Huang et al., 2020). These measurements revealed electron hopping between cytochromes traveling $\sim 100 \mu$m through the crystal lattice with apparent electron diffusion coefficient of $10^{-9}$ cm$^2$ s$^{-1}$ but
such crystals have not yet been probed using electrical characterization techniques (Huang et al., 2020). Mats of bovine serum album have also been shown to be proton conductors with proton conductivities of $10^{-5}$ S cm$^{-1}$ (Amdursky et al., 2016) and, when doped with an electron carrier like Fe-containing haem, they can reach electron conductivities of $10^{-3}$ S cm$^{-1}$ (Amdursky et al., 2017). Subsequent studies of BSA mats doped with porphyrins containing different metal centres or protoporphyrin-IX containing no metal at all were also found to increase electron conductivity to $10^{-3}$ S cm$^{-1}$ leading the authors to suggest this results from the conjugated $\pi$-system of the porphyrin ring and not hopping between metal centres (Agam et al., 2020). Hydrogels made from the aforementioned alpha-helical bundles have also been constructed. AC conductance measurements of these materials have returned values on the order of $10^{-7}$ S when measured using two-probe configuration under hydrated conditions (Ing, Spencer, et al., 2018).

From the above-mentioned review of supramolecular wires, it is possible to glean important trends that are influencing our understanding of charge transport and structure–function relations. One trend is an increasing emphasis on developing electron transport measurement techniques that go beyond dry, solid-state conditions to hydrated or in-solution electrochemical measurements. This development is key to understanding electron transport in living electronics under physiologically relevant conditions. Moreover, there is increasing emphasis on reporting the intrinsic conductivity (or electron diffusion coefficients), rather than conductance, finally allowing detailed comparisons across studies in a way that is not confounded by contact resistances or electrode/biomaterial geometries. Moreover, with the increasing availability of structures, it is finally possible to make the link between these structures and the key thermodynamic and quantum mechanical parameters that control electron transport (Jiang et al., 2020). This increasing emphasis on structure–function studies, and the emergence of synthetic structures that promise to rationally control these parameters, are providing unprecedented mechanistic insight into both the limitations and potential of biological wires.

Multicellular networks as micron-to-centimetre scale wires

Just as redox-active molecules and supramolecular structures can carry charge, groups of cells can themselves come together to form multicellular networks that act as charge-carrying wires over length scales from microns to centimetres (Table 3). Model electroactive organisms such as S. oneidensis and G. sulfurreducens naturally cluster together and form

<table>
<thead>
<tr>
<th>Organism</th>
<th>Charge carrier</th>
<th>Collector</th>
<th>Conductivity (S/cm$^{-1}$)</th>
<th>Conductance (S)</th>
<th>Measurement orientation</th>
<th>Measurement orientation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. sulfurreducens</td>
<td>Multihaem cytochromes</td>
<td>haem</td>
<td>$10^{-6}$</td>
<td>$10^{-9}$</td>
<td>hydrated, electrochemical gating</td>
<td>Transverse, cell-containing film</td>
<td>Yates et al. (2015)</td>
</tr>
<tr>
<td>S. oneidensis</td>
<td>Multihaem cytochromes</td>
<td>haem</td>
<td>$10^{-9}$</td>
<td>$10^{-3}$</td>
<td>hydrated, solid-state 2-electrode</td>
<td>Transverse, core/shell encapsulated cable</td>
<td>Hsu et al. (2018)</td>
</tr>
<tr>
<td></td>
<td>Multihaem cytochromes</td>
<td>haem</td>
<td>$10^{-3}$</td>
<td>$10^{-3}$</td>
<td>hydrated, solid-state 2-electrode</td>
<td>Transverse, cell-containing film</td>
<td>Zhao et al. (2022)</td>
</tr>
<tr>
<td>E. coli</td>
<td>AuNP Ni-NTA</td>
<td>Ni-NTA</td>
<td>$10^{-9}$</td>
<td>$10^{-2}$</td>
<td>dry, solid-state 2-electrode</td>
<td>Transverse, single-cable filament</td>
<td>Meyneman et al. (2019)</td>
</tr>
<tr>
<td>Cable bacteria (marine)</td>
<td>unknown</td>
<td>Ni-NTA</td>
<td>$10^{-2}$</td>
<td>$10^{-2}$</td>
<td>dry, solid-state 2-electrode</td>
<td>Transverse, single-cable filament</td>
<td>Meysman et al. (2019)</td>
</tr>
</tbody>
</table>

aUnspecified genus assignment.
bHypothesis from spectral studies of proposed metal centres (Boschker et al., 2021).
current-producing biofilms on electrodes. To probe in vivo biofilm conductance, biofilms have been cultivated on interdigitated arrays (IDA) of source and drain electrodes where electrochemical gating measurements have shown that these networks of cells form conductive channels capable of bridging the insulating gaps between the two electrodes (Xu et al., 2018; Yates et al., 2015). Temperature-dependent electrochemical gating measurements have revealed that these biofilms conduct electrons using a thermally activated redox hopping mechanism, where electrons travel through the biofilm, from source electrodes to drain electrodes, by hopping across the haem centres of the cells’ outer-membrane cytochromes (Xu et al., 2018; Yates et al., 2015). In vivo biofilm conductivity, a fundamental material parameter that requires a conduction channel of known dimensions in order to be calculated, has also been determined for *S. oneidensis* and *G. sulfurreducens*. By constraining the physical dimensions of the conduction channel during electrochemical gating measurements, using the defined IDA geometry in the case of *G. sulfurreducens* and using light-patterned biofilms in the case of *S. oneidensis*, conductivities were found to be on the order of $10^{-9}$ S cm$^{-1}$ for *S. oneidensis* and $10^{-8}$ S cm$^{-1}$ for *G. sulfurreducens* (Yates et al., 2015; Zhao et al., 2022). With these fundamental, quantitative electron transport results, more in depth characterization can be performed on these systems and they can inform the construction of microbial-based living electronics.

While conductive multicellular networks have typically been studied in the form of micron-scale 2-D and 3-D biofilms on surfaces, the study of 1-D conductive multicellular networks was recently enabled by the discovery of cable bacteria, centimetre-scale filamentous chains of conductive cells. Cable bacteria were first discovered in aquatic sediments in Denmark in 2012 and were found to link sulfide oxidation occurring centimetres below the sediment surface to oxygen reduction occurring at the surface (Pfeffer et al., 2012). To connect these spatially separated redox reactions, cable bacteria evolved to form 1.5 cm to 3 cm long chains of cells that contain thousands of cells linked end-to-end (Schauer et al., 2014). Ultrastructural studies using cryotomography and nanodissection revealed that each individual cell in the chain has an individual inner membrane while the entire chain of cells shares a common outer membrane and periplasmic space (Cornelissen et al., 2018; Jiang et al., 2018). Within this shared periplasmic space, cable bacteria contain a network of 15–60 parallel ridge-structures (Cornelissen et al., 2018; Jiang et al., 2018). These periplasmic ridges consist of 10–50 nm diameter fibres that run along the long-dimension of individual cells (~3 μm) and join together into cartwheel structures at cell–cell junctions forming a sheath-like cage around each cell within the cable and are continuously connected over the centimetre length of the cables (Cornelissen et al., 2018; Jiang et al., 2018). This conspicuous structure suggests that these fibres are the conductive components enabling long-distance electron transport in cable bacteria.

To determine the charge-transfer characteristics of cable bacteria, they have been interrogated using both solid-state and electrochemical techniques. As implied by their geochemical activity, solid-state measurements of intact cable bacteria confirmed that they are capable of long-distance electron transport along their length using a two-electrode configuration (Meysman et al., 2019). Both cyclic and differential pulse voltammetry of the biofilms on modified gold electrodes revealed redox-active molecules with potential near 0.155 V versus standard hydrogen electrode (V vs. SHE) that, based on resonance Raman spectroscopy, likely correspond to c-type cytochromes (Meysman et al., 2019). Conductive atomic force microscopy performed on the surface of intact cables directly implicated the periplasmic fibres as the conductive structure within the cable bacteria (Thiruvallur Eachambadi et al., 2020). The periplasmic fibre sheaths have been extracted from cables and directly studied using two-electrode and three-electrode field effect transistor (FET) measurements yielding conductivity values ranging from $10^{-2}$ to $10^{-4}$ S cm$^{-1}$ and electron mobility of $10^{-1}$ cm$^{2}$ V$^{-1}$ s$^{-1}$, making these fibres one of the most conductive biomaterials studied to date (Bonné et al., 2020; Boschker et al., 2021; Meysman et al., 2019). Spectroscopic analysis of the extracted fibre sheaths revealed that, unlike the intact cables, they do not have c-type cytochrome signatures (Meysman et al., 2019) and suggests that they may contain a novel sulfur-ligated nickel cofactor that is important for the fibre-based long-distance electron transport (Boschker et al., 2021). There is much work to be done to understand the charge transfer properties of cable bacteria including determining the structure of the charge-carrying periplasmic fibres and characterizing the properties of diverse cable bacteria species. Currently, these organisms are the only known examples of macroscale-leading conductors and present some of the most conductive biomolecular structures known.

In addition to studying multicellular assemblies that self-assemble, electroactive bacteria have been directed to assemble into different structures using material encapsulation. Approaches for encapsulating multicellular assemblies often incorporate additional charge carriers such as polypyrrole (Song et al., 2017), polydopamine (Yu et al., 2020), PEDOT:PSS (Zajdel et al., 2018) or metallic nanoparticles (Atkinson et al., 2022; Chen, Kang, et al., 2022) making it challenging to directly measure cell-mediated conductivity. However, in some cases, researchers have examined composites where cells are encapsulated only using non-conductive materials. Inspired by cable bacteria, Hsu et al. utilized calcium-alginate hydrogels to form
quasi-1D assemblies of *Shewanella loihica* packed into 2-cm-long core/shell cables and studied their conduc-
tivity using a two-electrode configuration revealing con-
ductivities of $-10^{-3}$ S cm$^{-1}$ depending on the density of
cells packed into the core/shell cable (Hsu et al., 2018).
Further exploration at this interface of materials science
and electronmicrobiology will yield generalizable plat-
forms for analysing microbial conductivity and generate
interesting new components for bioelectronic devices.

Conductive multicellular networks have been demon-
strated to transport electrons over micron (biofilms) and
centimetre (cable bacteria/encapsulated cells) length
scales. Further characterizing their fundamental elec-
tronic properties could lead to the development of novel
living electronics that leverage the unique biological
ability to sense and respond to environmental stimuli,
self-assemble and self-repair. In the context of cable
bacteria specifically, further electronic, spectroscopic
and structural characterization of their periplasmic fibre
network may reveal novel mechanisms responsible for
their unprecedented long-distance biological conduc-
tion. Additionally, the further study of cable bacteria
periplasmic fibres could lead to the development of bio-
mimetic materials that are both flexible and share the
long-distance electron transport efficiency of the fibres.
Such materials could act as biocompatible wires in the
development of new bioelectronic devices.

Biogenic synthesis of solid-state wires by
electroactive microorganisms

In addition to producing conductive biomolecules or
forming multicellular conductive networks, electroac-
tive organisms can enable the mineralization of meta-
llic and semiconductor nanomaterials by reducing metal
and chalcogenide ions in solution. While this biominer-
alization phenomenon is often studied in the context of
bioremediation and formation of catalytic nanoparticles,
reviewed elsewhere (De Corte et al., 2012; Gadd, 2010;
Shedbalkar et al., 2014) here, we discuss the relevance
of biomineralization to the formation of electronic
components. Biomineralizing microbes can produce
inorganic materials that can act as wires or as more
advanced electronic components such as photoresis-
tors and transistors. Biogenic formation of inorganic
nanomaterials can: (1) enable the synthesis of tech-
nologically relevant materials at ambient temperature,
pressure and near neutral pH, without the resource-
intensive conditions required for conventional chemi-
cal or solid-state material fabrication (Estroff, 2008); (2)
allow use of synthetic biology tools to control material
morphology and chemical composition (Chellamuthu,
Naughton, et al., 2019; Chellamuthu, Tran, et al., 2019)
and (3) allow the formation of cell-nanomaterial hybrids
that combine the properties of both microbes and solid-
state material (Ng et al., 2019).

Diverse strains of *Shewanellaceae*, such as HN-41,
ANA-3 and MR-1, have been used to generate micron-
long, nanoscale fibres consisting of metallic and semi-
conductor materials including selenium, goethite,
telluride, uranium and arsenic-sulfide (Ho et al., 2010,
2015; Jiang et al., 2009, 2011; Kim et al., 2012, 2013;
McFarlane et al., 2015; Tam et al., 2010). *Shewanella*
sp. HN-41 can reduce Se(IV) to form extracellular Se
nanoparticles when selenite is provided as the sole
electron acceptor (Tam et al., 2010). When mercury
chloride is present alongside Se(IV), HN-41 forms
Hg-Se nanoparticles (Ho et al., 2015). Se and Hg-Se
nanowires are formed when the cells and either type
of Se nanoparticles are washed and resuspended in
various solvents including acetone and DMSO (Ho
et al., 2010, 2015). HN-41 also reduces the Fe(III)-
-oxyhydroxide akaganeite to form goethite nanowires
that were evaluated as anodes materials for Li-ion
batteries (Jiang et al., 2013). *S. oneidensis* MR-1 was
demonstrated to reduce Te(IV) to Te(0) resulting in the
formation of intracellular Te nanorods, when sodium tel-
lurite was provided as the sole electron acceptor (Kim
et al., 2012). However, in the presence of Fe(III), Te(0)
nanorods were formed extracellularly (Kim et al., 2013).
Such bacterially derived elemental tellurium nanostruc-
tures have been explored for their unique photonic
properties (Wang, Zhang, et al., 2019). Similarly, when
uranyl acetate is supplied as the sole electron acceptor,
MR-1 formed U(VI) nanowires (Jiang et al., 2011).

HN-41, ANA-3 and MR-1 can form arsenic sul-
fide nanofibres by simultaneously reducing thiosul-
fate to sulfide and As(V) to As(III) (Jiang et al., 2009;
McFarlane et al., 2015). As-S nanofibres harvested
from HN-41 cultures demonstrated metallic-like con-
duction, with conductance decreasing with increasing
temperature (Lee et al., 2007). While HN-41, ANA-3
and MR-1 have been shown to be electroactive, only the
electrochemistry of MR-1 has been characterized in detail (Bretschger et al., 2010; Wu et al., 2013; Xu
et al., 2016, 2018). To improve methods for the bio-
genic generation of these materials and for creating
microbe-nanomaterial electroactive biomaterials the
electrochemical activity of additional strains should
be studied further. The electrochemical and biominer-
alterial capabilities of electroactive microbes are
often examined separately. However, a recent review
by Ostermeyer et al. discusses the potential for using
microbial fuel cell (MFC) and microbial electrolysis
cell (MEC) technology with biomineralizing micro-
organisms for the industrial-scale bioremediation of
metallurgic plant wastewater, with the additional po-
tential of generating some energy from this process
(Ostermeyer et al., 2022). In addition to wastewater
treatment, MFC and MEC technology combined with
microbial biomineralization should be used for the re-
clamation of precious metal and semiconductor materi-
als (Capeness & Horsfall, 2020).
Capacitors

In addition to charge-carrying wires, capacitors are another important electrical component (Figure 2). Capacitors are circuit elements that store electrical energy as accumulated charge. Redox molecules, including biomolecules like those outlined above, can function as pseudocapacitive components of supercapacitors. Monolayers of redox-active proteins including cytochrome c (González-Arribas et al., 2018) and rusticyanin (González-Arribas et al., 2018) adsorbed onto the surface of electrodes is a facile way to increase the areal capacitance to ~10^{-4} F cm^{-2}. Redox-active molecules can also be stored within supramolecular assemblies (Cao et al., 2017; Liba et al., 2013) and multicellular networks (Bonanni et al., 2012; Esteve-Núñez et al., 2008; Malvankar et al., 2012; Schrott et al., 2011; Uriá et al., 2011) resulting in increased capacitance. Supramolecular hydrogels of the aminopolysaccharide chitosan have been engineered to have capacitive moieties including catechol (Liba et al., 2013) and Li^{+}/Ag^{+} (Cao et al., 2017) reaching areal capacitances of 10^{-4} F cm^{-2}.

Similar to the redox moieties in supramolecular assemblies, the cytochromes associated with G. sulfurreducens (Bonanni et al., 2012; Malvankar et al., 2012; Schrott et al., 2011) and S. oneidensis (Uriá et al., 2011) have been shown to store charge when cells are starved of terminal electron acceptors. Individual G. sulfurreducens cells were found to be capable of storing ~10^{7} electrons cell^{-1} when cytochrome discharge of cells starved of terminal electron acceptor was measured.
using both fluorescence (Esteve-Núñez et al., 2008) and electrochemical (Bonanni et al., 2012; Malvankar et al., 2012) approaches. Using similar electrochemical charge–discharge cycles, S. oneidensis biofilms have been found to store up to ~10^{14} electrons cm^{-2} (Uria et al., 2011) and individual cells are estimated of storing ~10^5 electrons cell^{-1} within the haems of c-type cytochromes (Sturm et al., 2015). Leveraging this capacitive property of electroactive cells, Malvankar et al. constructed supercapacitors using multicellular biofilms of G. sulfurreducens that spanned a 50-μm gap between gold electrodes (Malvankar et al., 2012). Electrochemical impedance spectroscopy and equivalent capacitive circuit modelling revealed biofilm areal capacitance of 10^{-4} F cm^{-2} and a specific capacitance of 10^5 F g^{-1}. C-type cytochromes served as pseudocapacitive components within this biofilm-based supercapacitor, increasing the capacitance beyond the double-layer capacitance of the medium, and yielded charge–discharge cycles consistent with pseudocapacitive behaviour which could be eliminated when treated with a cytochrome inhibitor (Malvankar et al., 2012).

Living capacitors hold great promise as charge-storage devices that could be used as power sources for electrical devices. They can be easily coupled to enzymatic-power generation systems, making them self-powered energy storage devices for deployment in resource-limited environments including the human body and environmental settings (González-Arribas et al., 2018; Malvankar et al., 2012). Many of the previously mentioned wires function using a redox-hopping mechanism that also makes them potential capacitors and should be explored for their capacitive properties. While living capacitors have limited energy density relative to traditional battery chemistry, there is potential for improving on these early designs. Their limited resource requirements and facile construction make living capacitors an attractive option for energy storage devices to help address the need for renewable energy storage.

**Transistors**

Transistors are switchable circuit elements that can be controlled to either allow (on-state) or block (off-state) current from flowing through them (Figure 2). The on- and off-states of solid-state transistors, of which there are up to several billions of in modern computational devices, act as the physical bits that enable complex digital logic processes. Solid-state transistors are constructed from doped semiconductors, whose electronic properties enable gating of current flow through the device (Neamen, 2011). Soft-matter transistors in the form of organic electrochemical transistors (OECTs) have also been constructed in solution with conductive polymers acting as the switchable current channel (Friedlein et al., 2018; Rivnay et al., 2018). Additionally, some of the above-discussed conductive solid-state and biological materials produced by electroactive microorganisms have also been used as switchable current channels in dry, transistor device setups (Bonné et al., 2020; Leung et al., 2013; McFarlane et al., 2015). While transistors can enable digital logic processes, they can also be used to characterize fundamental electronic properties of the channel material, such as identifying the majority charge carrier type and charge mobility.

Transistor measurements where biogenic conductive materials serve as the conducting channel enable the extraction of additional fundamental properties, including charge carrier mobility. Measurements of the aforementioned As-S nanofibres harvested from ANA-3 cultures revealed a mixed population of both n-type (electron majority charge carrier) and p-type (hole majority charge carrier) semiconducting fibres (McFarlane et al., 2015). Leung et al. showed that current through the S. oneidensis outer-membrane extensions could be gated using an electric field, with the conduction interpreted to be p-type with an estimated hole mobility of 0.1 cm^2 V^{-1} s^{-1} (Leung et al., 2013). More recently, fibre sheaths extracted from marine cable bacteria were used as channels in transistor measurements, illustrating that current through these structures could be gated. From these measurements, the conduction was interpreted to be n-type and an electron mobility was estimated to be on the order of 0.1 cm^2 V^{-1} s^{-1} (Bonné et al., 2020), a value similar to charge mobility in some organic semiconductors and the hole mobility in S. oneidensis outer-membrane extensions. While these examples are few, the use of biologically synthesized solid-state semiconductors and biological conducting materials as transistor channels demonstrates their potential application in the construction of more complex bioelectronic devices.

Looking forward, transistor measurements offer an additional avenue to further characterize electron transport in biogenic materials. In addition to revealing the majority charge carrier and charge mobility of materials, transistor measurements can be used to investigate the speed at which the current flowing through channels can be switched on and off, as well as how many on/off cycles the channel can support before degrading. Since transistors can be used as amplifiers, transistor measurements can also be used to investigate how well current channels made of biogenic materials amplify input current and voltage signals. Thus far, conductive supramolecular assemblies and biogenic semiconducting materials have been the focus of transistor-based characterization; however, multicellular networks, such as the patterned biofilms discussed earlier, could also be used as device channels. Since electroactive biofilms act as redox conductors, when a constant applied source-drain potential offset is applied, there are...
applied gate potentials that enable current flow through a biofilm spanning source and drain electrodes that can be switched on and off. The characterization of biofilms as transistor channels may prove useful as multicellular networks can self-assemble, are patternable and may be engineered to respond to environmental stimuli. Taking inspiration from how solid-state semiconductors can be doped to tune electronic properties, biofilms can also be engineered for tunable expression of electron transport proteins (West et al., 2017) or with metal or semiconductor nanomaterial binding sites to modulate their conductivity (Seker et al., 2017).

**Diodes**

Diodes are useful components in electrical circuits because of their ability to control the direction through which current can flow. Recently, separate EET pathways in the model electroactive organism *G. sulfurreducens* have been identified that activate depending on the potential of an external electron acceptor (Figure 2). Similar to their diversity of extracellular nanowires that serve different functions depending on the biofilm stage, *G. sulfurreducens* also uses a variety of inner-membrane quinol oxidases to input electrons into the EET pathway. With higher potential (> −0.1 V vs. SHE) electron acceptors the inner-membrane cytochrome ImH is triggered (Levar et al., 2014), while use of the inner-membrane cytochrome CbcL is triggered when the biofilms exchanges electrons with lower potential (< −0.1 V vs. SHE) (Zacharoff et al., 2016), and at very low potentials (< −0.2 V vs. SHE), near the energetic limit of respiration, *G. sulfurreducens* use the inner-membrane cytochrome CbcBA (Joshi et al., 2021). The existence of such potential-dependent EET pathways raises the intriguing possibility of building living electronic components (at the cellular or biofilm scale) with asymmetric conductance features, in a manner analogous to traditional electron rectifiers and diodes.

**Optoelectronic components**

Adding to the above list of circuit elements, biomineralized and biological materials have been used for the development of a diverse set of living optoelectronic components. This can include hybrid components that use inorganic semiconductors that are photore sponsive and alter their material properties in response to light such as photoresistors. Other living optoelectronic components such as biophotovoltaic cells use biological structures to harvest energy from photoexcited electrons. Additionally, components can be built that utilize cells engineered with optogenetic circuits, allowing photon absorption to trigger alterations in gene expression such as the production of redox active biomolecules that can interact with electronic devices.

The earlier described As-S nanofibres biomineralized by *Shewanella* sp. HN-41 display photovoltaic properties and were used for the construction of a photosensor after being harvested from cell cultures. Increased current was observed when As-S fibres were illuminated with UV light while a DC voltage was applied across the fibres (Lee et al., 2007). In addition to being capable of natively biomineralizing photovoltaic nanomaterials, microbes have also been engineered to act as scaffolds for the abiotic formation of photoactive semiconductor networks. Recently, Wang et al. engineered *E. coli* to produce curl fibres with transition metal binding sites (Wang, Zhang, et al., 2022). This then allowed photoactive CdS nanoparticles to spontaneously form along the fibres when Cd$^{2+}$ and S$^{2−}$ ions were introduced to the cell culture. When formed on the working electrode surface of a three-electrode bio reactor setup, the cell and curl fibre scaffold network of CdS was shown to produce a photocurrent upon illumination with blue light. This system was also leveraged for the development of an artificial photosynthesis scheme in which electrons generated by the photoexcitation of the CdS-cell network were used to drive enzymatic chemical production (Wang, Zhang, et al., 2022). One limitation of using such semiconducting nanoparticles is the need for sacrificial electron donors (often exogenously supplied cytostine or unspecified cellular metabolites) to fill holes generated during semiconduc tor photoexcitation.

While interfacing cell networks with photovoltaic nanomaterials can confer optoelectronic properties, biophotovoltaic systems have been developed that harness the intrinsic light-harvesting and electroactive properties of photosynthetic microbes to harvest energy (Figure 2) (Tschörtner et al., 2019). For example, films of *Synechocystis* sp. PCC6803 (*Synechocystis*), a model photosynthetic bacterium, deposited onto the working electrodes of three-electrode reactors present enhanced EET currents when they are illuminated with light (Cereda et al., 2014; Thirumurthy et al., 2020; Zhang et al., 2018). Control experiments using photosystem II (PSII) inhibitors confirmed that electrons from this light-enhanced EET are derived from water oxidation rather than coming from another electron donor (Cereda et al., 2014; Zhang et al., 2018). While the mechanisms for how electrons from PSII cross the cell membrane and reach the electrode are not currently known, redox-active molecules have been implicated as soluble electron shuttles in this process (Cereda et al., 2014; Kusama et al., 2022; Saper et al., 2018; Shlosberg et al., 2021; Thirumurthy et al., 2020; Zhang et al., 2018). In addition to pursuing a better mechanistic understanding of the EET pathways in photosynthetic microbes, the field of biophotovoltaics has focused on optimizing these systems (Wey et al., 2019). This work has included enhancing biofilm formation (Wey et al., 2021), creating porous...
electrodes with increased surface area (Wenzel et al., 2018; Zhang et al., 2018), disrupting the cell surface (Kusama et al., 2022; Saper et al., 2018; Wey et al., 2021), introducing additional redox-active molecules (Clifford et al., 2021) or EET proteins (Meng et al., 2021; Schuergers et al., 2017; Sekar et al., 2016) and creating microbial consortia where photosynthetic microbes use light to produce electron donor chemicals that other electroactive microbes can use to produce current (Zhu et al., 2019). Biophotovoltaic devices using *Synechocystis* have proven functional as continuous power sources for low-powered electronics such as a digital clock (McCormick et al., 2011) and a microprocessor (Bombelli et al., 2022).

Producing living optoelectronic components by leveraging the ability for electroactive microbes to produce solid-state semiconductor materials and for photosynthetic microbes to harvest light has great potential for enabling new types of living electronic circuits that use light as both a stimulus and a power source. One unexplored avenue for living optoelectronics is using optogenetic tools, commonly used by synthetic biologists, to control EET protein expression or activity in electroactive cells (Baumslag & Khammash, 2021). Another interesting unexplored research area is using living electronic circuits to generate nanoparticles that undergo radiative emission of light when electrically stimulated to function as optical displays. Using electroactive microbes to both generate such semiconducting nanoparticles as well as functioning as living electrodes for interfacing these particles with electronics could provide a more sustainable route for manufacturing such devices.

**Spin filters**

Spintronic devices have revolutionized magnetic recording and memory (Bhatti et al., 2017; Wolf et al., 2001). Components that leverage the impact that spin has on electron transmission are typically constructed using inorganic metals, oxides, and semiconductors, but recently have begun to employ organic molecules (Naaman & Waldeck, 2015). Chiral molecules have been shown to preferentially transmit electrons with one spin orientation over the other. This phenomenon has been dubbed chirality-induced spin selectivity (CISS) (Naaman & Waldeck, 2015).

CISS was first reported in 1999, when Naaman, Waldeck and colleagues (Ray et al., 1999) observed a correlation between the transmission of polarized photoelectrons and the chirality of L- or D-stearoyl lysine thin films. The effect involves the breakdown of spin degeneracy in chiral molecules, leading to a coupling of the electron spin with its linear momentum (Naaman & Waldeck, 2012). The favoured spin depends on the handedness of the chirality, where if one enantiomer favours a particular spin along an axial vector, its mirror image will favour the other along the same vector (Zwang et al., 2016). In all cases, the spin polarization depends on the energy of the incident electrons, and has been observed to vary nonlinearly in current–voltage curves (Xie et al., 2011). Interestingly, the spin polarization observed in organic molecules can be very large relative to inorganic materials (Naaman & Waldeck, 2015). The extent of spin filtering due to CISS in some supramolecular organic polymer-based nanofibres has been observed to be as high as 85% (Kulkarni et al., 2020). Devices constructed using organic molecules displaying CISS include magnetoresistive random access memory (MRAM) (Ben Dor et al., 2013), memristors (Al-Bustami et al., 2018) and a 9-bit optical memory device (Al-Bustami et al., 2020).

In addition to organic molecules, the CISS effect has been observed in many biological materials, including DNA (Göhler et al., 2011; Mishra et al., 2020), oligopeptides (Kirian et al., 2017; Mishra et al., 2020) and proteins (Carmeli et al., 2014; Mishra et al., 2019). In the case of DNA and linear oligopeptides, spin selectivity is observed to increase with increasing molecular length while total electron transmission decreases (Mishra et al., 2020). Recently, spin selectivity was demonstrated in monolayers of metalloproteins including photosystem I from *Synechocystis* (Carmeli et al., 2014), azurin from *P. aeruginosa* (Sang et al., 2021) and the multihæm cytochromes OmC and MtrF from *S. onii densis* (Mishra et al., 2019). While the role that CISS plays in the physiological function of these metalloproteins remains unknown, the electron transport properties of these biomolecules make them a promising new material for the development of spintronic components.

**Sensors**

One powerful use for integrating living cells with electronics is for applications in sensing. To thrive in diverse and changing environments, microbes are capable of sensing, integrating and dynamically responding to a variety of physical, chemical, and biological stimuli (Del Valle et al., 2020; Wan et al., 2021). Efforts in synthetic biology have enabled the construction of microbial sensors.
that produce diverse phenotypic outputs including the production of electrical currents (Atkinson et al., 2022; Dundas et al., 2020; Golitsch et al., 2013; Hu et al., 2015; Li et al., 2020; Ueki et al., 2016; Webster et al., 2014; West et al., 2017; Zhou et al., 2017), redox molecules (Li et al., 2011; Terrell et al., 2021; VanArsdale et al., 2019; VanArsdale, Hörnström, et al., 2020; VanArsdale, Pitzer, et al., 2020) and light (Mimée et al., 2018) that can be integrated into microelectronic devices as sensor modules. Sensor cells can be engineered by introducing stimuli-regulated transcriptional promoters that control the concentration of proteins that facilitate electron flow or the production of redox-active molecules (Figure 2) (Del Valle et al., 2020; Wan et al., 2021). Additionally, the activity of these proteins can be directly controlled by stimuli through insertion of stimuli-responsive domains to generate allosteric protein switches (Atkinson, Wu et al., 2019; Kanwar et al., 2013; Stein & Alexandrov, 2015). To date, living electronic sensors have been developed to respond to various chemical stimuli (Table 4).

Signal transduction using redox mediators

Biological systems natively produce and respond to molecules that undergo redox processes (Falkowski et al., 2008). These redox-active molecules undergo electron transfer reactions making them well-suited mediators for enabling communication between cells and electrodes (VanArsdale, Pitzer, et al., 2020). Many bioelectronic sensors implemented that utilize redox mediator-based signal transduction require the addition of exogenous substrates that become redox active following enzymatic activation such as 4-amino-naphthol 1,2-quinone-oxidase (PAPG) which is cleaved by β-galactosidase (LacZ) to form the redox-active molecule p-aminophenol (PAP) that can be detected electrochemically (Tschirhart et al., 2016; VanArsdale et al., 2019). Efforts have also been made to engineer cells that self-produce electroactive molecules such as phenazines (Feng et al., 2018; Li et al., 2011), riboflavin (Khan et al., 2020; Zhou et al., 2021) and L-3,4-dihydroxyphenylalanine (L-DOPA) (VanArsdale, Hörnström, et al., 2020) that can function as redox mediators for biosensing. As additional pathways for synthesizing and interacting with extracellular redox mediators are discovered (Coates et al., 2018; Glasser et al., 2017; Hernandez et al., 2004; Light et al., 2018; Mevers et al., 2019) it opens the potential for enabling multiple channels of cell-electrode communication in a single device.

Signal transduction using direct electron transfer

An alternative approach for enabling communication between cells and electrodes is to use direct electron transfer machinery from electroactive microbes to generate or receive an electrical signal. Bioelectronic sensors using direct electron transfer for signal transduction often engineer electroactive microbes to enable control over the production of critical components within the extracellular electron transfer pathway such as the synthesis of multihaem cytochromes or dehydrogenases. Because the EET pathways in S. oneidensis are well-understood (Bretschger et al., 2007; Coursolle & Granick, 2012), it has been engineered into bioelectronic sensors for a variety of stimuli by controlling the ability of electrons to reach extracellular electrodes. This has been implemented by several labs over the years by controlling the transcription of various EET genes including the inner-membrane quinol-oxidase cymA (Dundas et al., 2020), the periplasmic decahaem cytochrome mtrA (Dundas et al., 2020; Golitsch et al., 2013), the porin mtrB (Golitsch et al., 2013; Webster et al., 2014), the outer-membrane cytochromes mtrF (Golitsch et al., 2013) and mtrC (Dundas et al., 2020) as well as the endogenous operon encoding mtrCAB (West et al., 2017) or an engineered operon encoding mtrFAB (Golitsch et al., 2013). This strategy works well in organisms with well-understood EET pathways and limited redundancy in electron transfer pathways (Coursolle & Granick, 2012). In contrast, G. sulfurreducens utilizes a more complicated EET pathway with many built in redundancies that make controlling any single EET component challenging (Jiménez Otero et al., 2018). To overcome this challenge, Ueki et al. engineered control over how electrons enter the EET pathway by controlling flux through acetate metabolism by gating transcription of citrate synthase and acetyl-CoA transferase as well as controlling flux through lactate metabolism by gating transcription of D-lactate dehydrogenase (Ueki et al., 2016). This same strategy has also been successfully applied to build sensors for acetate and lactate controlling their metabolic fluxes in Arcobacter butzleri, an electroactive organism with unknown EET components (Szydlowski et al., 2020).

Enabling sensors with rapid response times

While using gene networks to build microbial sensors allows for the facile design of new sensors based on the plug-and-play nature of transcriptional promoters, one limitation is that they require minute-hour long response times to allow for transcription and translation of output proteins (Olson & Tabor, 2012). To overcome this limitation, proteins can be engineered into stimuli-regulated switches (Atkinson, Wu et al., 2019; Kanwar et al., 2013; Stein & Alexandrov, 2015). This approach has been used to build living electronic sensors where intracellular electron transfer is controlled by ferredoxins that have been engineered to function as chemically gated allosteric switches (Atkinson, Campbell,
**TABLE 4** Bioelectronic sensors modules. A description of bacterial sensors that utilized electrical or redox signals as outputs to interface with electronics.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Organism</th>
<th>Receptor</th>
<th>Regulated component</th>
<th>Output type</th>
<th>Measurement</th>
<th>Limit of detection</th>
<th>Linear range</th>
<th>Response time</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>arsenite</td>
<td><em>S. oneidensis</em></td>
<td>Promoter (AsrR – P&lt;sub&gt;ars&lt;/sub&gt;)</td>
<td>MtrB</td>
<td>DET</td>
<td>Chronoamperometry</td>
<td>40 μM</td>
<td>100 μM</td>
<td>1.5 d</td>
<td>Webster et al. (2014)</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td><em>E. coli</em></td>
<td>Promoter (ZnR – P&lt;sub&gt;zntA&lt;/sub&gt;)</td>
<td>riboflavin synthesis</td>
<td>MET</td>
<td>Microbial Fuel Cell voltage</td>
<td>20 μM</td>
<td>100 μM</td>
<td>ND</td>
<td>Khan et al. (2020)</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;</td>
<td><em>E. coli</em></td>
<td>Promoter (CusR – P&lt;sub&gt;cusC&lt;/sub&gt;)</td>
<td>riboflavin synthesis</td>
<td>MET</td>
<td>Microbial Fuel Cell voltage</td>
<td>28 μM</td>
<td>500 μM</td>
<td>ND</td>
<td>Zhou et al. (2021)</td>
</tr>
<tr>
<td>IPTG</td>
<td><em>S. oneidensis</em></td>
<td>Promoter (LacI- P&lt;sub&gt;tac&lt;/sub&gt;)</td>
<td>MtrA</td>
<td>DET</td>
<td>Microbial Fuel Cell voltage</td>
<td>10 μM</td>
<td>ND</td>
<td>ND</td>
<td>Hu et al. (2015)</td>
</tr>
<tr>
<td>IPTG</td>
<td><em>G. sulfurreducens</em></td>
<td>Promoter (LacI- P&lt;sub&gt;tac&lt;/sub&gt;)</td>
<td>GfIA</td>
<td>DET</td>
<td>Chronoamperometry</td>
<td>1 mM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>Ueki et al. (2016)</td>
</tr>
<tr>
<td>IPTG</td>
<td><em>E. coli</em></td>
<td>Promoter (LacI- P&lt;sub&gt;tac&lt;/sub&gt;)</td>
<td>phenazine-1-carboxylic acid synthesis</td>
<td>MET</td>
<td>Microbial Fuel Cell voltage</td>
<td>0.5 mM</td>
<td>ND</td>
<td>ND</td>
<td>Feng et al. (2018)</td>
</tr>
<tr>
<td>3-oxo-C6-HSL</td>
<td><em>S. oneidensis</em></td>
<td>Promoter (LuxR – P&lt;sub&gt;lux&lt;/sub&gt;)</td>
<td>MtrA</td>
<td>DET</td>
<td>Microbial Fuel Cell voltage</td>
<td>10 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>Hu et al. (2015)</td>
</tr>
<tr>
<td>3-oxo-C12-HSL</td>
<td><em>E. coli</em></td>
<td>Promoter (LasR – P&lt;sub&gt;luxI&lt;/sub&gt;)</td>
<td>L-DOPA synthesis</td>
<td>MET</td>
<td>Cyclic voltammetry</td>
<td>ND</td>
<td>10 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>VanArsdale, Hörnström, et al. (2020), VanArsdale, Pitzer, et al. (2020)</td>
</tr>
<tr>
<td>Autoinducer-2</td>
<td><em>E. coli</em></td>
<td>Promoter (LasR – P&lt;sub&gt;luxI&lt;/sub&gt;)</td>
<td>p-aminophenol synthesis</td>
<td>MET</td>
<td>Cyclic voltammetry</td>
<td>5 μM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40 μM</td>
<td>2 h</td>
<td>Tschirhart et al. (2016)</td>
</tr>
<tr>
<td>Pyocyanin</td>
<td><em>E. coli</em></td>
<td>Promoter (SoxR – P&lt;sub&gt;soxS&lt;/sub&gt;)</td>
<td>L-DOPA synthesis</td>
<td>MET</td>
<td>Cyclic voltammetry</td>
<td>ND</td>
<td>0.5 μM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>VanArsdale, Hörnström, et al. (2020), VanArsdale, Pitzer, et al. (2020)</td>
</tr>
<tr>
<td>Paraquat</td>
<td><em>E. coli</em></td>
<td>Promoter (SoxR - P&lt;sub&gt;soxS&lt;/sub&gt;)</td>
<td>L-DOPA synthesis</td>
<td>MET</td>
<td>Cyclic voltammetry</td>
<td>ND</td>
<td>1 μM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>VanArsdale, Hörnström, et al. (2020), VanArsdale, Pitzer, et al. (2020)</td>
</tr>
<tr>
<td>Dicamba</td>
<td><em>E. coli</em></td>
<td>Promoter (SoxR - P&lt;sub&gt;soxS&lt;/sub&gt;)</td>
<td>p-aminophenol synthesis</td>
<td>MET</td>
<td>Cyclic voltammetry</td>
<td>0.9 mM</td>
<td>4.5 mM</td>
<td>0.5 h&lt;sup&gt;b&lt;/sup&gt;</td>
<td>VanArsdale et al. (2019)</td>
</tr>
<tr>
<td>Diquat</td>
<td><em>E. coli</em></td>
<td>Promoter (SoxR - P&lt;sub&gt;soxS&lt;/sub&gt;)</td>
<td>p-aminophenol synthesis</td>
<td>MET</td>
<td>Cyclic voltammetry</td>
<td>0.1 μM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 μM</td>
<td>0.5 h&lt;sup&gt;b&lt;/sup&gt;</td>
<td>VanArsdale et al. (2019)</td>
</tr>
<tr>
<td>4-hydroxytamoxifen</td>
<td><em>E. coli</em></td>
<td>Protein Switch (sFd-ER-55)</td>
<td>Relative electron flux</td>
<td>DET</td>
<td>Chronoamperometry</td>
<td>12.5 μM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>5–10 min</td>
<td>Atkinson et al. (2022)</td>
</tr>
<tr>
<td>Thiosulfate</td>
<td><em>E. coli</em></td>
<td>Thiosulfate metabolism</td>
<td>Relative electron flux</td>
<td>DET</td>
<td>Chronoamperometry</td>
<td>0.28 mM</td>
<td>20 mM</td>
<td>3–10 min</td>
<td>Atkinson et al. (2022)</td>
</tr>
<tr>
<td>L-araabinose</td>
<td><em>S. oneidensis</em></td>
<td>Promoter (AraC- P&lt;sub&gt;BAD&lt;/sub&gt;)</td>
<td>MtrFAB</td>
<td>DET</td>
<td>Chronopotentiometry</td>
<td>0.2 μM</td>
<td>1 mM</td>
<td>2 h</td>
<td>Golitsch et al. (2013)</td>
</tr>
<tr>
<td>Lactate</td>
<td><em>S. oneidensis</em></td>
<td>Lactate metabolism</td>
<td>Relative electron flux</td>
<td>DET</td>
<td>Chronoamperometry</td>
<td>40 μM</td>
<td>ND</td>
<td>ND</td>
<td>Zhou et al. (2017)</td>
</tr>
<tr>
<td>Lactate</td>
<td><em>A. butzleri</em></td>
<td>Lactate metabolism</td>
<td>Relative electron flux</td>
<td>DET</td>
<td>Microbial Fuel Cell voltage</td>
<td>450 μM</td>
<td>ND</td>
<td>ND</td>
<td>Szylowska et al. (2020)</td>
</tr>
<tr>
<td>Acetate</td>
<td><em>A. butzleri</em></td>
<td>Acetate metabolism</td>
<td>Relative electron flux</td>
<td>DET</td>
<td>Microbial Fuel Cell voltage</td>
<td>600 μM</td>
<td>ND</td>
<td>ND</td>
<td>Szylowska et al. (2020)</td>
</tr>
<tr>
<td>Trimethylamine N-oxide</td>
<td><em>S. oneidensis</em></td>
<td>Promoter (TorR-P&lt;sub&gt;tor&lt;/sub&gt;)</td>
<td>MtrCAB</td>
<td>DET</td>
<td>Chronoamperometry</td>
<td>5 mM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>West et al. (2017)</td>
</tr>
<tr>
<td>Fumarate</td>
<td><em>S. oneidensis</em></td>
<td>Fumarate metabolism</td>
<td>Relative electron flux</td>
<td>DET</td>
<td>Chronoamperometry</td>
<td>1 mM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.8 h</td>
<td>Zhou et al. (2017)</td>
</tr>
</tbody>
</table>

Abbreviations: DET, Direct electron transfer; HSL, homoserine lactone; IPTG, isopropyl β-D-1-thiogalactopyranoside; MET, Mediated electron transfer; ND, Not determined.

<sup>a</sup>LOD not formally determined, minimum tested yielding response.

<sup>b</sup>Minimum time tested.
et al., 2019; Wu, Atkinson, et al., 2020). These switches allowed for metabolic pathways to be rapidly turned on only in the presence of small-molecule signals. Coupling the metabolic pathway controlled by these ferredoxin electron transfer switches to an extracellular electron transport pathway enabled engineered cells to report on chemical concentrations in the cellular environment to electrodes with mass transport limited kinetics (Atkinson et al., 2022). A recent study probing the insertional tolerance of the multihaem cytochrome MtrA suggests that it may also be possible to build such allosteric switches from native EET proteins (Campbell et al., 2022).

Contrasting signal transduction mechanisms

Each of these signal transduction mechanisms have unique advantages and disadvantages. One advantage of mediator-based signal transduction is that the sensor output does not require metabolically derived electrons to generate a detectable signal. Rather, the quantity of the accumulated molecule is assessed using voltammetry techniques (current vs. electrode potential) which are sensitive to very low concentrations of redox-active molecules. The use of voltammetry however limits the frequency of measurements and the response time of the overall device. In contrast, signal transduction using direct electron transfer can be monitored using chronoamperometry (current vs. time) but requires metabolic flux that is sufficiently high to generate detectable currents. Chronoamperometry allows for real-time recording of signalling outputs allowing for rapid analyte detection. Building out miniature platforms that are compatible with both signal modalities will enable the use of living electronic sensors to monitor chemicals that can be deployed into the environment and human body.

Logic processor

In addition to functioning as sensors and logic-processing units, the ability to interface living cells with electrical devices opens the door to controlling the behaviours of cells using electrical stimuli, through what is called ‘electrogenetics’ (Mansouri & Fussenegger, 2022; Weber et al., 2009). This was first illustrated in 2009 by Fussenegger and colleagues when they showed it was possible to control gene expression in mammalian cells by modulating an electrical signal (Weber et al., 2009). In their system, the electrochemical oxidation of ethanol to acetaldehyde was used to induce gene expression from an acetaldehyde-responsive transcriptional promoter. By controlling the expression of the human placental secreted alkaline phosphatase (SEAP) and using a photodiode to monitor the production of a colorimetric indicator of phosphatase activity they showed that cells functioned as electronic current integrators that report on the total charge driven into the system using either direct or alternating currents. Additionally, cells could be engineered into frequency generators by introducing this electrogenetic system in cardiomyocytes to control expression of human bone morphogenetic protein 2 (BMP-2) which tunes the frequency of cellular contractions (Weber et al., 2009).

In Tschirhart et al., 2017, Tschirhart, Bentley and colleagues extended electrogenetics to microbes using redox signalling to trigger gene expression in E. coli (Tschirhart et al., 2017). This system is based on electrochemical control of the redox state of ferro/ferri-cyanide which in turn controls the oxidation state of pyocyanin, a redox-active molecule that when oxidized induces gene expression from an oxidative-stress promoter, $P_{\text{soxS}}$ (Tschirhart et al., 2017). Similar to the mammalian cell example, this system enabled E. coli cells to function as electronic current integrators yielding gene expression that was dependent on the total charge extracted from the system by oxidation of electronic signals could enable biohybrid computing architectures (Sarkar, Bonnerjee, et al., 2021; Sarkar, Chakraborty, et al., 2021; Sexton & Tabor, 2020; Shin et al., 2020; Wong et al., 2015). Some work has been done implementing logic gate operations to control electronic signals. This allows cells to digitally respond to multiple stimuli for control of electronic signal production. To date, AND (Hu et al., 2015; Ueki et al., 2016), Buffer (Dundas et al., 2020) and NOT (Dundas et al., 2020) gates have been implemented to control electronic signals from living cells. There is great promise for further engineering of bacteria in living electronics to perform these more complex layered DNA-based logic processing operations outlined above that can be directly integrated into electrical circuits.

Bioactuator

In addition to using cells as sensors and logic-processing units, the ability to interface living cells with electrical devices opens the door to controlling the behaviours of cells using electrical stimuli, through what is called ‘electrogenetics’ (Mansouri & Fussenegger, 2022; Weber et al., 2009). This was first illustrated in 2009 by Fussenegger and colleagues when they showed it was possible to control gene expression in mammalian cells by modulating an electrical signal (Weber et al., 2009). In their system, the electrochemical oxidation of ethanol to acetaldehyde was used to induce gene expression from an acetaldehyde-responsive transcriptional promoter. By controlling the expression of the human placental secreted alkaline phosphatase (SEAP) and using a photodiode to monitor the production of a colorimetric indicator of phosphatase activity they showed that cells functioned as electronic current integrators that report on the total charge driven into the system using either direct or alternating currents. Additionally, cells could be engineered into frequency generators by introducing this electrogenetic system in cardiomyocytes to control expression of human bone morphogenetic protein 2 (BMP-2) which tunes the frequency of cellular contractions (Weber et al., 2009).

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ferrocyanide at the electrode. This system was used for electronic control of fluorescent protein expression, cell motility and cell-to-cell communication (Tschirhart et al., 2017). Subsequently, electrode-controlled transcription was extended to genes not directly under the control of $P_{\text{soxS}}$ by using this system to drive expression of guide RNAs used for targeting CRISPR-based activation (dCas9-uu) or inhibition (dCas9), which enabled genome-scale electronic control over gene expression (Bokhisham et al., 2020). The $P_{\text{soxS}}$ promoter has been recently engineered to have a higher dynamic range, drive transcription in a single direction, and was tested with a diversity of redox-active molecules (Lawrence et al., 2022).

The synthetic biology toolbox for microbial electrogenetics is rapidly expanding. Another oxidative stress promoter, $P_{\text{oxyR}}$, has also been leveraged for electrogenetics (Stephens et al., 2021; Terrell et al., 2021). Rather than relying on the introduction of an exogenous mediator to electrically connect cells to an electrode, instead systems using $P_{\text{oxyR}}$ rely on the electrochemical production of hydrogen peroxide at the surface of electrodes under aerobic conditions. Hydrogen peroxide activates gene expression from $P_{\text{oxyR}}$ but is quickly enzymatically consumed by the cells. This results in heterogeneous gene expression when cells are in planktonic conditions, but results in homogenous expression in the electrode-localized cells (Terrell et al., 2021). To extend these electrical signals to the planktonic cells, $P_{\text{oxyR}}$ was used to control the production of acyl-homoserine lactone enabling cell-to-cell chemical communication generating a biological local area network (BioLAN) (Terrell et al., 2021). BioLAN was used to control the activity of two different strains: a ‘verifier’ strain that produces electrical signals to verify the input and an ‘actuator’ strain that excretes a fluorescent protein and granulocyte macrophage colony-stimulating factor, a therapeutic protein (Terrell et al., 2021). In a similar illustration, planktonic ‘relay’ cells used $P_{\text{oxyR}}$ to respond to electronic signals and control the growth rate of ‘controller’ cells changing the relative composition of the community (Stephens et al., 2021). This illustrated that electrogenetics coupled to cell-to-cell signalling can enable the electronic control of a population of diverse strains. Additionally, there are active efforts to enable electrogenetics in natively electroactive bacteria to also enable closed circuit actuation-verification (Hirose et al., 2019).

An alternative to using redox-mediated communication between electrodes and cells to control biological systems, ionic signals that are endogenously produced in some bacterial biofilms can be used to control microbial behaviour (Prindle et al., 2015). Similar to how neurons electrically communicate, some bacteria have evolved to respond to stimuli by using ion channels to control the local concentration of ions in their environment. These ions can serve as electrical signals that propagate through the biofilm community (Larkin et al., 2018; Prindle et al., 2015) and to recruit other diverse microbes to form multispecies biofilms (Humphries et al., 2017). Electrodes have been used to exogenously control the concentration of such ionic signals which resulted in control over membrane potential and the transcriptomic differentiation of motile and biofilm cells near the electrode (Comerci et al., 2022). It remains to be seen how portable such ionic signalling systems are in other microbial hosts that do not natively respond to such ionic signals. Regardless, ionic signalling is an exciting avenue to further explore for using electrical stimuli to control biological behaviour.

In addition to controlling gene expression, the metabolic activity of cells can be controlled using external electrodes. While not a major focus of this review as they have been extensively reviewed elsewhere (Rabaey & Rozendal, 2010; Schievano et al., 2016), electrosynthesis and electrofermentation have been a major focus of efforts in the electromicrobiology field and are both quintessential examples of bioactuation by using electrodes to tune the metabolic profile of both native and engineered cells. In the case of electro synthesis, cells are grown in the presence of cathodes that provide electrons for the reduction of $CO_2$ into more reduced (e.g. formate, methane) and longer-chain organic molecules (e.g. acetate, butyrate). In the case of electrofermentation, cells are grown in the presence of an anode to facilitate the recycling of intracellular reduced cofactors resulting in shifts in the fermentative products generated and allowing for unbalanced fermentation pathways (Askitosari et al., 2019; Bursac et al., 2017; Förster et al., 2017; Tejedor-Sanz et al., 2022; TerAvest et al., 2014). Recently, there has also been an interest in both electro synthesis and electrofermentation to control microbial-based food production (Tejedor-Sanz et al., 2022; Wise et al., 2022).

The extensive use of microbes in both chemical and food production makes this particular type of electrical bioactuation particularly exciting.

The ability to use electrical stimuli to control the genetic and metabolic states of microbial cells holds great potential as a tool for synthetic biology. The development of generalizable strategies for controlling the behaviour of cells using electrical stimuli will open avenues to new biohybrid devices. Electrical bioactuation systems in microbes utilize promoters that are natively used to respond to stress signals including oxidative stress and starvation and incidentally control the global transcriptome of the cell. Strategies for using electrical stimuli-responsive promoters that are orthogonal to cell stress response systems and do not consequently alter the global transcriptome of the cell would be useful to develop.

### Information storage

DNA is the primary information storage medium of living organisms and is an attractive energy efficient and
high-density data medium for long-term archival data storage applications. One challenge with using DNA storage is the requirement for in vitro synthesis (column- or array-based) and sequencing of DNA (Sanger-, short-read-, long-read or nanopore-based). While the price of DNA synthesis and sequencing has dropped precipitously over the past two decades, the price of DNA storage remains 7–8 orders of magnitude higher than conventional tape-based archival storage (Ceze et al., 2019). One approach to solving this cost challenge would be for living cells to synthesize DNA. Digital-to-biological data storage has been implemented in living bacterial populations using an engineered redox-sensitive CRISPR system (Yim et al., 2021). This technology enables digital data to be directly encoded into the genomes of living cells by using electrical signals to change concentrations of redox molecules (ferricyanide and phenazine methosulfate) that control the activity of Cas1-Cas2 spacer acquisition machinery. While this is a powerful illustration of using genomic DNA as an electronically writable data storage system, it ultimately requires extraction and in vitro sequencing of the DNA. Developing systems for cells to electronically report on the state of information stored in DNA could close this loop leading to living data storage and retrieval systems.

In addition to DNA, other biomolecules have been used to develop short-term rewritable memory systems. Charge stored in monolayers of the redox-active molecules such as ferredoxin (Yagati et al., 2009b) and azurin (Yagati et al., 2009a) has been used as rewritable memory bits. In such devices, bits are written by applying either oxidative or reductive potentials to shift the redox state of the electrode-attached redox-active molecules electrode between oxidized and reduced state, while open circuit potential measurements are used to read the state of the device. Similarly, the previously described chitosan-catechol capacitors have been used as memory devices for temporal integration of the production of mediators by bacteria and enzymes by storing charge in the catechol moieties (Wu et al., 2019; Wu, Kim, et al., 2020). Neuromorphic memristors have also been constructed using protein nanowires derived from G. sulfurreducens (Fu et al., 2020). In these devices, the nanowires catalyse the voltage-dependent reduction of Ag nanoparticles that form a conductive metal channel between electrodes resulting in changes in conductance. These changes in conductance function to temporally integrate charge flux from electrode pulses are experienced by the device (Fu et al., 2020).

In addition to biomolecules, cells can also function as memory-storing bits. The aforementioned biofilms that produce ionic electrical signals can store a history of their ionic signalling in their membrane potential (Yang et al., 2020). In this study, light is used to activate ionic signalling in discrete regions of a biofilm resulting in those cells becoming hyperpolarized (i.e. generating a high ionic potential across their cell membrane). This shift to a hyperpolarized state results in a phase shift relative to the remaining biofilm as they exhibit oscillatory ionic signalling behaviour (Prindl et al., 2015) and this anti-phase behaviour is maintained over several hours as a form of electrical memory. Since electrodes have been used to interact with biofilm ionic signalling (Comerci et al., 2022; Stratford et al., 2019), it is possible to envision exploiting this concept of electrical memory in a living electronic circuit.

Assembly of living electronic circuits

To leverage the diversity of living electronic components to build living electronic circuits, there is a need for methods to control the spatial arrangement of these components. A variety of strategies have been utilized to localize both biomolecules and cells onto electrode surfaces and non-conductive surfaces including chemical-conjugation, light-patterned adhesion and electrodeposition. Development of generalizable approaches that can be applied to the diversity of living electronic components that span a range of length scales will enable the arrangement of these components into living electronic circuits that can integrate the multitude of electrical properties outlined in the previous sections.

There have been extensive efforts for the immobilization of biomolecules onto electrode surfaces (Yates et al., 2018). Biomolecular components are often immobilized by making surface modifications to the electrode to introduce molecular handles that enable chemical-coupling of the biomolecule with the electrode (Yates et al., 2018). Proteins can be covalently bound to gold electrodes by selective introduction of the thiol-containing amino acid cysteine, which enables Au-S bond formation (Algov & Alfonta, 2022). Diverse electrode materials can be bound by using approaches from protein engineering and chemical biology to site-specifically introduce non-canonical amino acids that include chemical-handles specific for the material of interest (Algov & Alfonta, 2022). Similarly, polyhistidine tags have also been used to bind diverse materials by using functionalized Ni2+-NTA as adapter molecules. For example, Ni2+-NTA covalently linked to pyrene can be used to attach redox-active molecules to carbon-based electrodes (Sakamoto et al., 2019). In addition to chemical-conjugation approaches, DNA hybridization has been used to spatially localize and arrange multiple redox-active proteins on an electrode surface (Tepper, 2010). Recently, three-dimensional DNA nanostructures were utilized to selectively orient horse-heart cytochrome C on an electrode surface to facilitate electron transfer (Ge et al., 2019). These diverse approaches allow for the selective binding of redox-active molecules to electrode surfaces.

There have been a great deal of efforts to localize and enhance the formation of electroactive biofilms, which...
have been reviewed elsewhere (Catania et al., 2021). Early efforts utilized chemically modified surfaces to selectively bind electroactive bacteria (Lienemann et al., 2018; Young et al., 2020). Synthetic biology has enabled genetic control over biofilm formation such as engineering the expression of adhesive cell surface proteins that enable the binding of specific materials. To enhance electrode colonization on specific metal surfaces, cells have been engineered to display extracellular matrix proteins fused to metal-binding peptides (Suo et al., 2020). Additionally, cells have been engineered to display DNA sequences that could hybridize with complementary sequences that have been selectively deposited onto surfaces (Furst et al., 2018).

The expression or activity of adhesins has been placed under optogenetic control enabling biofilm lithography using light to pattern cells onto surfaces (Jin & Riedel-Kruse, 2018; Moser et al., 2019; Zhao et al., 2022). Recently, this approach was used to tune the conductance of electroactive biofilms by controlling biofilm dimensions using light (Zhao et al., 2022). While not used to pattern cells, optogenetic control over cyclic-di-GMP synthesis in S. oneidensis leads to increased electrode colonization and current production (Hu et al., 2017). Similar optogenetic approaches for controlling cyclic-di-GMP synthesis have been used to spatially pattern P. aeruginosa, another electroactive organism, indicating this could be an alternative strategy for light-patterning of conductive biofilms (Huang et al., 2018). Taking advantage of biofilm lithography and combining it with controllable cytochrome expression can lead to the living electronics equivalent of solid-state photolithography and dopant implantation for modulating material conductivity, techniques ubiquitous in semiconductor fabrication.

In addition to biofilm lithography, a range of electrobiofabrication approaches can be used to spatially pattern electroactive biofilms (Li et al., 2019). Dielectrophoretic deposition (DEP) has been used to spatially sort electroactive bacteria based on their cytochrome content which is related to the cell surface polarizability used to generate the electrokinetic forces that enable the deposition (Wang, Jones, et al., 2019). DEP also shows great promise for guiding the spatial arrangement of cells onto surfaces (Albrecht et al., 2006). Electrodeposition of multiple strains of electroactive bacteria in a single device can be achieved by spatial patterning of conductive polymers as individually addressable electrodes and selectively applying oxidative potentials to individual electrodes (Tseng et al., 2022). DEP and electrodeposition are two diverse approaches to control surface deposition of electroactive cells by using electric fields or electrode potential respectively. Such electrodeposition approaches could complement chemical-patterning and lithography.

Harnessing these biofilm patterning techniques ahead of biomineralization could be used for the controlled surface deposition of biogenic nanomaterials for various potential electronic device applications. Additionally, these techniques can be used to create hybrid biomaterials that can combine the unique properties of cells, such as sensing and self-assembly, and those of synthesized nanomaterials, such as photoactivity. Using biomineralization-capable electroactive microbes as the base for such biomaterials allows the biofilm to be conductive without relying on the nanomaterials and it allows for materials to be natively synthesized, simplifying the engineered system. Thus, by combining the biomineralization capabilities of electroactive microbes, as well as with synthetic biology biofilm engineering strategies, novel biohybrid living electronic circuits can be developed.

**CONCLUSION**

Recent works in the fields of electromicrobiology, synthetic biology and bioelectronics have yielded a wealth of information about the electrical properties of cells and biomolecular components. It is an exciting time where rapid advances in knowledge and engineering of such components are enabling the integration of these biological entities with electronic devices to generate living electronics. It is encouraging to see the recent trend for reporting on the intrinsic parameters (e.g. conductivity and charge carrier mobility) of conductive biomolecules and multicular networks. As knowledge in these areas continues to expand, it is important for the community to continue, whenever possible, to communicate these intrinsic properties so that they can be directly compared and evaluated across studies. Taking advantage of the natural and engineered sensing capabilities of these electroactive biomolecules and microbes has led to the development of new biosensors directly capable of providing an electronic readout. The development of bioactuator components for electrically controlling the behaviour of biomolecules and microbes is allowing for closed-loop biohybrid devices that electrically communicate enabling them to sense, compute and actuate in response. Additionally, the recent trends for controlling the assembly of biomolecules and cells hold great promise for integrating multiple disparate living electronic components into more complex electrical circuits with predictable and tunable behaviours. By combining these assemblies with biofabricated metallic and semiconductor nanomaterials, living electronic systems with both solid-state and biological functionalities can be developed. The bodies of work discussed herein show the different avenues in which living electronic components can be constructed and characterized, highlighting the diverse specialities and points of view required to push the overall field forward. However, this then creates new space for these specialities and their resulting disparate developments to converge in...
overcoming challenges and creating new technologies thought impossible when attempted separately. By combining the different facets of electromicrobiology, synthetic biology and bioelectronics, perhaps novel living electronics with improved biocompatibility, lower environmental footprints and hybrid biological and solid-state capabilities can be constructed.

**AUTHOR CONTRIBUTIONS**

Joshua T Atkinson: conceptualization (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). Marko S Chavez: conceptualization (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). Christina M. Niman: conceptualization (supporting); writing – original draft (supporting); writing – review and editing (equal). Moh El-Naggar: conceptualization (supporting); writing – review and editing (equal).

**ACKNOWLEDGMENTS**

Writing of this review was supported by the US Office of Naval Research Multidisciplinary University Research Initiative Grant No. N00014-18-1-2632. J.T.A. was supported by the NSF Postdoctoral Research Fellowships in Biology Program under Grant No. 2010604.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

**FUNDING INFORMATION**

Writing of this review was supported by the US Office of Naval Research Multidisciplinary University Research Initiative Grant No. N00014-18-1-2632. J.T.A. was supported by the NSF Postdoctoral Research Fellowships in Biology Program under Grant No. 2010604.

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