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Electron transfer at the microbe–mineral interface: a grand challenge in biogeochemistry

James K. Fredrickson
Pacific Northwest National Laboratory, jim.fredrickson@pnl.gov

John M. Zachara
Pacific Northwest National Laboratory, john.zachara@pnl.gov

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Electron transfer at the microbe–mineral interface: a grand challenge in biogeochemistry

J. K. FREDRICKSON AND J. M. ZACHARA

Pacific Northwest National Laboratory, PO Box 999, Richland, WA 99352, USA

ABSTRACT

The interplay between microorganisms and minerals is a complex and dynamic process that has sculpted the geosphere for nearly the entire history of the Earth. The work of Dr Terry Beveridge and colleagues provided some of the first insights into metal–microbe and mineral–microbe interactions and established a foundation for subsequent detailed investigations of interactions between microorganisms and minerals. Beveridge also envisioned that interdisciplinary approaches and teams would be required to explain how individual microbial cells interact with their immediate environment at nano- or microscopic scales and that through such approaches and using emerging technologies that the details of such interactions would be revealed at the molecular level. With this vision as incentive and inspiration, a multidisciplinary, collaborative team-based investigation was initiated to probe the process of electron transfer (ET) at the microbe–mineral interface. The grand challenge to this team was to address the hypothesis that multiheme *c*-type cytochromes of dissimilatory metal-reducing bacteria localized to the cell exterior function as the terminal reductases in ET to Fe(III) and Mn(IV) oxides. This question has been the subject of extensive investigation for years, yet the answer has remained elusive. The team involves an integrated group of experimental and computational capabilities at US Department of Energy's Environmental Molecular Sciences Laboratory, a national scientific user facility, as the collaborative focal point. The approach involves a combination of *in vitro* and *in vivo* biologic and biogeochemical experiments and computational analyses that, when integrated, provide a conceptual model of the ET process. The resulting conceptual model will be evaluated by integrating and comparing various experimental, i.e. *in vitro* and *in vivo* ET kinetics, and theoretical results. Collectively, the grand challenge will provide a detailed view of how organisms engage with mineral surfaces to exchange energy and electron density as required for life function.

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Corresponding author: J. K. Fredrickson. Tel: 509-376-7063; fax: 509-376-4909; e-mail: jim.fredrickson@pnl.gov.

CHALLENGING TERRAIN: THE MICROBE–MINERAL INTERFACE

Microbial life has been closely intertwined with the geosphere for the entire history of the Earth. Microorganisms, because of their small size, high surface area to volume ratio and incredibly diverse metabolism, have a tremendous influence on their environment through the transfer of energy and materials across complex biologic–solvent–solid interfaces. Although the microbial 'sculpting' of the geosphere is often evident at large scales over the surface of the planet, the interplay between microbes and geological materials is dominated by processes at the molecular and microscopic scales. The microbe–mineral interface is a prime example of this interplay and represents a complex, and relatively unexplored domain. Many of the details that are currently known about this domain can be attributed to the collective scientific contributions of Terry Beveridge, his students, and colleagues.

Among the first quantitative descriptions of the interactions between metal ions and bacteria were reports by Beveridge and colleagues investigating the binding of aqueous metal cations to various microorganisms including *Bacillus subtilis* and *Escherichia coli* and their specific cellular components (Beveridge & Murray, 1976, 1980; Beveridge & Koval, 1981; Beveridge *et al.*, 1982; Hoyle & Beveridge, 1983; Hoyle & Beveridge, 1984; Ferris & Beveridge, 1986; Ferris *et al.*, 1988; Mayers & Beveridge, 1989; Mera & Beveridge, 1993). These reports revealed the mechanistic nature of these interactions and led to a number of important conclusions regarding the specificity of binding sites on cells for various types of ions. Recognizing the importance of the binding of metal ions to microbial cells and cell components, Beveridge and colleagues

subsequently probed the nature of resulting biogenic minerals and the mechanisms by which they formed via a combination of observations and characterization of mineral matter from natural environments, and laboratory-based investigations (Beveridge *et al.*, 1983; Ferris *et al.*, 1986; Ferris *et al.*, 1987; Schultze-Lam & Beveridge, 1994; Urrutia & Beveridge, 1994; Schultze-Lam *et al.*, 1996; Thompson *et al.*, 1997; Douglas & Beveridge, 1998). Collectively, this body of research has demonstrated the importance and bacteria and bacterial surfaces in the formation of many mineral types with often unique properties, and the diagenesis of sediments.

Transmission electron microscopy has been used extensively in geobiology to define the architecture of the cell envelope, the location and nature of the interactions with metal ions, and the structure and composition of associated biominerals. Many of the advances in this area can be directly attributed to the pioneering work conducted by the Beveridge laboratory. These investigations visually revealed, for the first time, the intricate and complex structure of the bacterial cell envelope, and the specific sites and structures where metals bind and minerals nucleate. Electron microscopy (EM) and associated sample preparation techniques were developed and perfected by Terry and colleagues to enable study of these complex systems (Graham & Beveridge, 1990a,b; Graham *et al.*, 1991; Matias *et al.*, 2003), evolving to the point where the University of Guelph has become a leading institution for EM-based analyses of microbial cell architecture and interactions between microbial cells and metals.

The interfacial region between microorganisms and minerals is dynamic with chemistry and structure determined by interplay and response. The molecular workings and linkages across this complex region remain poorly characterized and the science required for their resolution spans broad fields in biology and the physical sciences. Furthermore, in a colloquium sponsored by the American Academy of Microbiology in 2000, 'Geobiology: Exploring the Interface between the Biosphere and the Geosphere', it was concluded that the 'real action' in geobiology happens at the level of individual cells or groups of cells. In the ensuing report from the colloquium (Nealson & Ghiorse, 2001), it was emphasized that the details of such processes would only be revealed by observations and measurements made at small scales. New advances in microscopy, such as those pioneered in the Beveridge laboratory at the University of Guelph, spectroscopy, and computational chemistry are providing unprecedented opportunities to probe, characterize, and resolve fundamental biologic and chemical phenomena that occur in this important and unique microscopic domain. Biogeochemical phenomena driven by fundamental biologic and chemical interactions at the microbe–mineral interface are significant to major environmental and geoscience research areas including: rock weathering and soil formation; contaminant fate and transport; environmental mineralogy and surface chemistry; biogeochemical cycling of C, Mn, Fe, and other elements; biotransformation of organic and inorganic contaminants; environmental sustainability; enhanced oil recovery; and radioactive waste storage and disposal.

GRAND CHALLENGE CONCEPT

The William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a US Department of Energy (DOE) national scientific user facility at Pacific Northwest National Laboratory, initiated a grand challenge research effort in biogeochemistry in 2005 to align with research programs in the Environmental Remediation Sciences (ERSD) and Life Sciences Divisions (LSD) of the DOE's Office of Biological and Environmental Research (BER). The Biogeochemistry Grand Challenge, one of two initiated by EMSL, is a coordinated, multi-investigator research effort focused on resolving a major scientific issue in biogeochemistry not accessible to the single investigator. The science themes, debated and identified by a group of experts at a workshop titled 'Earth-Life Interaction at the Microbe–Mineral Interface', held at the Pacific Northwest National Laboratory (PNNL) from November 4–6, 2003 in Richland, Washington, were cutting edge research topics with potentially broad impacts. As part of the grand challenge concept, advanced experimental and computational capabilities in EMSL and other DOE user facilities were to be leveraged to resolve complex science issues and questions associated with the grand challenge.

After considerable deliberation with input from a group of experts in the field, including Terry Beveridge, the topic of electron transfer (ET) at the microbe–mineral interface was selected. This process involves interplay between microorganisms and the surface, structural, and physicochemical properties of minerals, with the colligative biogeochemical behavior of the microbe–mineral association being of primary importance. Poorly recognized and understood is the interplay between organisms and solids that occurs via the coupling of the electron and proton transport systems in microorganisms with the surface chemical and bulk electronic properties of the solid. Oxides of Fe and Mn are semiconductors with very different band gaps and electrokinetic properties, meaning that they respond differently as electrons and protons are added or withdrawn from their surfaces and structures. Changes in electron density induced by microorganisms may be localized to the surface or structurally dispersed depending on these properties and the size of the mineral particle. Accordingly, the associated mineral phase may modulate bacterial activity in complex fashion through these properties, or through structural rearrangements or surface chemical reactions that dissipate energy or alter electron or proton density. An important facet of this topic is the role of surface and structural defects in the mineral phase that typically represents focal points for reactivity (e.g. (Brown *et al.*, 1999)). These defects may be chemical or physical and often represent microscopic domains in the mineral phase that contain excess

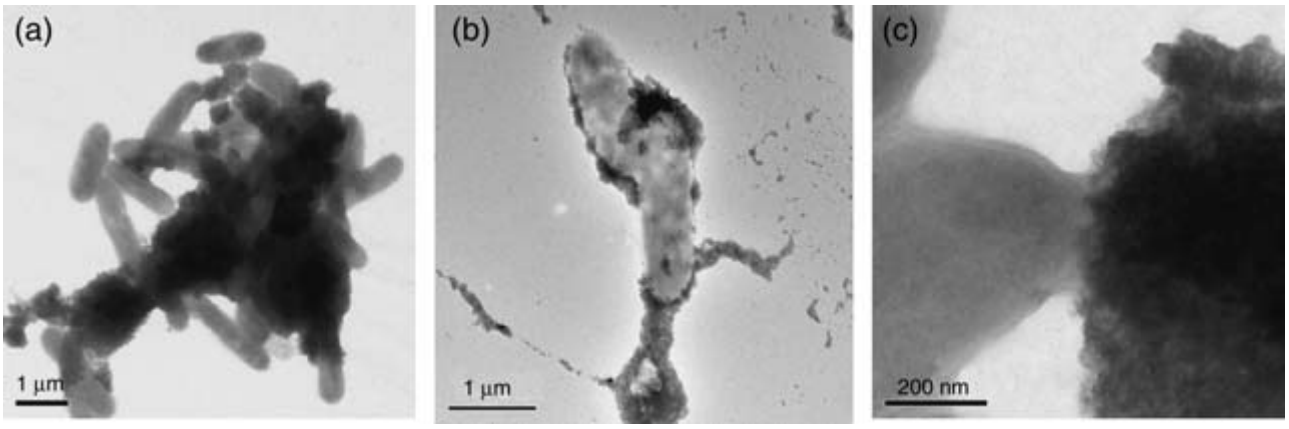

Fig. 1 Transmission electron microscopy images illustrating the interfacial contact between bacteria cells and minerals, specifically the engagement of *Shewanella oneidensis* MR-1 cells with hydrous ferric oxide (HFO). MR-1 cells rapidly attach to HFO and can form multicellular aggregates in cell–mineral suspensions (a). The engagement of HFO with MR-1 cells is a dynamic process in that with time, other Fe minerals may form as a result of bioreduction and the HFO as well as transformation products can associate with extracellular polymeric substances (EPS, b, c) in addition to being in direct contact with cell surfaces. (Images courtesy of Alice Dohnalkova, Pacific Northwest National Laboratory.)

energy that may be more favorable for microbial exploitation. Alternatively, through their spatially directed microscopic activity, microorganisms may generate defects on or within the mineral phase that alter mineral stability and subsequent reactivity with other system components. Included also in this topic is the important issue of biomineralization. The close spatial association of active bacterial cells with minerals (Fig. 1) creates chemical gradients that drive the formation of new biominerals through recrystallization, solid-state transformation, and/or heterogeneous nucleation (Fredrickson *et al.*, 1998; Zachara *et al.*, 2002), including reactions on mineral surfaces and cell components that serve as templates (Fortin *et al.*, 1997). These oxidative and reductive biomineralization products typically exhibit small particle size and are among the most reactive mineral phases found in the environment.

The biochemical mechanisms by which bacteria exchange electrons with poorly soluble metal oxides have a profound impact on the electron flux from cells to solids and vice versa; bacteria utilize Fe(III) or Mn(IV) containing solids as electron acceptors, others may recover energy from structural Fe(II) or Mn(II) in mineral solids by enzymatic oxidation, while still others seek structural phosphorous or trace metals to satisfy nutrient requirements. Common to all of these microbiologic processes is the bacterial need to access and react with near-surface and structural ions through microbe-mediated phenomena at the mineral–water interface. Such access may be gained by slow dissolution processes mediated by biogenic organic acids or complexants that facilitate release the target ions (Taillefert *et al.*, 2007), or by chemical transfer between cell surface components and bound, inorganic structural constituents of the mineral medium.

A common tenet in mineral surface chemistry is that structural and chemical defects are focal points for both surface and bulk reaction. Defects may occur as structural vacancies (i.e. where an O or Fe is missing in an iron oxide structure), as foreign chemical substituents (i.e. where Al^{3+} substitutes for Fe^{3+} in an iron oxide), or as structural discontinuities (e.g. screw, step, or edge dislocations) (see Fig. 2). Defects may occur at the surface or within the bulk, and represent points or regions in the solid where energy is perturbed. Important research questions are whether organisms: (i) exploit the energetic perturbations present in defects to gain access to structural ions needed as energy or nutritional sources, and (ii) generate defects by their own action that regulate the overall biogeochemical behavior of the microbemineral association. Published research has shown striking patterns of bioreductive dissolution on mineral surfaces that contrast with cell-shaped 'footprints' (Rosso *et al.*, 2003) that might be expected from direct contact mechanisms in the absence of electron diffusion within the mineral structure. The dissolution features of haematite observed by Rosso *et al.* (2003) displayed crystallographic control and alignment with structural defects associated with screw and step dislocations. Implied is the preferential dissolution of high-energy structural regions associated with the defects promoted by a nonlocal ET process, or electron migration to defect sites from the point of biologic ET. Regardless, the mechanisms remain speculative, and resolving them would provide insights into how microorganisms extract energy from complex natural Fe(III) oxides.

Electron transfer between microbial cells and mineral solids is a fundamental process that controls energy exchange throughout the geosphere, yet the mechanisms by which this occurs remain obscure. A prime example is the problem faced by bacterial ET coupled to poorly soluble extracellular electron acceptors such as Fe(III) and Mn(III, IV) oxides, as illustrated in Fig. 3, a simplified conceptual model for the dissimilatory metal-reducing bacterium *Shewanella oneidensis*

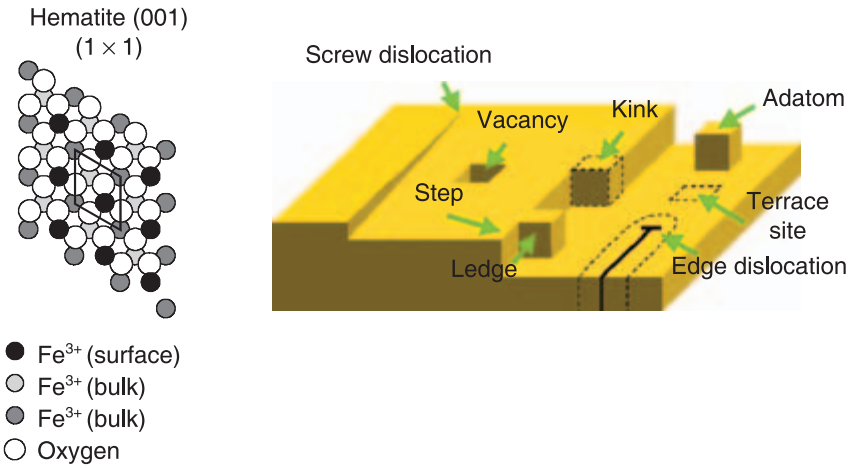

Fig. 2 There are many forms of surface defects in minerals that range in size from subnanometer (vacancies) to micron (structural dislocations in size). These are sites that exhibit different energetic properties from the bulk. Theoretical and experimental lines of evidence confirm that the reactivity of these sites can vary from the bulk phase. (Courtesy of Kevin Rosso, Pacific Northwest National Laboratory.)

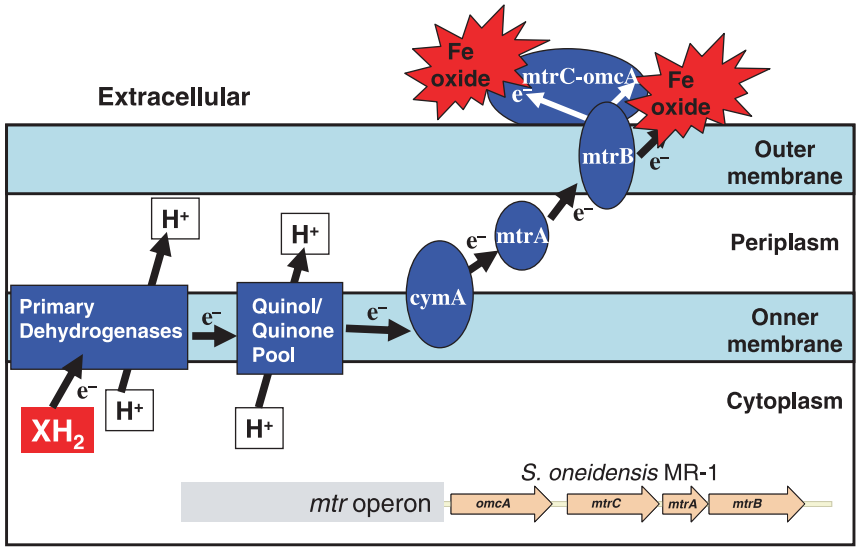

Fig. 3 Model of electron transfer to extracellular Fe(III) oxides proposed for metal-reducing *Shewanella* species. Electrons are transferred from primary hydrogenases through an electron transport chain that extends from the cytoplasm and through the inner membrane and quinol pool and subsequently to the tetraheme cytochrome CymA to the periplasm. At this point the electrons are believed to be transferred via the periplasmic decaheme cytochrome MtrA, across the OM via MtrB, an integral OM protein, by an unknown mechanism. The terminal electron transfer mechanism is hypothesized to be via outer membrane multiheme cytochromes MtrC and OmcA by a mechanism involving MtrB. (Courtesy of David Richardson, U. of East Anglia.)

MR-1. For respiratory processes involving O_2, nitrate, sulfate, or CO_2, the substrates freely move across the outer membrane into the periplasm, typically through porins or ion channels, where they engage with various ET proteins. In the case of Fe and Mn oxides, direct engagement of the periplasmic proteins with Fe(III) or Mn(III,IV) is not possible because these exist as poorly soluble solids external to the cell surface and are unable to pass the outer membrane under most circumstances. Certain bacteria that are proficient at metal oxide reduction appear to have solved this problem by localizing multiheme cytochromes to the exterior of the outer membrane where the proteins can potentially engage directly with oxide surfaces and transfer electrons. Although this is an attractive model, many aspects remain unresolved including the fundamental problem of how electrons are moved from inside the cell to the cell exterior, a process termed solid-state respiration (Nealson & Little, 1997). Among other mechanisms that may be involved include the biosynthesis of water-soluble organic compounds with electron-donating and -accepting properties that facilitate ET via a shuttle-type mechanism (Newman & Kolter, 2000), and the biosynthesis of conductive nanowires (Reguera *et al.*, 2005; Gorby *et al.*, 2006). More recently, metal-reducing strains of *Shewanella* were shown to excrete riboflavin and flavin mononucleotide (FMN) that could function as electron shuttles and accelerate the reduction of poorly crystalline Fe(III) oxide (von Canstein *et al.*, 2008). These processes are not mutually exclusive and may simultaneously be at play, depending on organism type and environmental conditions. The intent of the biogeochemistry grand challenge was not to broadly address these various mechanisms but rather to focus on evaluating the hypothesis that multiheme cytochromes, implicated in oxide reduction via genetic analyses (Beliaev *et al.*, 2001) and localized to the outer face of the outer membrane in the dissimilatory metal-reducing bacterium *Shewanella oneidensis* MR-1 (Myers & Myers, 1992; Myers & Myers, 2003), can directly transfer

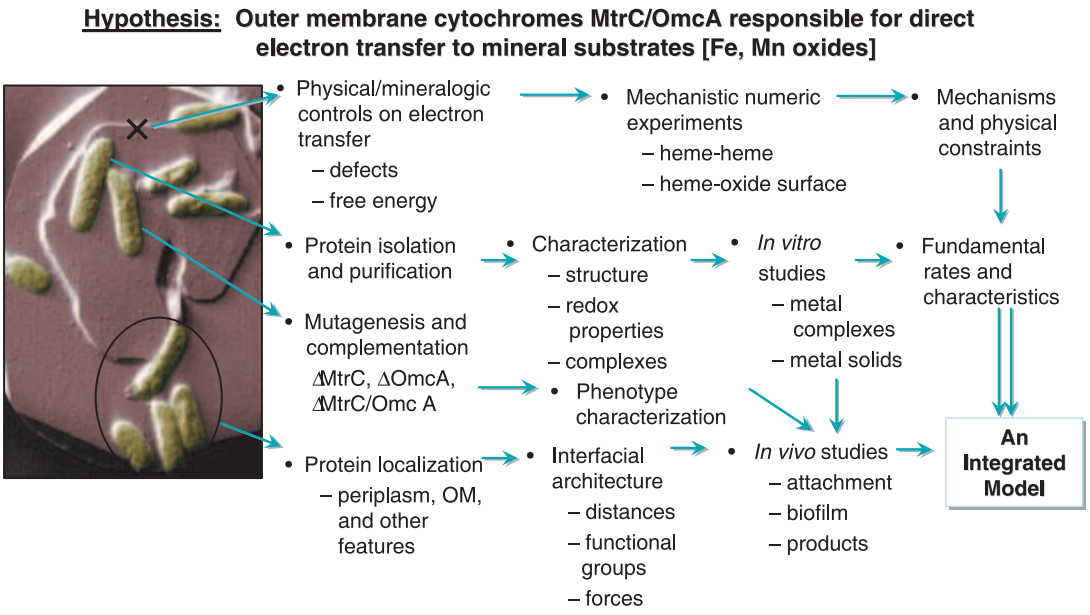

Fig. 4 Technical organization and information flow for the Biogeochemistry Grand Challenge to test the hypothesis that the outer membrane cytochromes MtrC/OmcA in *Shewanella oneidensis* MR-1 are responsible for direct electron transfer to mineral substrates (Fe, Mn oxides).

electrons to Fe and Mn oxide mineral surfaces. Although the grand challenge was initially focused on dissimilatory metal-reducing bacterium *Shewanella*, there is evidence to support that this feature is not unique to *Shewanella* but is likely widespread among other metal-reducing bacteria including *Geobacter* (Mehta *et al.*, 2005).

INTERDISCIPLINARY APPROACH TO MICROBE–MINERAL ELECTRON TRANSFER

In a visionary, forward-looking perspective, Terry Beveridge noted that 'truly interdisciplinary approaches and teams would be required to explain how individual microbial cells interact with their immediate environment at nano- or submolecular scales' (Beveridge, 2002). To this end, a multidisciplinary approach was developed for the grand challenge to evaluate the hypothesis that the outer membrane cytochromes (OMCs) MtrC (locus tag SO1778) and OmcA (SO1779) in the dissimilatory metal-reducing organism *Shewanella oneidensis* MR-1 transfer electrons to Fe and Mn oxide mineral substrates. The approach involves a combination of *in vitro* and *in vivo* biologic and biogeochemical experiments and computational analyses that, when integrated, provide a conceptual model of the ET process (Fig. 4). The *in vitro* studies involve the purification and characterization of proteins and protein complexes and determination of structural and electrochemical properties by redox titration, scanning tunneling microscopy, thin-film voltammetry on different relevant surfaces, and electron paramagnetic spectroscopy. Experiments with purified proteins have included binding experiments and force measurements to establish mineral–protein interactions and kinetic studies to determine ET rates as well as evaluation of the effect of metal ion structural environment on ET rate. *In vivo* experiments complement the *in vitro* studies by establishing the phenotype of specific mutants; probing the location of the OMCs with respect to the cell–mineral interfacial environment; determining the architectural features of the interface including distances, functional groups and biomolecules involved; and by quantifying whole-cell ET kinetics and the apparent governing factors. Numeric experiments and modelling are probing the influence of mineral surface defects, free energy, and electron diffusion within the oxide; and heme orientation, approach distance, and redox potential effects on ET from cytochrome groups to Fe(III) oxide surfaces (Fig. 5). The resulting conceptual model will be evaluated by integrating and comparing various experimental, i.e. *in vitro* and *in vivo* ET kinetics, and theoretical results.

IN VITRO FUNCTIONAL CHARACTERIZATION OF *S. ONEIDENSIS* MR-1 OMCS

In vitro OMC characterization efforts were initiated with the expression, isolation, and purification of MtrC and OmcA from membrane fractions of a *S. oneidensis* MR-1 double mutant lacking endogenous *mtrC* and *omcA* but expressing recombinant, C-terminus-tagged MtrC and OmcA (Shi *et al.*, 2006). The purified proteins were observed to: contain the predicted number of hemes (10); form a high-affinity complex with each other; and rapidly reduce Fe(III)-NTA. Subsequent investigations by Ross *et al.* (2007) extended these results by

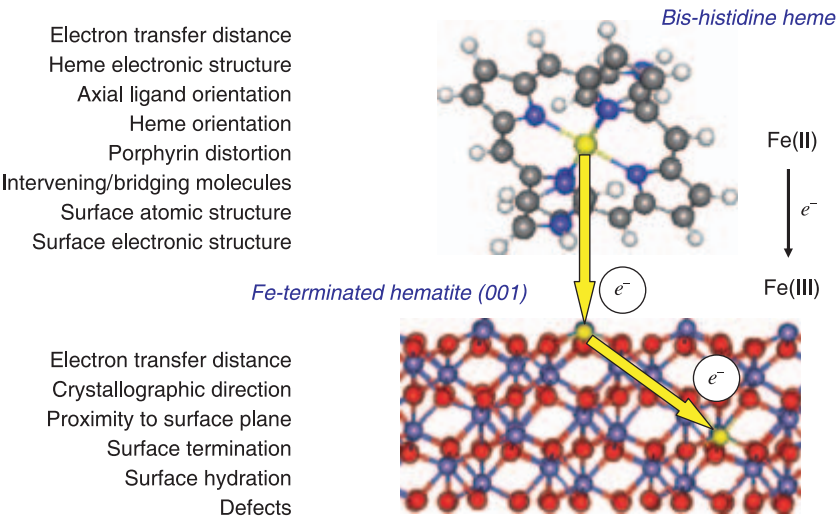

Fig. 5 Factors potentially influencing molecular-scale electron transfer kinetics at the cytochrome/heme–mineral surface interface and targeted for analysis via numeric experiments. (Courtesy of Sebastien Kerisit and Kevin Rosso, Pacific Northwest National Laboratory.)

demonstrating that MtrC, MtrB, and MtrA also co-purify as a functional complex and that MtrC and OmcA are cross-linked by formaldehyde, confirming their physical association. MtrA, another *c*-type cytochrome predicted to be localized to the periplasm, and MtrB, a nonheme integral OM protein, co-migrated through a native gel and were also cross-linked by formaldehyde. MtrB was previously shown to be essential for the reduction of Fe(III) and Mn(IV) (Beliaev & Saffarini, 1998).

Electron transfer between OMCs and their mineral substrates represents a physical challenge to microorganisms because of the strong dependence of ET kinetics on donating heme distance and orientation with respect to the accepting surface plane (Kerisit *et al.*, 2007). *In vitro* investigations by Xiong *et al.* (2006) with OmcA and haematite (Fe_2O_3) nanoparticles (~11 nm) demonstrated a rapid, high-affinity binding interaction between the purified cytochrome and the mineral concurrent with an electron flux on the order of 0.1 electrons per second per cytochrome. Recombinant MtrC and OmcA covalently linked to a gold substrate through a tetracysteine tag and probed by atomic force microscopy (AFM) with a haematite-functionalized tip, revealed force spectra with unique signatures indicative of specific bonding between each OMC and the haematite surface (Lower *et al.*, 2007), providing further support that the OMCs are capable of direct interaction with metal oxide surfaces (mechanism unspecified). Their comparison of *in vitro* and *in vivo* (i.e. whole cell) measurements showed a strong correlation between the various force spectra, allowing the authors to speculate that the unique binding attributes of each OMC are complementary, allowing both proteins to play a role in the transfer of electrons to Fe(III) mineral surfaces. Optical waveguide lightmode spectroscopy also showed that OmcA adsorbs to isostructural Al_2O_3 and Fe_2O_3 in similar amounts, and that the adsorption is ionic strength- and pH-dependent (Eggleston *et al.*, 2007). In this same study, electrochemical interaction between adsorbed OmcA and Fe_2O_3 was observed using cyclic voltammetry with a haematite electrode. Observed variations in the cathodic peak positions were suggested to result from redox-linked conformational or molecular orientation changes of the adsorbed cytochrome at the haematite interface.

Ruebush *et al.* (2006) used total membrane fractions of *S. oneidensis* MR-1 cells to demonstrate formate-dependent reduction of goethite, haematite, birnessite, and ramsdellite/pyrolusite. Although these authors did not identify the terminal ET agent(s) associated with the membranes, they did conclude that electron shuttles and iron chelators are not needed for the *in vitro* reduction of the metal oxides, confirming that reduction can occur by direct contact between the mineral oxides and ET molecules associated with the membranes. Collectively, these results are consistent with previous findings demonstrating that MtrC and OmcA associated with intact cells of *S. oneidensis* MR-1 were degraded by proteinase K and could be detected by immunofluorescence microscopy, confirming at least a partial cell-surface exposure for these proteins (Myers & Myers, 2003).

Protein thin-film voltammetry (PFV), used to probe the interfacial redox reactivity of sorbed MtrC on a conductive substrate, revealed facile ET between the cytochrome and the graphite electrode surface and catalytic ET behavior in presence of soluble Fe(III) complexes (Harthshorne *et al.*, 2007). The hemes within MtrC were observed to titrate over a broad potential from approximately +100 to –500 mV (SHE), demonstrating thermodynamic power for ET to Mn(III/IV) and most Fe(III) oxides. Electron paramagnetic resonance spectra were consistent with the presence of magnetically spin-coupled, low spin *c*-hemes. These results indicate that the redox and structural properties of MtrC hemes (e.g. alignment, electrostatic environment, and solvent exposure) are compatible with direct, intermolecular electron exchange to solid Fe(III) and Mn(III/IV) surfaces. Scanning tunneling microscopy and tunneling spectroscopy have also

been used to probe the ET properties of MtrC and OmcA complexed to the Au(111) surface (Wigginton *et al.*, 2007). Current–voltage TS of individual cytochrome molecules revealed that OmcA and MtrC have different abilities to mediate tunneling current despite exhibiting some similar biochemical properties suggesting that the two OMCs may have different roles in metal oxide reduction.

Collectively, this experimental and theoretical evidence and other grand challenge results in publication support the hypothesis that MtrC and OmcA can function as the terminal components of an extracellular metal-reduction pathway in *S. oneidensis* MR-1. These cytochromes exhibit high-affinity binding to a range of mineral and electrode surfaces and engage in rapid ET reactions with them. Despite these significant findings that have clearly contributed to hypothesis resolution, many outstanding scientific issues remain. The details of the biophysical interactions between the OMCs and the electron-accepting surfaces as well as intraprotein ET reactions will ultimately require high-resolution crystal structures to constrain the relative positions of the individual hemes in the context of the overall protein structure, and distances from and alignment with the metal oxide surface. Membrane proteins are particularly challenging in regard to structural characterization and we are unaware of any homologs to the OMCs for which structures are currently available that could be used to gain insight. Research has also revealed that the OMCs are components of a relatively large, complex protein assembly that facilitates ET from the CM, across the periplasm and OM, and ultimately past the LPS to accomplish extracellular ET. The assembly components, at minimum, contain MtrA, MtrB, and the OMCs but are likely to include other components that may be involved in scaffolding and proper localization and positioning within the cell envelope. The nature of these interactions and components of the assembly is likely dynamic, subject to reconfiguration by the cell in response to environmental change. This multiprotein complex may constitute a true molecular 'wire' that facilitates long-distance (e.g. tens of nanometers) ET. In the context of the molecular assembly, we believe that MtrB plays a key role. This protein is known to be essential for Fe(III) and Mn(IV) reduction (Beliaev & Saffarini, 1998), but its function remains poorly understood. Evidence for OMC mislocalization to the inner membrane in an *mtrB* mutant led to the suggestion that MtrB is involved in the proper insertion of the cytochromes in the OM (Myers & Myers, 2002). Because of their intimate physical association (Ross *et al.*, 2007), we suggest that MtrB plays a direct role in facilitating ET across the OM, either by providing a conduit for physical contact between MtrA and MtrC/OmcA or by directing electrons across the OM between via an unknown mechanism.

CONCLUDING REMARKS

The early field and experimental observations by Terry Beveridge and colleagues of microbes interacting with aqueous metal ions, and dissolving and precipitating various types of mineral phases have led to important insights and understandings regarding the underlying mechanisms. With the increasingly sophisticated techniques for probing, at high resolution and sensitivity, the chemical and physical attributes of solids and solutions in combination with the emerging biologic details of microbes being afforded by genomics and new analytical techniques, mechanistic details of microbe–mineral interactions are being revealed. Results generated from the grand challenge are anticipated to lend new insights into other important geomicrobial processes such as the microbial colonization and weathering of basaltic glass, an important rock weathering process on a global basis. The potential energy and nutrients within basalts may also support chemolithotrophic microbial communities in aphotic environments such as the deep terrestrial subsurface (Stevens, 1997). Similar approaches could be applied to develop an understanding of how microbes contribute to the dissolution and precipitation of carbonate minerals that have implications to global carbon budgets, and for biocorrosion, a serious economic problem. With regard to anaerobic corrosion, a *Desulfobacterium*-like organism was isolated that could reduce sulfate with metallic iron much faster than H_2-scavenging sulfate-reducing bacteria such as *Desulfovibrio* (Dinh *et al.*, 2004). These authors suggested that the efficient use of metallic iron linked to sulfate reduction could be facilitated by direct electron uptake via a cell-surface-associated redox-active component, a process potentially analogous to but in the reverse direction of electron flow from OMCs to metal oxides in some Fe(III)-reducing bacteria.

The collective investigations of Terry Beveridge and colleagues in the field of geomicrobiology have provided a technical foundation for the 'electron transfer at the microbe–mineral interface' grand challenge. Moreover, Terry Beveridge was a key proponent of the grand challenge concept and served on the advisory committee that helped to identify the science topic. He was also an important technical contributor to tasks focused on protein localization and characterization of interfacial architecture. Terry also served as an inspiration in developing a fully multidisciplinary, integrated approach to this problem and continuously challenged the team to 'think outside the box' and to not become too comfortable with a specific mechanism or interpretation. Finally, we wish to acknowledge Terry as one of the true gentlemen in science. With his quick wit, well-developed sense of humor, and understated recognition of his own accomplishments, he was a pleasure to interact with. We feel privileged to have known Terry both as a colleague and as a friend.

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