
An Ultra-stable Protein Nanowire Made by Bacteria Provides Clues to Combating Climate Change

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Bacteria deep in the soil need "nanowires" made up of cytochrome OmcZ to export electrons to extracellular acceptors in order to survive without oxygen. Our cryo-EM OmcZ architecture explains how bacteria make nanowires on demand and why only OmcZ nanowires show extremely high electron conductivity.

Accelerated climate change is a major and acute threat to life on Earth. Rising temperatures are caused by the atmospheric methane, which is 30-times more potent than CO₂ at trapping heat. Microbes are responsible for generating half of this methane. Elevated temperatures are also accelerating microbial growth and thus producing more greenhouse gases than can be used by plants, thus weakening the earth's ability to function as a carbon sink – ability of environments to absorb carbon dioxide (CO₂) from the atmosphere. Due to rising temperatures, soil microbes are growing faster, producing CO₂ at rate much faster than that is used by land plants. Thus, the global warming due to human-made CO₂ is also causing the land surface to release more CO₂.

A potential solution to this vicious circle could be another kind of microbes that eat up to 80% of methane flux from ocean sediments that protects the Earth. How microbes serve as both the biggest producers as well as consumers of methane has remained a mystery because they are very difficult to study in the laboratory. In Nature Microbiology, surprising wire-like properties of a protein highly similar to the protein used by methane-eating microbes, is reported by our protein nanowire team led by Yangqi Gu.

Common soil bacteria *Geobacter* need nanowires to produce electricity and survive without oxygen. Some methane-eating microbes from ocean sediments do produce electricity but exactly how is not known. As they are very difficult to study in the laboratory, we chose *Geobacter* as a model system because it produces wires of cytochrome proteins called OmcZ that are similar to those of methane-eating microbes.

Our team had previously shown that OmcZ nanowires show the highest electron conductivity known to date where structural information is available, even 1000-times higher than the nanowires of another cytochrome OmcS made by the same bacteria. It allows bacteria to produce the highest electric power reported possible so far. But to date, no one had discovered how bacteria make them and why show such extremely high conductivity.

Using high-resolution cryo-electron microscopy and purifying nanowires from a new strain genetically engineered by Cong Shen, Yangqi, working with Fadel Samatey and Vishok Srikanth, was able to see the nanowire's atomic structure and discover that hemes packed closely to move electrons very fast with ultra-high stability. We found that these nanowires are highly conductive because of a unique arrangement of metal-containing molecules, called hemes, that line up in a straight line to create a continuous path along which electrons travel. Previously nobody had suspected such arrangement. With advice from Prof. Victor Batista, Matthew Guberman-Pfeffer's calculations explained how this heme arrangement confers distinct excitonic coupling measured by Yangqi.



Figure 1. Many methane-eating microbes could be using OmcZ-like nanowires

Yangqi's structure explain OmcZ nanowire's physiological role in bacterial survival in extreme environments that lack oxygen-like soluble, membrane-ingestible molecules by allowing bacteria to move electrons over 100-times their size. It also explains nanowire's ecological role in forming microbial communities in biofilms.

Yangqi also found that these nanowires are ultrastable due to their negatively charged surface and their ability to form networks which prevents binding of many protein-breaking chemicals which are also negatively charged and cannot attack the nanowire surface.

Yangqi is also the first to achieve synthetic assembly of cytochrome nanowires without *Geobacter*. Using the E-coli strain genetically engineered by Yuri Londer, Yangqi built nanowires synthetically to explain how bacteria make nanowires on demand. Prof. Kallol Gupta and Fabian Giska helped with mass spectrometry to ensure that synthetic OmcZ is

similar to that made by *Geobacter*. This achievement will allow production of nanowires using *E. coli* both at large yield and high purity.

So why should we care about these findings? These are the only known nanowires *Geobacter* absolutely need in order to convert waste into electricity. In addition, even without bacteria, some protein nanowires can generate electricity from ambient humidity. Our team is working on if these nanowires achieve the same feat, but more efficiently, and figure out the underlying mechanism. The continuous energy-harvesting strategy by the nanowires is robust, works over months and it is less restricted by location or environmental conditions than other solar or wind approaches.

This discovery of high conductivity in microbial nanowires might mean that a variety of other reactions in environments are also mediated by rapid electron flow, such as the oxidation of organic carbon, or of inorganic electron acceptors. Therefore, rather than relying on diffusion of small molecules as electron carriers, this in turn would implicate a new model of direct electron flow via nanowires in processes such as bioremediation, corrosion and carbon sequestration.

So how can these nanowires help to counter global warming? Both methane-consuming and methane-producing microbes need partners to drive an otherwise slow or thermodynamically unfavourable reaction performed by another. In addition to methane-eating microbes that produce proteins similar to *Geobacter* nanowires, methane-producing microbes also thought to partner up with *Geobacter* and share electricity via nanowires. Although many processes such as anaerobic methane oxidation are thought to use such a mechanism, the components and pathways involved have yet been identified. Microbial electron transfer via nanowires could accomplish the same catalytic feat, perhaps faster and more specifically (so that electrons are transferred directly between only the right kinds of organisms) than via rapid diffusion of small molecules.

Indeed, Yangqi's analysis using bioinformatics and AlphaFold models suggest that nanowire machinery could be widespread in diverse bacteria and archaea, including that regulate climate (Fig. 1). Our team is now working on understanding how methane-consuming

microbes use similar heme wires. Therefore, it could be possible to inhibit methane production and promote methane consumption by controlling electricity in such nanowires which requires figuring out what makes electrons move so fast in these proteins and how microbes charge these nanowires with electrons.

Discovery of nanowires used by diverse microbes will thus lead to new paradigms in the fields of microbial energetics and metabolism, as well as in ecology and evolution. This is truly an exciting time for working on the interface and microbiology and biophysics!

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