

## METALLOPROTEINS

livewires: uncovering nature's electronics.

Thomas A. Clarke, Marcus J. Edwards

**The electrogenic bacterium *Geobacter* synthesizes conductive extracellular nanowires to facilitate electron transfer that powers respiration. A highly conductive form of these nanowires is now revealed to be composed of oligomers of an 8-heme cytochrome, OmcZ.**

In the absence of oxygen, some bacteria use extracellular electron acceptors in order to support respiration. Collectively these bacteria are known as dissimilatory metal reducing bacteria, of which *Geobacter* and *Shewanella* are the most well-studied members<sup>1</sup>. Functional extracellular electron acceptors include not only Fe(III)/Mn(IV) minerals but also electrodes, which form the key component of microbial fuel cells that can generate electrical energy. Many of the most proficient energy-generating organisms are from the *Geobacter* genus, which form thick biofilms on the electrode surface<sup>2</sup>. These electrogenic bacteria transport intracellularly generated electrons to the electrode using conductive nanowires to facilitate electron transfer over large distances. In a new study, Yalcin et al. identified a new type of conductive nanowire produced by *Geobacter* which displays enhanced physical and electrical properties compared to previously identified cytochrome nanowires<sup>3</sup>. This study not only increases our understanding of the diverse mechanisms of bacterial extracellular electron transfer (EET) but also provides exciting opportunities for the development of bioelectronics.

Transferring electrons to extracellular acceptors from the cell interior of Gram-negative bacteria is no small feat. In order to achieve this, electrons have to be transferred from the quinol pool in the inner membrane, across the periplasm, across the outer membrane and from the cell surface to the terminal electron acceptor. It is well established that porin-cytochrome complexes that span the outer membrane represent the most likely route for electron transfer from the periplasm to the cell surface<sup>4</sup>. In the case of *Geobacter*, the bacterium also synthesises protein-based conductive nanowires to transport electrons from the cell surface to the electrode. The mechanisms of long-range electron transfer are of interest not only from an environmental microbiology standpoint but also for potential applications in biotechnology e.g. the development of bio-electronic devices/interfaces.

Until recently, a single form of conductive nanowires, bacterial pili consisting of PilA oligomers, were proposed to be responsible for connecting cells to electrode surfaces. However, in 2019 the first structure of a conductive nanowire from *Geobacter sulfurreducens* was solved using cryo-EM<sup>5</sup>. This nanowire was shown to be a polymer of a hexaheme cytochrome, called OmcS, that formed a continuous chain of hemes located no more than 0.60 nm apart. These OmcS nanowires were shown to be conductive and comparable to conductivity measurements recorded for isolated *G. sulfurreducens* PilA nanowires (Fig. 1a)<sup>6</sup>.

In this new study, Yalcin et al. utilize a range of biophysical techniques to identify a second type of conductive nanowire produced by *G. sulfurreducens*, composed of oligomers of the octaheme OmcZ<sup>3</sup>. A structural model of OmcZ was developed with a diameter of ~ 2.5 nm, similar to the PilA nanowires (Fig. 1b,c). The model shows that the OmcZ hemes are more closely packed than the hemes of OmcS, which may explain why the OmcZ nanowires have approximately 1000-fold greater conductivity than the OmcS nanowires. The authors further demonstrated that exposing

OmcZ nanowires to low pH leads to irreversible structural changes that result in enhanced conductivity (400 S/cm). Surprisingly, the authors also found that mutating the *pilA* gene changed the extracellular concentrations of OmcZ on the surface of the cell, revealing a complex relationship between PilA and cytochrome nanowires.

To date, there is no experimentally derived structure available that conclusively demonstrates how *Geobacter* PilA monomers assemble to form conductive nanowires. In contrast, we now have structural evidence for two cytochrome nanowires with properties similar to previously measured nanowires, meaning that some previous experimental studies may have been complicated due to a mixture of PilA, OmcS and OmcZ nanowires. However, there is still a wealth of experimental data supporting the existence of conductive pilin nanowires<sup>7</sup>, and it would be unwise to discount them as an additional mechanism for long-range EET.

In any case, the existence of cytochrome nanowires raises many more interesting questions; there is no evidence yet to explain how they assemble, and it is not clear how electrons are passed to them – whether they are anchored by a porin-cytochrome complex or if an intermediate cytochrome exchanges electrons between the complex and nanowire. It is also curious to consider what might be at the end of a cytochrome nanowire – does it end with an exposed OmcS/OmcZ terminal, or is there a further protein adaptor that connects to the end? Such a protein would be hard to find, as it might be present only at a thousandth of the OmcS/OmcZ monomer concentration. Related electron-transfer proteins have shown electron spin-state selectivity, suggesting that it may even be possible to manipulate electrons at a quantum level using these systems<sup>8</sup>.

Perhaps more excitingly, these cytochrome nanowires are electron conduits that allow only a single electron to move across distances further than a micrometer. They provide a way to connect both cells and other redox proteins/electrodes together allowing for the assembly of biocircuits, potentially opening the door to a new era in the development of bioelectronic devices.

Thomas Clarke is in the School of Biological Sciences, University of East Anglia, Norwich, Norfolk, UK. Marcus Edwards is at the School of Life Sciences, University of Essex, Colchester, Essex, UK. e-mail: [tom.clarke@uea.ac.uk](mailto:tom.clarke@uea.ac.uk), [m.edwards@essex.ac.uk](mailto:m.edwards@essex.ac.uk)

**Figure 1: Comparison of *Geobacter* nanowires.** **a**, The OmcS nanowire (PDB id: 6EF8) is composed of multiple 6-heme subunits that form an electron transfer chain. The hemes are colored red throughout the subunits. **b**, The PilA nanowires are proposed to be assembled from multiple PilA subunits containing closely packed aromatic residues (shown as red and orange respectively). The PilA structure was developed through molecular simulations<sup>9</sup>. **c**, A possible OmcZ nanowire structure was assembled from the 8-heme OmcZ monomer<sup>3</sup>. The subunits form a chain for electron transfer with hemes colored red throughout the subunits.

## References

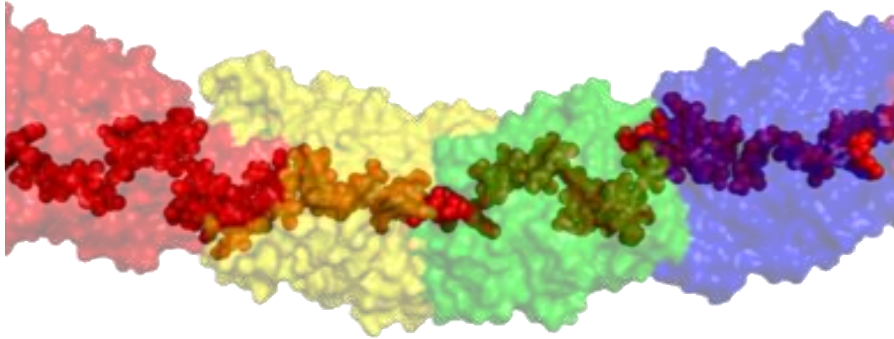
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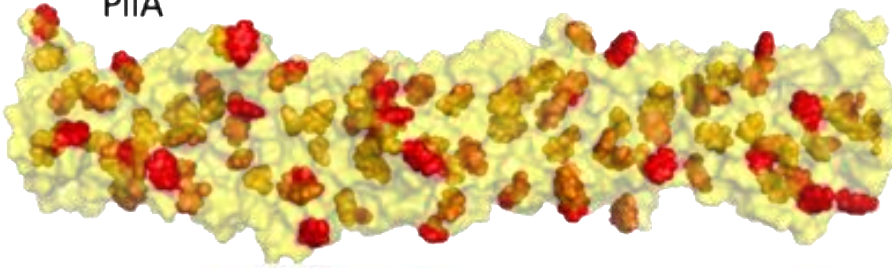
COMPETING FINANCIAL INTEREST:

There is NO competing interest.

OmcS



PilA



OmcZ

