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**Article** *in* Nature Reviews Microbiology · August 2016

DOI: 10.1038/nrmicro.2016.93



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# Extracellular electron transfer mechanisms between microorganisms and minerals

*Liang Shi[1], Hailiang Dong[2,3], Gemma Reguera[4], Haluk Beyenal[5], Anhuai Lu[6], Juan Liu[7], Han-Qing Yu[8] and James K. Fredrickson[9]*

Abstract | Electrons can be transferred from microorganisms to multivalent metal ions that are associated with minerals and vice versa. As the microbial cell envelope is neither physically permeable to minerals nor electrically conductive, microorganisms have evolved strategies to exchange electrons with extracellular minerals. In this Review, we discuss the molecular mechanisms that underlie the ability of microorganisms to exchange electrons, such as *c*-type cytochromes and microbial nanowires, with extracellular minerals and with microorganisms of the same or different species. Microorganisms that have extracellular electron transfer capability can be used for biotechnological applications, including bioremediation, biomining and the production of biofuels and nanomaterials.

Autotrophic growth
A metabolic process that supports microbial growth and uses light or chemical energy to fix $CO_2$ for the synthesis of organic matter.

Respiratory terminal electron acceptors
A group of oxidizing agents, such as $O_2$, $NO_3^-$ and Fe(III), that receive the electrons that are released from the metabolic oxidation of organic and inorganic substrates by microorganisms.

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doi:10.1038/nrmicro.2016.93
Published online 30 Aug 2016

Redox-active minerals, such as those that contain iron (Fe(II) and/or Fe(III)) and manganese (Mn(III) or Mn(IV)), are abundant in soils and in aquatic and subsurface sediments, in which they electrically support microbial growth in at least four different ways: as electron sinks for heterotrophy-based respiration, as energy sources for autotrophic growth, by enabling cell-to-cell transfer of electrons, and as electron storage materials (FIG. 1). In the absence of molecular oxygen ($O_2$) and other respiratory terminal electron acceptors, dissimilatory metal-reducing microorganisms, such as *Geobacter metallireducens* GS-15 (REFS 1,2) and *Shewanella oneidensis* MR-1 (REFS 3,4), oxidize organic matter or hydrogen ($H_2$) and then transfer the released electrons to minerals that contain Fe(III) or Mn(III) or Mn(IV) for respiration (FIG. 1a). By contrast, metal-oxidizing microorganisms, including *Rhodopseudomonas palustris* TIE-1 and *Sideroxydans lithotrophicus* ES-1, use structural and soluble metal ions as electron and/or energy sources to reduce $O_2$, carbon dioxide ($CO_2$) and nitrate ($NO_3^-$) for growth[5–8] (FIG. 1b). Furthermore, semiconductive minerals, including haematite ($\alpha$-$Fe_2O_3$) and magnetite (Fe(II) $Fe(III)_2O_4$), can function as conductors to transfer electrons between different microbial species[9] (FIG. 1c). For example, haematite and magnetite facilitate the transfer of electrons that are released from the oxidation of acetate by *Geobacter sulfurreducens* PCA to *Thiobacillus denitrificans*, which uses the received electrons to reduce $NO_3^-$ to nitrite ($NO_2^-$). Finally, minerals such as magnetite and clay minerals that contain Fe(II) and Fe(III), act as electron-storage materials, or 'batteries', that receive electrons from electron-releasing microorganisms (for example, *G. sulfurreducens* PCA and *S. oneidensis* MR-1) when no other terminal electron acceptors are available and then donate the received electrons to other microorganisms (for example, *R. palustris* TIE-1 and *Pseudogulbenkiania* sp. strain 2002) when conditions change[10,11] (FIG. 1d). Therefore, electrical interplay between microorganisms and minerals links the redox transformation of metal ions in minerals to the oxidation of organic carbon compounds and fixation of $CO_2$ to organic compounds through photosynthesis[5] and $NO_3^-$ reduction[7].

The cell envelopes of microorganisms have a cytoplasmic membrane that is the primary barrier to the external environment and the centre of electron transfer, which is essential for microbial energy generation. However, the microbial cell envelope often includes other external structural components, such as peptidoglycan, the outer membrane and the S-layer, which are electrically nonconductive and physically impermeable to minerals[12,13]. The transfer of electrons between redox carriers in the cytoplasmic membrane and extracellular minerals is often referred to as microbial extracellular electron transfer. To overcome the electrical and physical barrier of the cell envelope, microorganisms have evolved specialized mechanisms for the exchange of electrons[14,15]. For example, *S. oneidensis* MR-1 uses the metal-reducing (Mtr) pathway to transfer electrons from the quinone and quinol pool in the cytoplasmic membrane,

Dissimilatory metal-reducing microorganisms
These microorganisms carry out a biochemical reaction during which metal ions are reduced but not incorporated into the cells.

Semiconductive minerals
Minerals the conductivity of which is between that of insulators and that of most metals.

S-Layer
A cell envelope structure that consists of proteins or glycoproteins and is on the cell surface.

Quinone and quinol
A group of aromatic compounds that act as mobile components of the electron transport chain in the cytoplasmic membrane and can receive and donate electrons (quinone is the oxidized form and quinol the reduced form).

Photoreaction centre
A pigment–protein complex in the inner cytoplasmic membrane of photosynthetic bacterial cells, where it converts solar energy to chemical energy to support bacterial growth.

Multistep hopping mechanism
A redox conduction process in which electrons transfer sequentially from one charge-localizing redox centre (for example, the haem iron) to an adjacent one, such as along the haem chain of multihaem *c*-type cytochromes (*c*-Cyts).

through the periplasm and across the outer membrane to the surface of minerals that contain Fe(III)[15]. Similarly, *G. sulfurreducens* PCA is hypothesized to use different sets of proteins, including porin–cytochrome proteins, to transfer electrons to the cell surface[16]. Opposite to the direction of electron transfer in the Mtr-mediated and porin–cytochrome-mediated pathways, the proposed phototrophic iron oxidation (Pio) pathway of *R. palustris* TIE-1 and the metal-oxidizing (Mto) pathway of *S. lithotrophicus* ES-1 are thought to oxidize Fe(II) extracellularly and then transfer the released electrons to the photoreaction centre in the inner cytoplasmic membrane and to the quinone and quinol pool in the cytoplasmic membrane, respectively[5,17,18].

Microorganisms have also evolved unique mechanisms to transfer electrons to minerals that are distant from the cell surface. For example, *G. sulfurreducens* PCA forms extracellular nanowires to transfer electrons to minerals and between cells[19]. Moreover, *G. sulfurreducens* PCA also uses nanowires and OmcS, a multihaem *c*-type cytochrome (*c*-Cyt) that is associated with the nanowires, to accept electrons from *G. metallireducens* GS-15 (REF. 20). In addition, *G. metallireducens* GS-15 transfers electrons directly to methanogenic archaea through their nanowires[21,22]. Furthermore, cable bacteria of the family Desulfobulbaceae form multicellular filaments along which electrons can be transferred[23]. Recently, direct intercellular electron transfer from archaea to bacteria has also been proposed[24,25]. Furthermore, microorganisms can exchange electrons with other extracellular substrates, such as humic acids[26,27], soluble metal ions[28], dimethyl sulfoxide[29], electrodes[30] and electrically conductive carbon materials[31]. The distance of microbial extracellular electron transfer varies, ranging from nanometres, as in electron transfer across the microbial cell envelope, to more than a centimetre, as in electron transfer mediated by cable bacteria[13,14,23].

Microorganisms that have extracellular electron transfer capability have been harnessed for various biotechnological applications. For example, stimulation of the activity of indigenous *Geobacter* spp. helps immobilize uranium at contaminated sites[32]. In addition, Fe(II)-oxidizing microorganisms are essential for recovering copper, gold and other metals from low-grade ores[33]. Recent results also demonstrate the direct involvement of microbial extracellular electron transfer in the production of methane biofuel[21,34].

Over the past decade, substantial progress has been made in understanding the mechanisms of microbial extracellular electron transfer. In this Review, we summarize these advances, including the molecular identification and functional characterization of the electron transfer pathways, the discovery of electron transfer that extends over centimetres and the biotechnological applications of microbial extracellular electron transfer.

## Microbial extracellular electron transfer pathways

Many microorganisms are thought to transfer electrons between their cytoplasmic membranes and extracellular minerals through a network of redox and structural proteins. Some of these proteins are well characterized in a few model microorganisms. These proteins often form pathways that electrically and physically connect intracellular metabolic processes with redox transformations of extracellular mineral-associated metal ions. However, the components of these pathways are phylogenetically diverse[13] and cannot always be identified from genomic data. The development of a mechanistic understanding of microbial extracellular electron transfer pathways requires the identification and functional characterization of their components.

***The metal-reducing pathway of* S. oneidensis *MR-1.*** *S. oneidensis* MR-1 was among the first identified microorganisms capable of using minerals that contain Fe(III), Mn(III) or Mn(IV) as terminal electron acceptors[3]. Genetic studies of this bacterium revealed the direct involvement of six multihaem *c*-Cyts (BOX 1) — CymA, $Fcc_3$ (also known as FccA), MtrA, MtrC, OmcA and small tetrahaem cytochrome (STC) — and the porin-like outer membrane protein MtrB in the extracellular reduction of minerals that contain Fe(III)[35–40] (FIG. 2). Functional characterization has confirmed that CymA oxidizes quinol in the cytoplasmic membrane and transfers the released electrons to the periplasmic *c*-Cyts $Fcc_3$ and STC[41–44]. Because a mutant without $Fcc_3$ and STC has an impaired ability to reduce Fe(III) oxides or oxyhydroxides, both $Fcc_3$ and STC are proposed to transport electrons from CymA to MtrA[40,45,46]. MtrA, MtrB and MtrC form a trans-outer membrane protein complex that transfers electrons from the periplasmic proteins to the bacterial surface[47–50]. Finally, on the bacterial surface, MtrC and OmcA can physically interact with each other and transfer electrons directly to minerals that contain Fe(III), probably through solvent-exposed haems[51–58] (BOX 1; FIG. 2a). Notably, MtrC and OmcA also associate with extracellular structures that were previously referred to as 'nanowires' (REF. 59). Recent results have demonstrated that nanowires are extensions of the outer membrane that contain MtrC and OmcA and which can make physical connections with neighbouring cells[59,60]. These outer membrane extensions are proposed to mediate the transfer of electrons to minerals and other *S. oneidensis* MR-1 cells through a multistep hopping mechanism[60,61].

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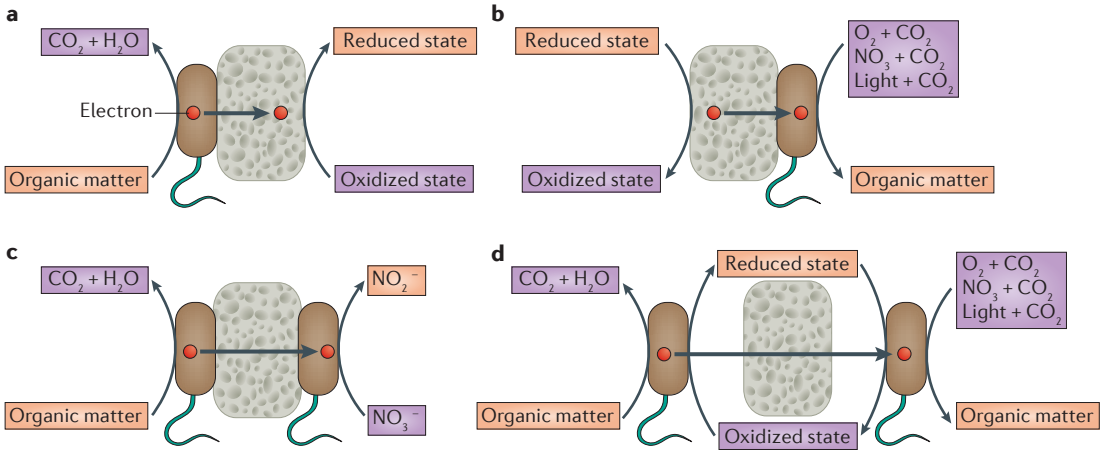



Figure 1 | **Electrical interplay between microorganisms and minerals.** Microorganisms use minerals that contain metal ions as terminal electron acceptors for respiration (part **a**), electron and/or energy sources for growth (part **b**), electrical conductors that facilitate electron transfer between microbial cells of the same and different species (part **c**) and electron-storage materials, or batteries, to support microbial metabolism (part **d**).

*S. oneidensis* MR-1 also extracellularly releases flavins that facilitate electron transfer from bacteria to minerals and electrodes[62–65]. Chemically reduced flavins can transfer electrons directly to minerals that contain Fe(III)[66,67]. Deletion of *bfe*, a gene that controls the extracellular release of flavins, severely impairs the reduction of ferrihydrite ($Fe(OH)_3$) by *S. oneidensis* MR-1 (REF. 65). Therefore, the released flavins are proposed to function as diffusive electron shuttles that transport electrons from MtrC and OmcA to mineral surfaces[62,63]. MtrC and OmcA might also bind to flavins as cofactors to facilitate electron transfer. Indeed, MtrC, OmcA and related *c*-Cyts have solvent-exposed haems that can transfer electrons directly to mineral surfaces[68–72] (BOX 1; inset of FIG. 2a). Under anoxic conditions, MtrC and OmcA bind to flavins, and these *c*-Cyt-bound flavins exist as semiquinone forms that have increased redox potentials, which may enhance the rates of electron transfer[71,73]. Furthermore, the reduction rates of Fe(III)-containing minerals by flavins alone are much slower than those of the MtrABC complex alone or the MtrABC complex with flavins[18,48,50,67,74].

To summarize, CymA, $Fcc_3$, MtrA, MtrB, MtrC, OmcA and STC form a pathway that oxidizes quinol in the cytoplasmic membrane and transfers the released electrons across the entire width of the cell envelope to the surface of minerals (FIG. 2a). Although the mechanism of electron transfer across the outer membrane remains undetermined, the Mtr pathway of *S. oneidensis* MR-1 is the best-characterized microbial extracellular electron transfer pathway. Notably, Mtr homologues are found in all sequenced metal-reducing *Shewanella* spp. and have been identified in other metal-reducing and Fe(II)-oxidizing bacteria, such as *Rhodoferax ferrireducens*, *R. palustris* TIE-1 and *S. lithotrophicus* ES-1 (REFS 48,75–78) (see below).

***The porin–cytochrome-mediated pathways of* G. sulfurreducens**. Multihaem *c*-Cyts also have a key role in electron transfer across the cell envelope during the extracellular reduction of Fe(III)-containing minerals by *G. sulfurreducens* DL-1 and *G. sulfurreducens* PCA. The identified multihaem *c*-Cyts include the putative quinol oxidases ImcH and CbcL in the cytoplasmic membrane[79,80], PpcA and PpcD in the periplasm[81,82], and OmaB, OmaC, OmcB and OmcC in the outer membrane. The latter form porin–cytochrome trans-outer membrane protein complexes with the porin-like outer membrane proteins OmbB and OmbC[16,83–85]. Together, these *c*-Cyts and porin-like proteins are thought to transfer electrons from the quinone and quinol pool in the cytoplasmic membrane, through the periplasm and across the outer membrane to the bacterial surface (FIG. 2b). Moreover, both *G. sulfurreducens* DL-1 and *G. sulfurreducens* PCA contain three additional PpcA and PpcD homologues in the periplasm and two additional homologues of porin–cytochrome trans-outer membrane complexes[16,79,82,85–87]. Thus, these strains have multiple and parallel electron transfer pathways that are crucial for the reduction of minerals that contain Fe(III)[16,85] (FIG. 2b).

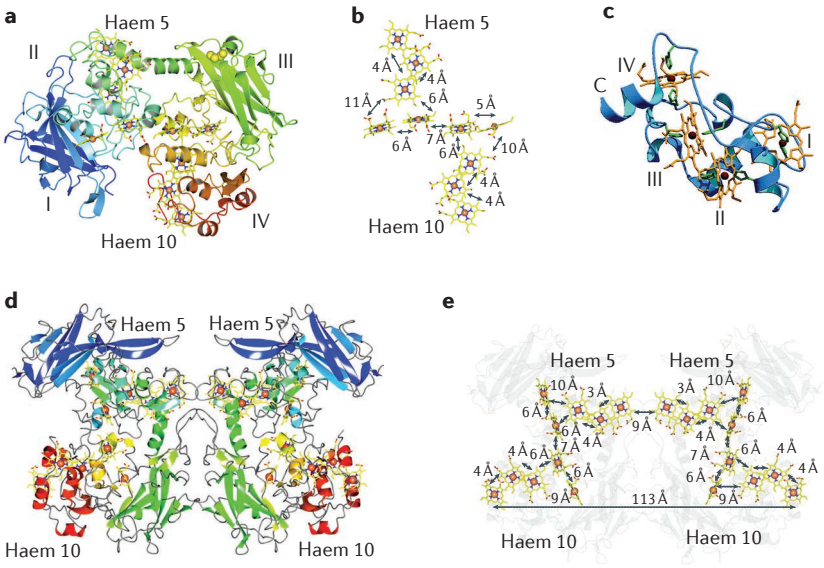
Although they all form trans-outer membrane complexes, the MtrA, MtrB and MtrC proteins of *S. oneidensis* MR-1 and the porin–cytochrome proteins of *G. sulfurreducens* are phylogenetically unrelated, which suggests that they evolved independently to provide a similar function[87]. Furthermore, porin–cytochrome homologues have been identified in all sequenced *Geobacter* species and in bacteria from six different phyla, such as *Anaeromyxobacter dehalogenans* 2CP-1, '*Candidatus* Kuenenia stuttgartiensis', *Denitrovibrio acetiphilus* DSM12809, *Desulfurispirillum indicum* S5, *Ignavibacterium album* JCM16511 and *Thermovibrio ammonificans* HB-1, which suggests that porin–cytochrome-like proteins in these bacteria are involved in the extracellular reduction of minerals that contain Fe(III) and other substrates, such as selenium (Se(IV) and/or Se(VI))[16,87]. Therefore, MtrABC and porin–cytochrome protein complexes represent a general design principle for transferring electrons.

Flavins
A group of organic compounds with a tricyclic aromatic moiety that can exist in three different redox states: oxidized (0 electron), semiquinone (1 electron) and reduced (2 electrons).

Box 1 | **Multihaem *c*-Cyts and their structural roles in microbial extracellular electron transfer**

Electron-transferring *c*-type cytochromes (*c*-Cyts) contain several haems that are electron transfer centres composed of porphyrin rings and iron atoms. The figure below shows the molecular structures of the multihaem *c*-Cyts MtrC, OmcA and STC of the metal-reducing (Mtr) extracellular electron transfer pathway of *Shewanella oneidensis* MR-1. MtrC folds into four domains: domain I and domain III each contain a Greek key split-barrel structure, whereas domain II and domain IV each bind to five haems covalently. Together, domain II and domain IV form a central core with domain I and domain III flanking either side (see the figure, part **a**). The haems of MtrC are arranged into a unique 'staggered cross' of short and long chains and the solvent-exposed haem 5 and haem 10 are the termini of the long chain (see the figure, part **b**; porphyrin rings are in yellow and blue, iron atoms are in brown). All of these solvent-exposed haems can transfer electrons directly to a mineral surface, which confirms the terminal mineral reductase role of MtrC. All of the haems of MtrC are closely packed, each within 7 Å. This enables rapid electron transfer through a multistep hopping mechanism.

All of the haems of STC are solvent-exposed (see the figure, part **c**), which maximizes rapid electron transfer between STC and its redox partners CymA and MtrA. OmcA and MtrC share similar structural folds and haem arrangements. Two OmcA monomers interact with each other in the area close to haem 5 of domain II to form a dimer. The distance between two haem 5 moieties is 9 Å. Therefore, the formation of an OmcA dimer results in a branched, 20-haem network that is 169 Å long, which supports the role of OmcA in long-distance electron transfer along the surface of *Shewanella* spp. cells (see the figure, part **d** and part **e**).




Part **a** and part **b** are from REF. 71, Nature Publishing Group. Part **c** is reproduced from REF. 45 © (2002) the American Society for Biochemistry and Molecular Biology. Part **d** and part **e** are adapted with permission from REF. 69, Elsevier.

***The phototrophic iron oxidation pathway of* R. palustris *TIE-1.*** The phototrophic Fe(II)-oxidizing bacterium *R. palustris* TIE-1 uses light as an energy source and Fe(II) as an electron source to fix $CO_2$ (REF. 5). The genome of *R. palustris* TIE-1 contains a *pio* gene cluster that consists of *pioA* (an *mtrA* homologue), *pioB* (an *mtrB* homologue) and *pioC* (a gene that encodes an iron–sulfur protein with a high redox potential). Deletions of *pioA*, *pioB*, *pioC* or the entire *pio* gene cluster all decreased the ability of *R. palustris* TIE-1 to oxidize and/or grow on Fe(II) as well as to accept electrons from electrodes[8,75]. Therefore, PioA and PioB are proposed to oxidize Fe(II) extracellularly and then transfer the released electrons across the outer membrane to PioC, which is hypothesized to be in the periplasm. PioC is thought to relay the electrons to the photoreaction centre in the inner cytoplasmic membrane[17] (FIG. 2c). Consistent with this hypothesis, PioC transfers electrons to the photoreaction centre in a light-dependent manner *in vitro*[88].

***The metal-oxidizing pathway of* S. lithotrophicus *ES-1.*** At approximately pH 7, *S. lithotrophicus* ES-1 produces energy from Fe(II) oxidation for autotrophic growth[6]. The genome of *S. lithotrophicus* contains an *mto* gene cluster that comprises *cymA*, *mtoA* (an *mtrA* homologue), *mtoB* (an *mtrB* homologue) and *mtoD* (a gene that encodes a mono-haem *c*-Cyt)[18,77]. MtoA directly oxidizes Fe(II), including minerals that contain Fe(II), and MtoD is a periplasmic *c*-Cyt that has a possible role in transferring electrons from MtoA in the outer membrane to CymA in the cytoplasmic membrane[18,89,90]. Although no genetic evidence is available, these results support the notion that MtoA, MtoB, MtoD and CymA form a pathway that couples the extracellular oxidation of Fe(II) to the reduction of quinone to quinol in the cytoplasmic membrane of *S. lithotrophicus* ES-1 (REFS 77,90) (FIG. 2d).

***Bidirectional transfer of electrons.*** The involvement of Mtr and possible CymA homologues in both Fe(II) oxidation and Fe(III) reduction not only suggests that

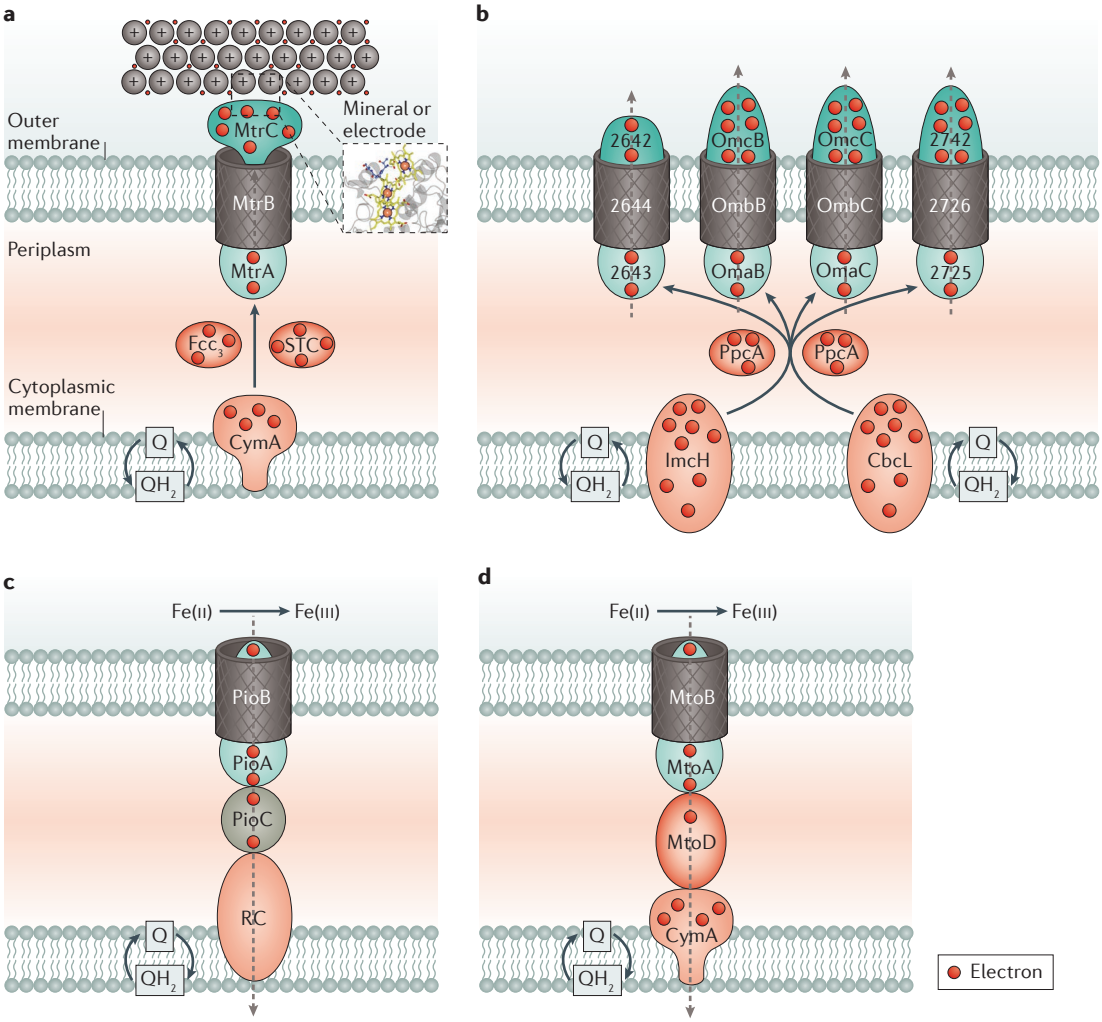



Figure 2 | **The proposed Mtr, Pcc, Pio and Mto extracellular electron transfer pathways.** In the metal-reducing (Mtr) pathway of *Shewanella oneidensis* MR-1 (part **a**) and the porin–cytochrome (Pcc) pathways of *Geobacter sulfurreducens* (part **b**), electrons are transferred from quinol ($QH_2$) in the cytoplasmic membrane, through the periplasm, and across the outer membrane to the bacterial surface, where MtrC transfers electrons to surface iron atoms directly through its solvent-exposed haem iron atom (inset of part **a**; brown sphere). For simplicity, OmcA on the bacterial surface and flavins are not shown in part **a**. In the phototrophic iron oxidation (Pio) pathway of *Rhodopseudomonas palustris* TIE-1 (part **c**) and the metal-oxidizing pathway (Mto) pathway of *Sideroxydans lithotrophicus* ES-1 (part **d**), electrons are transferred from extracellular Fe(II) to the quinone (Q) in the inner cytoplasmic membrane and cytoplasmic membrane, respectively. RC, photoreaction centre. The lower portion of the inset in part **a** is adapted with permission from REF. 69, Elsevier.

MtrA, MtrB, PioA, PioB, MtoA, MtoB and the CymA homologues in Fe(II)-oxidizing and Fe(III)-reducing bacteria are the result of divergent evolution, respectively, but also reflects the bidirectional nature of these electron transfer pathways[77]. Indeed, the MtrABC complex can transfer electrons in and out of liposomes across the lipid bilayer[48,50]. Furthermore, the Mtr pathway of *S. oneidensis* MR-1, which transfers electrons from CymA in the cytoplasmic membrane and through the cell envelope to the mineral surface, can also transfer electrons in the opposite direction, from extracellular electrodes to CymA[91]. Finally, MtrF, an MtrC homologue, is predicted by computational simulations to transfer electrons from one end of its long haem chain to the other and vice versa at almost identical efficiency[92] (BOXES 1,2).

***Other pathways.*** Although other microbial extracellular electron transfer pathways have been proposed[7,25,77,78,93–95], they are much less well characterized than those described above. For example, several proteins, including multihaem *c*-Cyts, were detected on the surface of the Gram-positive, Fe(III)-reducing bacterium *Thermincola potens* JR. Enzymatic degradation of these surface-exposed proteins decreased the ability of *T. potens* JR to reduce Fe(III) oxides or oxyhydroxides. Based on these results, a pathway of at least four different multihaem *c*-Cyts (that is, TherJR_0333, TherJR_1117, TherJR_1122 and TherJR_2595) is proposed to transfer electrons across the whole cell envelope to Fe(III) oxides or oxyhydroxides[96].

Similarly, a complex of more than 14 different proteins was isolated from the acidophilic, Fe(II)-oxidizing bacterium *Acidithiobacillus ferrooxidans*[97]. This complex

Box 2 | **Simulations and models of microbial extracellular electron transfer mechanisms**

Computational simulations are essential complements to the experimental tools that are currently available. For example, molecular dynamic simulations of MtrF (an MtrC homologue) reveal that its calculated free energy is nearly symmetrical along the haem chain, which permits electron transfer at nearly identical rates from haem 5 to haem 10 and from haem 10 to haem 5 (REF. 92). Quantum mechanics and molecular mechanics simulations also suggest that the maximum one-electron transfer rate along haem chains in MtrF is $10^4$–$10^5$ $s^{-1}$ (REF. 72). The predicted bidirectional electron transfer capability and calculated electron transfer rate are consistent with measurements in the MtrABC proteoliposome system[50]. Moreover, the simulations reveal that rapid electron transfer by MtrF is achieved, in part, because the haem pairs are stacked, which increases the electronic coupling and thus the electron transfer probability in regions of haem chains in which electron transfer is thermodynamically unfavourable. This design principle of balancing the redox potentials and the strengths of electronic coupling between haem pairs probably also applies to MtrC, OmcA and UndA (an OmcA homologue), which have structural folds and haem arrangements that are similar to those of MtrF[72].

Several mathematical models have been developed to describe and predict extracellular electron transfer and its associated processes in biofilms, including models that predict the electrical current production, the roles of proton transfer in current production, and the electron transfer mechanisms in biofilms[143]. Kinetic models, including the Monod, Nernst–Monod, Butler–Volmer and Butler–Volmer–Monod models, have also been proposed to evaluate various electron transfer mechanisms of biofilms[144,145]. In biofilms of *Shewanella oneidensis* MR-1, two different mechanisms (diffusion and conductance) have been proposed for microbial extracellular electron transfer. Simulations in a dual electron transfer mathematical model suggested that the proposed diffusion mechanism was much less efficient than the proposed conductive mechanism. The simulations also suggested a synergistic effect of conductive and diffusion electron transfer mechanisms in supporting the metabolic activity of microorganisms[146].

consists of the trans-outer membrane *c*-Cyt, Cyc2, which oxidizes Fe(II); the cytoplasmic membrane protein complex consisting of CoxA and CoxB (CoxAB), which reduces $O_2$; and the periplasmic *c*-Cyts, Cyc1 and $Cyc_{42}$, and copper-bearing protein RcY, which are proposed to transport electrons from Cyc2 to the CoxAB complex and other cytoplasmic membrane proteins. Collectively, these proteins are thought to couple the extracellular oxidation of Fe(II) to the reduction of $O_2$ and probably NAD in the cytoplasm[97].

## Direct electron transfer between microbial cells

Some microorganisms can exchange electrons through nanowires[19–22,24,98] or nanowire-independent cell–cell connections[23,25] to cells of the same or different species or even of different domains. They transfer electrons to minerals[9–11,34,99,100] and through electrically conductive carbon materials, such as activated carbon[22,31], biochar[101] and carbon cloth[102]. This not only extends the ability of microorganisms to transfer electrons beyond the boundaries of individual cells but also electrically couples the metabolic activities of two different microorganisms.

**Geobacter *spp. nanowires.*** *Geobacter* spp. nanowires are pili that are formed by protein filaments that are anchored in the cell envelope[19] (FIG. 3). They are required for the transfer of electrons between cells of *G. sulfurreducens* PCA and to Fe(III) oxides or oxyhydroxides[19], as well as from *G. metallireducens* GS-15 (REF. 98) to *G. sulfurreducens* PCA[20] and to the methanogenic archaea *Methanosaeta harundinacea*[21] and *Methanosarcina barkeri*[22]. The latter interspecies electron transfers couple the oxidation of ethanol by *G. metallireducens* GS-15 to the reduction of fumarate by *G. sulfurreducens* PCA[20] and to the reduction of $CO_2$ to methane by *M. harundinacea*[21] and *M. barkeri*[22].

Conductive pili of *G. sulfurreducens* PCA are assemblies of the pilin protein PilA[86]. Similar to other bacterial type IVa pilins, maturation of PilA of *G. sulfurreducens* PCA (GSu PilA) involves the recognition and processing of the pre-polypeptide of PilA by a dedicated signal peptidase and assembly by the type IVa pilus apparatus[19]. However, the remaining structure and amino acid composition of GSu PilA diverges from other pilins[103–105]. Importantly, the GSu PilA polypeptide is much shorter than nonconductive PilA polypeptides in other microorganisms, such as *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*, and contains up to five aromatic amino acids (or aromatics) in its non-conserved region[103–105]. Substituting these aromatic amino acids with alanine in GSu PilA makes the nanowire nonconductive and impairs the bacterial reduction of Fe(III) oxides or oxyhydroxides and the generation of electrical current, which suggests that these aromatic amino acids are essential for electrical conductivity[106].

The conductivity of *Geobacter* spp. nanowires is measured using various methods[105,107–109]. When temperature or pH decreases, the measured conductivity increases[105,107–109]. Given that this type of temperature-dependent and pH-dependent conductivity is a property that is shared with some conductive polymers[110,111], *Geobacter* spp. nanowires are proposed to transfer electrons by a metallic-like electron transfer mechanism[107]. It has been suggested that this mechanism is mediated by a continuous aromatic–aromatic interaction chain that is formed by close stacking of the conserved aromatic amino acids of GSu PilA[105,107]. Although the molecular structure of *Geobacter* spp. nanowires has not been experimentally solved, several structural models indeed suggest that the conserved aromatic amino acids form a closely packed (approximately 3.2 Å) aromatic–aromatic chain on the nanowire surface[105,112,113] (FIG. 3b). Furthermore, measurements of isolated *Geobacter* spp. nanowires with synchrotron X-ray microdiffraction and rocking-curve X-ray diffraction detected a 3.2 Å periodic spacing of the aromatic amino acids, which is lost if the conserved aromatic amino acids are mutated. In addition, decreasing the pH increased the

Bacterial type IVa pilins
Pilins that have the following characteristics: a leader peptide of 5–6 amino acids, an average polypeptide of 150 amino acids, an *N*-methylated amino-terminal residue of phenylalanine and an average disulfide-bonded region (D-region) of 22 amino acids.

Metallic-like electron transfer
A conduction process in which electrons are delocalized and can move freely without thermal activation, as in metals and conductive polymers.

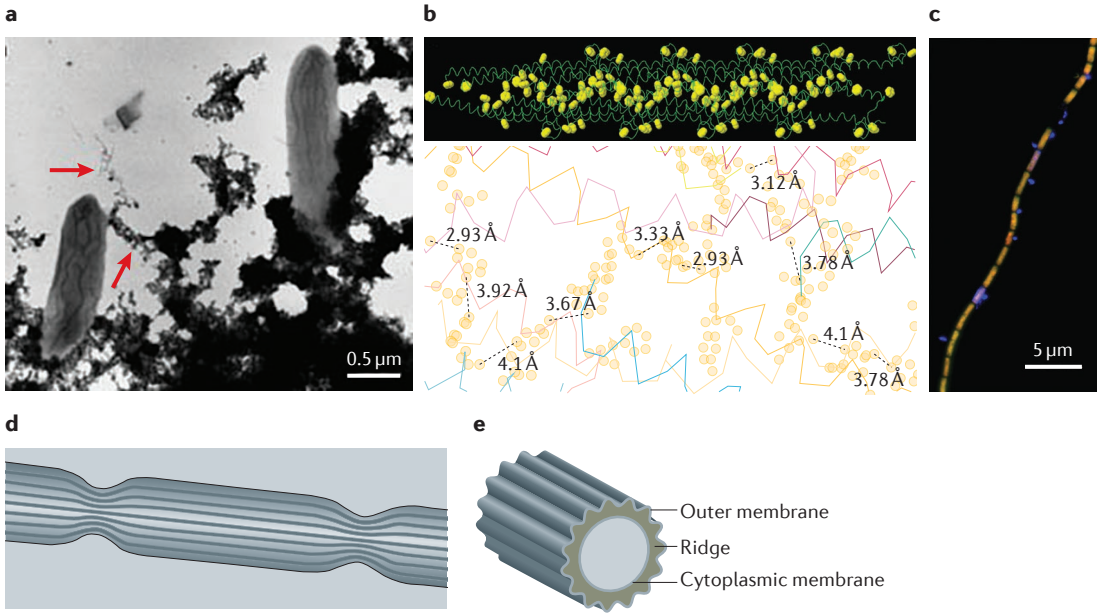



Figure 3 | **The proposed microbial structures for intercellular electron transfer. a** | Transmission electron micrographs of *Geobacter sulfurreducens* PCA cells and their pili are indicated by arrows. **b** | Structural model of a metallic-like electron transfer mechanism for *Geobacter* spp. nanowires through aromatic amino acids (side-chain rings are highlighted in yellow; top panel: side view of the proposed structure; bottom panel: inter-aromatic distances). **c** | Filamentous bacteria in the Desulfobulbaceae family that were identified using fluorescence *in situ* hybridization targeting 16S rRNA. **d** | A schematic representation showing that each filament contains 15 or 17 ridges. **e** | A cross section showing that these ridges are formed between the cytoplasmic membrane and the outer membrane. Part **a** is reproduced from REF. 19, Nature Publishing Group. Part **b** is republished with permission of the American Society for Microbiology, from Structural Basis for Metallic-Like Conductivity in Microbial Nanowires, Malvankar, S. *et al.*, **6**, 2 (2015); permission conveyed through Copyright Clearance Center, Inc.. Part **c** is reproduced from REF. 23, Nature Publishing Group.

3.2 Å periodic spacing, which might increase the conductivity of *Geobacter* spp. nanowires by promoting electron delocalization. Indeed, the increased spacing positively correlated with increased conductivity of *Geobacter* spp. nanowires[105,107]. Together, these results support a metallic-like electron transfer mechanism of *Geobacter* spp. nanowires[105].

However, other structural models predict that the distance between the conserved aromatic amino acids of Gsu PilA is 3.5–8.5 Å and thus is too large for a metallic-like electron transfer mechanism[104,114,115]. One of these models and its related measurements instead suggest that electrons are transferred along the conserved aromatic amino acids of Gsu PilA on the *Geobacter* spp. nanowire surface through a multistep hopping mechanism[115,116]. As no experimental structure of *Geobacter* spp. nanowires at atomic resolution is currently available, it remains unsolved how closely the conserved aromatic amino acids of Gsu PilA are positioned.

***Cable bacteria of Desulfobulbaceae.*** It has been suggested that intercellular electron transfer is important in filamentous, multicellular bacteria (also known as 'cable bacteria') in the family Desulfobulbaceae. Cable bacteria couple the oxidation of sulfide at depths of more than 1 cm in marine sediments to the reduction of $O_2$ at the sediment surface[23,117,118]. This decreases the pH in the sediments, which facilitates the dissolution of FeS minerals and the release of soluble Fe(II)[119]. Subsequent oxidation of the released Fe(II) by $O_2$ or Mn(IV) oxides results in the formation of solid-phase iron oxides or oxyhydroxides[119–121]. These iron oxides or oxyhydroxides function as a 'firewall', which protects life in the sediments by adsorbing toxic, free sulfide[120], and as a 'sink', which sequesters nutrient phosphorus[121]. Cable bacteria are widespread in marine and freshwater sediments and their activities are influenced by other microorganisms, such as photosynthetic algae[122–124].

The average cell length of members of the Desulfobulbaceae is 3 µm, but the cells seem to join end to end to form filaments up to 1.5 cm long (FIG. 3c). Each multicellular filament contains 15 or 17 unique ridges. These ridges are tunnel-like cellular structures that are 70–100 nm wide and are formed between the cytoplasmic and outer membranes (FIG. 3d,e). The ridges continue from one cell to the next, and in gaps between cells the ridges are wrapped up in the outer membrane, encasing the entire filament in the outer membrane[23]. The outer membrane might function as an insulator to prevent electrons 'leaking out' of the cells and to enable transfer beneath the outer membrane and along the ridges. Consistent with this hypothesis, measurements of the bacterial filaments using electrostatic force microscopy revealed a larger electrostatic force on the ridges than in the areas between the ridges, which indicates the electron storage capability of the ridges beneath the outer

membrane[23]. Furthermore, no electrical current was detected on the filaments of cable bacteria using methods that detect electron transfer on the surface of biological materials, which is consistent with an insulator role for the outer membrane[23]. Although direct evidence is still required, a mechanistic model predicts that cable bacteria transfer electrons along their ridges through a multistep hopping mechanism[125]. However, it should be noted that the molecular mechanisms for intercellular electron transfer by cable bacteria remain unknown.

***Transfer from archaea to bacteria.*** In ocean sediments, the oxidation of methane by anaerobic methanotrophic archaea (ANME) is coupled to sulfate reduction by sulfate-reducing bacteria (SRB), which have a key role in controlling the emission of the greenhouse gas methane. ANME and SRB aggregate to form granule-like structures that facilitate electron exchange between these two different domains of microorganism[126].

Recent investigations of consortia that consist of ANME of clade 1 (ANME-1) and SRB HotSeep-1, and of ANME-2 and SRB suggest direct intercellular electron transfer between cells[24,25]. These studies show no involvement of diffusive electron carriers, such as $H_2$, in shuttling electrons from ANME to SRB. However, both ANME and SRB have genes that encode multihaem *c*-Cyts and some of these *c*-Cyts are predicted to associate with the extracellular S-layer of ANME[24,25]. Furthermore, the SRB HotSeep-1 genome also contains a *pilA* gene. In the consortium of ANME-1 and SRB HotSeep-1, the genes that encode PilA and multihaem *c*-Cyts that are predicted to transfer electrons extracellularly are highly expressed under conditions of methane oxidation. Furthermore, nanowire-like structures are observed between the cells of ANME-1 and SRB HotSeep-1 under the same conditions, which suggests that nanowires and multihaem *c*-Cyts are responsible for transferring electrons[24]. Although no nanowire was detected in the consortia of ANME-2 and SRB, haem staining suggests that multihaem *c*-Cyts localize in the cytoplasmic membranes of both ANME-2 and SRB and in the extracellular matrix between cells. Multihaem *c*-Cyts might form pathways that are similar to those described above to transfer electrons from the cytoplasmic membrane of ANME-2 to the cytoplasmic membrane of SRB[25]. As ANME-2 couple intracellular oxidation of methane to the extracellular reduction of minerals that contain Fe(III) and Mn(IV)[127], the proposed multihaem *c*-Cyt pathways of ANME-2 may also transfer electrons to extracellular minerals[25].

***Minerals as electrical conductors and batteries.*** Minerals that contain iron can also function as electrical conductors that facilitate intercellular electron transfer. For example, magnetite facilitates electron transfer from *G. metallireducens* GS-15 to *G. sulfurreducens* DL-1. In this system, magnetite is proposed to function as an equivalent of OmcS (a multihaem *c*-Cyt that is associated with *Geobacter* spp. nanowires) to enhance the conductivity[34]. Similarly, magnetite is thought to promote interspecies electron transfer from bacteria, such as *Geobacter* spp., to methanogens, which enhances the production of methane[99,100]. Analogous to magnetite, granular activated carbon[31], biochar[101] and carbon cloth[102] promote the transfer of electrons from *G. metallireducens* GS-15 to *G. sulfurreducens* DL-1, *G. sulfurreducens* PCA and *M. barkeri*, which shows that intercellular electron transfer by these materials can affect the physiology of many microorganisms.

Redox-active minerals can also function as environmental batteries. For example, in a study of *R. palustris* TIE-1 and *G. sulfurreducens* PCA grown with magnetite, the Fe(II) in the magnetite was oxidized by *R. palustris* TIE-1, whereas the Fe(III) in the magnetite was reduced by *G. sulfurreducens* PCA, which reversed the oxidation process mediated by *R. palustris* TIE-1. Thus, mixed-valence Fe(II) and Fe(III) minerals, such as magnetite, green rust and clay, function as environmental batteries that support microbial metabolism[10]. Indeed, structural iron atoms in the clay mineral nontronite (NAu-2) were reversibly reduced and oxidized for up to three cycles by *S. oneidensis* MR-1 and the nitrate-dependent Fe(II)-oxidizing bacterium *Pseudogulbenkiania* sp. strain 2002, respectively. During these redox cycles, no substantial dissolution of mineral was observed[11]. This suggests that iron-containing clay minerals can function as environmental batteries.

## Biotechnological applications

Microorganisms that have extracellular electron transfer capability have been explored for the bioremediation of environmental contaminants, the production of nanomaterials with novel properties, biomining and the production of bioenergy.

In addition to metal ions in minerals, metal-reducing microorganisms reduce water-soluble metal ion contaminants, such as Cr(VI), Se(IV) and/or Se(VI), Tc(VII) and U(VI), to water-insoluble Cr(III), Se(0), Tc(IV) and U(IV), respectively. These types of reduction have been suggested for the remediation of such contaminants[128] (BOX 3). In addition to metal ion contaminants, Fe(III)-reducing microorganisms can degrade organic contaminants either directly or indirectly[129,130] (BOX 3). The microbial extracellular reduction of Fe(III)[1], Pd(II)[131] and Se(IV) and/or Se(VI)[132,133], and the extracellular oxidation of Fe(II)[134,135] also often result in the formation of nanomaterials that contain Fe(II), Pd(0), Se(0) and Fe(III), respectively. These biogenic nanomaterials have broad applications, ranging from catalysis and the remediation of environmental contaminants to manufacturing semiconductors and cancer treatment[131–133,136]. In addition, Fe(II)-oxidizing microorganisms have been used for biomining by extracting copper, gold, nickel and zinc from ore at industrial scales[33] (BOX 3).

As described above, the oxidation of organic substrates by *G. metallireducens* GS-15 can be electrically coupled to the reduction of $CO_2$ to produce methane biofuel by methanogenic archaea through nanowires[21,22] and conductive carbon materials[31,101,102]. Conductive minerals also stimulate the production of methane, probably by facilitating direct interspecies electron transfer from *Geobacter* spp. to methanogenic archaea[99].

Box 3 | **Bioremediation and biomining**

Both *Geobacter metallireducens* GS-15 and *Shewanella oneidensis* MR-1 can respire on contaminant uranium (U(VI)), reducing the water-soluble U(VI) to water-insoluble U(IV) and thus immobilizing it[28]. This has been used for uranium bioremediation at contaminated sites. For example, the injection of acetate (an electron donor for *Geobacter* spp.) into a site that was contaminated with uranium increased the activity of native *Geobacter* spp., which resulted in a decrease in the level of U(VI) in the groundwater, most probably through the direct reduction of U(VI) to U(IV) by the bacteria[28,32]. As minerals that contain Fe(II) produced from the reduction of Fe(III) by *Geobacter sulfurreducens* PCA also reduce Cr(VI) and Tc(VII) to Cr(III) and Tc(IV), respectively, and that some of the Cr(III) is further immobilized by being incorporated into the minerals[147–150], *in situ* stimulation of the activity of *Geobacter* spp. may also help remediate Cr(VI) and Tc(VII).

In addition, *G. metallireducens* GS-15 couples the oxidative degradation of aromatic hydrocarbon contaminants, such as benzoate, toluene, phenol and *p*-cresol, to the reduction of Fe(III), including minerals that contain Fe(III)[129]. *G. metallireducens* GS-15 and *Geobacter* strain Ben oxidize the aromatic contaminant benzene with Fe(III)-citrate and Fe(III) oxide or oxyhydroxide as the terminal electron acceptors, respectively[151]. Further investigations revealed that *G. metallireducens* GS-15 oxidizes benzene through the hydroxylation of benzene to phenol, which is then oxidized to $CO_2$ (REF. 152). As the genes that are involved in the oxidation of benzene to phenol share no homology to the genes that were previously proposed for the anaerobic hydroxylation of benzene, *G. metallireducens* GS-15 uses a new mechanism for the anaerobic degradation of benzene[153]. Acetate oxidation by *G. sulfurreducens* PCA is also electrically coupled to the reductive degradation of contaminant trichloroethene by *Desulfitobacterium* spp. and *Dehalococcoides* spp. through conductive minerals[130]. Moreover, Fe(II) reacts with hydrogen peroxide ($H_2O_2$) to produce hydroxyl ions ($OH^-$) and hydroxyl radicals ($HO^•$) through the Fenton reaction ($Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + HO^•$). The produced $HO^•$ oxidatively degrades a diverse group of organic contaminants, including 1,4-dioxane. Notably, *S. oneidensis* MR-1 reduces Fe(III) to Fe(II) under anoxic conditions and produces $H_2O_2$ under oxic conditions. Therefore, by alternating between growth under anoxic and growth under oxic conditions, *S. oneidensis* MR-1 couples lactate oxidation to the oxidative degradation of 1,4-dioxane[154].

Fe(II)-oxidizing microorganisms have been used to extract copper, gold and other metals from low-grade deposits[33]. For example, the Fe(II)-oxidizing and sulfur-oxidizing bacterium *Acidithiobacillus ferrooxidans* is a key member of microbial consortia that are used for the biomining of copper ores, during which *A. ferrooxidans* is proposed to dissolve ores that contain Cu(I), Fe(II) and sulfur by oxidizing Fe(II) to Fe(III)[155,156]. The produced Fe(III) then oxidizes Cu(I) to the more water-soluble Cu(II). The release, or 'bioleaching' of Cu(II) from the ores is also facilitated by sulfuric acid, a by-product of oxidation of the reduced sulfur compounds by *A. ferrooxidans*[156]. The solubilized Cu(II) is finally recovered from the solution abiotically[33]. Similarly, to recover gold from gold-bearing arsenopyrite ores, *A. ferrooxidans* is suggested to decompose the ore matrix by oxidizing the reduced sulfur compounds directly and indirectly through Fe(III) formed after Fe(II) oxidation[157]. Following the dissolution of sulfur and iron in the ores, the exposed gold can be extracted using chemical methods[33]. It should be noted that gold is neither redox-modified nor solubilized during the dissolution of ores by *A. ferrooxidans*[157]. This contrasts with the biomining of copper-bearing ores, during which water-insoluble Cu(I) is oxidized to water-soluble Cu(II)[156].

Collectively, these studies demonstrate a crucial role of microbial extracellular electron transfer in the production of methane. Microorganisms that exchange electrons with minerals also exchange electrons with electrodes. For example, *Geobacter* spp.[30,137] and *S. oneidensis* MR-1 (REF. 138) oxidize organic matter and transfer electrons to anodes for generating electricity in microbial fuel cells. It should be noted that, because of their low power output, microbial fuel cells are not ready to be used as an alternative energy source[139], although they can be used to provide electricity for low-power instruments[140]. Microbial electrosynthesis is an emerging field in biotechnology applications, in which microorganisms accept electrons from cathodes as an energy source to produce organic compounds with $CO_2$ as feedstock. However, microbial electrosynthesis is still in its infancy and has not become a major production process[141,142].

Anodes
An electrode that accepts electrons.

Cathodes
An electrode that donates electrons.

## Outlook

Over the past decade, several proteins that are crucial for microbial extracellular electron transfer have been identified, and some of them have been characterized in detail. Collectively, the results suggest that these proteins form pathways to exchange electrons between the cytoplasmic membrane and extracellular mineral-associated metal ions. Microbial intercellular electron transfer has also been discovered in various microorganisms during the past 10 years. Despite these advances, key knowledge gaps remain in our understanding of the electrical interplay between microorganisms and minerals. For example, it remains unknown how electrons are transferred from extracellular minerals to terminal electron acceptors inside microbial cells and how energy from these processes is conserved. Compared with the relatively well-characterized outward mechanisms of electron transfer across the cell envelope to extracellular minerals, these inward electron transfer mechanisms are not well understood. Additional *in vivo* and *in vitro* characterization are required to provide direct evidence that shows the functional capability of these proposed pathways.

The molecular mechanisms by which microorganisms transfer electrons across the outer membrane to exchange electrons with extracellular minerals also remain unknown. The outer-membrane lipid bilayer of Gram-negative bacteria is an electron barrier. To overcome this barrier, bacteria use phylogenetically diverse proteins. All of these proteins and protein complexes consist of a trans-outer membrane protein (or protein domain) and a redox protein (or protein domain; FIG. 2). It is hypothesized that the trans-outer membrane proteins act as sheaths and the redox proteins are embedded inside the sheaths and transfer electrons across the outer membrane[16,49,87]. This hypothesis can be examined best by determining the molecular structures of the proteins and protein complexes at atomic resolution. Detailed structural insights will not only reveal how these proteins and protein complexes fold, but also lay the foundation for revealing the electron transfer properties and design principles of these trans-outer membrane redox proteins and protein complexes through computational simulations (BOX 2). However, such molecular structures have not been experimentally determined to date.

Another key gap in our knowledge is the molecular mechanism that is used by microorganisms for intercellular electron transfer. For example, it remains unclear how *Geobacter* spp. nanowires interact with the porin–cytochrome-mediated extracellular electron transfer pathways. Moreover, although cable bacteria from the Desulfobulbaceae family have ridges, which are unique cellular structures that provide contiguous physical connections, their function in intercellular electron transfer remains unknown. Finally, direct evidence is still required to support the roles of nanowires and multihaem *c*-Cyts in transferring electrons from archaea to bacteria.

A mechanistic understanding of these electron transfer processes at the molecular level will lay the foundation for improving the biotechnological applications of microorganisms with extracellular electron transfer capability. This is especially true for the use of microbial electrosynthesis to convert the greenhouse gas $CO_2$ to various chemicals, such as advanced biofuels. At present, lack of a mechanistic understanding of electron transfer from extracellular electrodes to intracellular acceptors impedes the development of microbial electrosynthesis into a major bioproduction process[141].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### Acknowledgements
The authors acknowledge research grant funding from the Office of Biological and Environmental Research/Subsurface Biogeochemical Research Program of the US Department of Energy (L.S., H.D., G.R., H.B. and J.K.F.), the US National Science Foundation (H.D., G.R. and H.B.), the US National Institutes of Health and National Institute of Environmental Health Sciences (L.S. and G.R.), the US Office of Naval Research (H.B.), the National Natural Science Foundation of China (H.D., A.L., J.L. and H.Q.Y.), the 973 program of China (A.L.) and the One-Hundred Talented Researchers Project of the Chinese Academy of Science (H.Q.Y.). The authors also thank D. Lovley, J. Gralnick and an anonymous reviewer for their constructive comments.


### Competing interest statement
The authors declare no competing interests.