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Electron transfer and transport through multi-heme proteins: recent progress and future directions

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I review recent experimental measurements probing electron transfer (ET) and electron transport (ETp) through multi-heme cytochromes (MHCs) as well as their theoretical interpretation. Examples include pump-probe spectroscopy of Ru-labeled MHCs aimed at determining heme–heme ET rates in MHCs and the measurement of the *I–V* characteristics of MHCs in bioelectronic junctions. While the ET mechanism appears to be well established for MHCs in aqueous solution, the ETp mechanism in bioelectronic junctions such as STM remains elusive partly due to the complexities of the electrode–protein interface.

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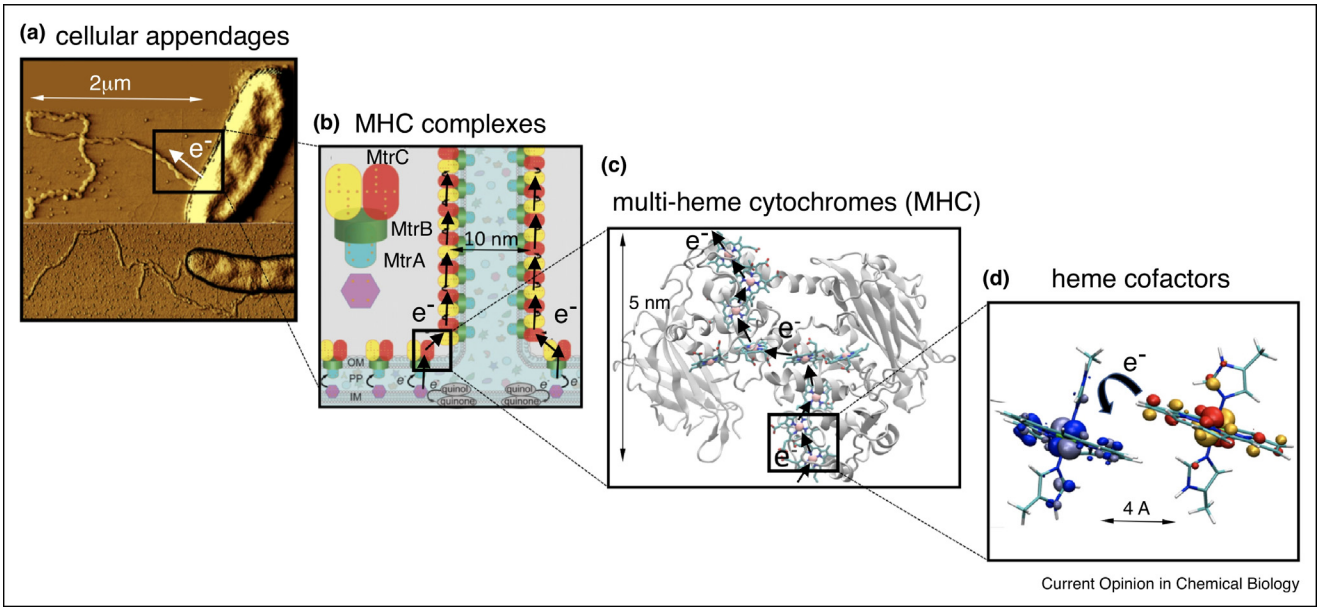
Introduction

The bacterium *Shewanella oneidensis* has evolved one of the most astonishing survival mechanisms in response to low oxygen concentrations [1]. When cytoplasmatic O_2 becomes scarce it starts to grow μm-long electrically conducting cellular appendages, which allows the bacterium to export electrons from the cytoplasm to extracellular space for reduction of extracellular electron acceptors in place of O_2 (see Figure 1a) [2,3•,4]. Until not too long ago it was speculated that these conducting appendages are bacterial pili, believed to be relevant for extracellular electron transport (ETp) in other organisms, for example *Geobacter sulfurreducens* [5–7]. However, recent studies on *S. oneidensis* demonstrated that the rigid fibers protruding from the cell surface are in fact extensions of the outer membrane and the periplasm into tubular vesicles [3•,4,8••]. Their electric conductivity, which rivals the one of man-made organic semiconductors [9•], is thought to be conferred to these structures by multi-heme cytochromes (MHCs) [10–13] that form wire-like complexes spanning the outer membrane (Figure 1b–d) [3•,14,15••].

MHCs have attracted much interest for some time, as they are thought to facilitate ETp in mediatorless microbial fuel cells [16,17], in microbial electrosynthesis [18–20], in the decontamination of water and soil containing radioactive isotopes [21] and in bionanotechnological applications [9•]. However, many fundamental properties of these fascinating biomolecules are still not well understood. What is the magnitude of the intrinsic electron flow that these biomolecules support? What parts of the protein limit the electron flow? How does conductivity change when moving from an aqueous to a non-polar environment or to air? What is the mechanism of conduction? Can we design mutations that allow us to control magnitude and directionality of electron flow? None of these questions can be currently answered with certainty, although efforts by several groups have been undertaken in recent years that have given us first insights and useful clues.

Here I start with a brief review of recently resolved structures of MHCs. Then I place focus on experimental measurements of electronic properties of single MHCs [22••,23,24••] and their complexes (nm length scale) [15••] as well as their interpretation by theory and computation [24••,25•,26,27••,28•,29•,30••,31]. In this respect I distinguish between electron transfer (ET) and ETp measurements. In ET an electron transfers between an electron donor and an electron acceptor resulting in a change of their net charge, which is accompanied by dielectric relaxation of the environment if present [32•]. This process is usually characterized by a chemical (here ET) rate constant. In biology it is often the case that several successive ET events occur along a chain of redox cofactors with each ET event characterized by a rate constant. An example that will be discussed here is electron injection in MHCs via molecular donor groups and the subsequent ET through the protein [15••,22••]. On the other hand, ETp is defined as the flow of electrons through a molecule (or biomaterial) without a change of the net charge of that molecule or donor/acceptor groups (e.g. redox cofactors) within that molecule [32•]. In practice, ETp is realized by application of a bias voltage to the termini of the molecule and the electric current replaces the ET rate constant as the experimental observable. In this review we will discuss ETp through MHCs that are sandwiched between a metal substrate and an STM tip [23,24••,33].

Figure 1



Electron transfer over multiple length scales in the bacterium *Shewanella oneidensis*. **(a)** Electronically conducting, micrometer-long cellular appendages. Adapted with permission from Ref. [3•]. **(b)** Model of a cellular appendage as an extension of the outer-membrane and the periplasm (light green). MtrCAB complexes comprised of the deca-heme proteins MtrC and MtrA and the non-heme protein MtrA are inserted in the outer membrane and form a connected electron transport path along the appendage. Adapted with permission from Ref. [3•]. **(c)** Crystal structure of MtrC (pdb code 4LM8 [13]), with heme cofactors shown in green and Fe atoms in pink. The assumed electron input site, heme 10, is shown at the bottom and the assumed electron egress site, heme 5, at the top. **(d)** Close-up on the heme 10–heme 9?pair forming a 'stacked motif' with heme edge-to-edge distance of about 4 Å. Two orbitals that contribute to electronic coupling between the two hemes are indicated by blue/gray and red/yellow positive/negative isosurfaces. The orbitals belong to the redox active $Fe^{2+}d(t_{2g})$ manifold of states that mix weakly with orbitals of the heme ring and axial His ligands. Adapted with permission from Ref. [27••].

A more comprehensive review on ET in MHCs covering developments until 2014 can be found in Ref. [34••] and a review on protein ETp and bioelectronics has been published very recently [32•]. For ETp measurements on cellular length scales and beyond, for example on appendages of *S. oneidensis* [35] and in biofilms of *G. sulfurreducens* [36,37,6,38,5,7], and for modeling work thereof [39,40], I refer to the recent literature.

Structures of MHCs and cellular appendages

High resolution structures have been resolved for numerous MHCs from the organism *S. oneidensis* MR-1, binding 4 hemes (quinol oxidase Cym A [41], small tetraheme cytochrome STC [42], fumerate reductase FccA [43]), 5 and 8 hemes (oxoanion reductases NrfA [44] and OTR [45]), 10 hemes (outer-membrane cytochromes MtrF [46] and MtrC [47]) and 11 hemes (UndA) [11]. While some of these proteins catalyze redox chemistry (Cym A, FccA, NrfA, OTR), others are thought to function as electron storage (STC) and electron transport proteins (MtrC, MtrF). The first crystal structure of a deca-heme protein of was resolved in 2011 (MtrF) [46], though with relatively low resolution. The most recent addition came in 2015 with the structure of MtrC [47], obtained at much improved resolution, see Figure 1c.

The MHC crystal structures reveal close-packed, chain-like, bis-His coordinated Fe-heme arrangements on the 1–10 nm scale, which is why they are sometimes referred to as 'nanowires'. Each heme contains iron coordinated by protoporphyrin IX (c-heme) that is covalently linked to the peptide by two thioether bonds arising from a Cys-X1-X2-Cys-His heme-binding motif that provides a His axial ligand to the heme iron and where X can be any amino acid. In most structures a second axial His ligand is provided by the peptide chain. The bis-His coordination results in a low-spin configuration of Fe in reduced (Fe^{2+}) and oxidized states (Fe^{3+}). Despite the different protein folds and molecular weights of these proteins, the hemes arrange in remarkably few motifs that differ in their heme edge-to-edge distances and hence the ET rate constants they support, the latter usually decreasing from 'stacked' (Figure 1d) to 'T-shaped' to 'coplanar' motif [27••].

Electron transfer from the cytoplasm to extracellular space requires a sophisticated transport system involving multiple soluble MHCs in the periplasm as well as protein complexes made of MHCs that transport electrons across the outer membrane. Examples for the latter are the MHC protein complexes MtrCAB and MtrFDE of *S. oneidensis* [14] as well as the recently identified