

The Molecular Density of States in Bacterial Nanowires

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ABSTRACT The recent discovery of electrically conductive bacterial appendages has significant physiological, ecological, and biotechnological implications, but the mechanism of electron transport in these nanostructures remains unclear. We here report quantitative measurements of transport across bacterial nanowires produced by the dissimilatory metal-reducing bacterium, *Shewanella oneidensis* MR-1, whose electron transport system is being investigated for renewable energy recovery in microbial fuel cells and bioremediation of heavy metals and radionuclides. The *Shewanella* nanowires display a surprising nonlinear electrical transport behavior, where the voltage dependence of the conductance reveals peaks indicating discrete energy levels with higher electronic density of states. Our results indicate that the molecular constituents along the *Shewanella* nanowires possess an intricate electronic structure that plays a role in mediating transport.

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Organisms extract electrons from many sources and the ensuing flow of these electrons through the cell's electron transport system charges a "biological capacitor" that can be used directly to power processes such as motility, or indirectly to drive the synthesis of biologically useful energy, such as ATP. This process, known as oxidative phosphorylation, requires a terminal electron acceptor to serve as the virtual ground. Prokaryotes use a variety of dissolved electron acceptors, such as oxygen, nitrate, and sulfate that are freely accessible to intracellular enzymes. However, dissimilatory metal-reducing bacteria are challenged by the poor accessibility of solid phase iron and manganese oxides that can serve as terminal electron acceptors, and therefore extracellular electron transfer takes place (1). Various strategies of extracellular transfer are reported for metal-reducing bacteria (2–4), the most recent of which is via electrically conductive pilus-like appendages, called bacterial nanowires (5,6). The mechanism of transport in these biological nanostructures, however, remains unclear.

We have focused our attention on the nanowires produced by *Shewanella oneidensis* MR-1, a dissimilatory metal-reducing bacteria whose electron transport system holds practical promise for renewable energy recovery in microbial fuel cells (7) and bioremediation of heavy metals and radionuclides (8). The redox-active *c*-type cytochromes thought to be present in *Shewanella* nanowires (6) are known to contribute to the electron transfer chain by undergoing oxidation and reduction, with the heme iron ions serving as sources or sinks of electrons during electron transfer. Molecular densities of states have previously been observed in redox molecules by resonant tunneling studies (9). The prospect of such an electronic structure being present in the nanowires is exciting, especially if it is present in an organized manner along the entire supramolecular assembly.

Most known electron transfer mechanisms that sustain living systems involve tunneling between sites of biological redox

chains or superexchange-mediated tunneling that takes into account the structural complexity of the proteins involved (10,11). The past decade has brought about considerable interest in electron transport over longer distances in biomolecular assemblies, driven by experimental observations of transport in DNA (12). While still debated, various mechanisms have been proposed for long-range transport in DNA, including sequential multistep hopping and band-mediated conduction where the energy states are delocalized over the length scales involved. This idea of common energy bands with various densities of electronic states, similar to those available in semiconductors, may appear to represent a new paradigm in biological electron flow, although it was first suggested in 1941 by Szent-Györgyi (13). Such thoughts have motivated this study of the electronic density of states in *Shewanella* nanowires. Cells of *Shewanella oneidensis* strain MR-1 (wild-type) were cultured in continuous flow bioreactors, and the nanowires' conductance was probed by conductive atomic force microscopy. Detailed methods can be found in the Supplementary Material, [Data S1](#), associated with this article.

Contact mode AFM revealed high numbers of bacterial nanowires, extending well beyond a cell's length (Fig. 1). The electrical properties of the bacterial nanowires were investigated using the configuration shown schematically in Fig. 2 *a*. After topographic scanning, the conductive AFM tip is moved to rest over a nanowire, in the low force regime (a few nN). The current response is measured as the voltage across the nanowire is swept using the highly ordered pyrolytic graphite (HOPG) support as the bottom electrode and the tip as the top electrode.

A typical I-V curve from a bacterial nanowire is shown in Fig. 2 *b*. The current response to applied voltage is nonlinear;

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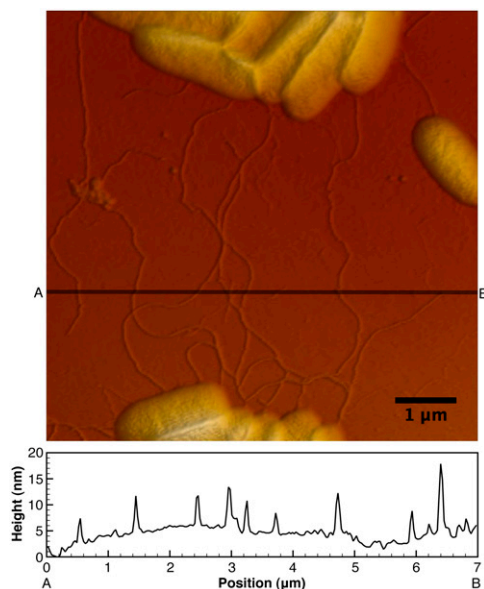


FIGURE 1 Contact mode AFM image of a chemostat-grown MR-1 culture with a high density of bacterial nanowires. The cells and nanowires are supported on a freshly cleaved HOPG surface.

increasing at a higher rate in the higher bias regime ($V > 0.5V$), and displaying various irregular, but repeatable (Fig. 3) features that represent small fluctuations in conductance. Sweeping the bias voltage changes the Fermi level of the tip with respect to the nanowire under investigation. For example, as the Fermi level comes into resonance with an available molecular state, an increase in the current between the tip and nanowire is observed. Further sweeping of the voltage moves the Fermi level away from this specific state, resulting in lower conductance or even a decrease of current (negative differential resistance). Conductance fluctuations, therefore, give us information about the molecular density of states (DOS) of the sample. However, this information may be obscured in the I-V curves by the lack of a robust contact between the sample and the tip, since they can be separated by a few Angstroms in the low force regime. Under these conditions, a clearer representation of the DOS emerges by computing the more invariant quantity, $(dI/dV)(I/V)^{-1}$, known as the normalized differential conductance (11,14,15). The normalized differential conductance (NDC) spectra provide a more direct measure of even complicated DOS structures, particularly at positive sample bias (16). Therefore, we apply the NDC analysis to the data of Figs. 2 and 3 as well as some other measurements from different nanowires. The NDC versus sample bias results are shown in Fig. 4.

The conductance measurements in Fig. 4 contain peaks indicating discrete energy levels with higher DOS. These features cannot be attributed to the underlying graphite, which did not exhibit these peaks, but are due to the nanowires themselves. The unknown nature of the contact resistance between the nanowires and the electrodes may contribute to differences in the overall resistance from point to point, even from adjacent points

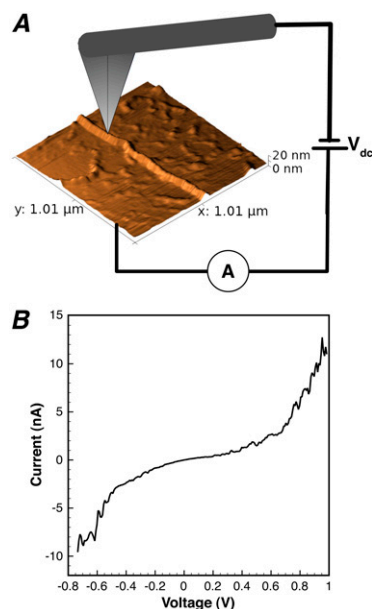


FIGURE 2 (A) Schematic of a typical measurement setup of a single bacterial nanowire using the conductive AFM probe as a top electrode and the HOPG support as bottom electrode. (B) A typical current-voltage curve from the nanowire pictured in panel A. The behavior is nonlinear and displays small fluctuations in conductance.

on the same nanowire (Fig. 3). However, the true features reflective of the electronic structure (kinks and bumps in the I-V curves) can be seen clearly in Figs. 3 and 4. One particular peak, at $V \sim 0.55V$, is highly reproducible in all the measurements of Fig. 4, including from different nanowires.

A previous report associated the conductivity in MR-1 nanowires with the decaheme cytochrome MtrC (6). Furthermore, previous tunneling spectroscopy studies on MtrC

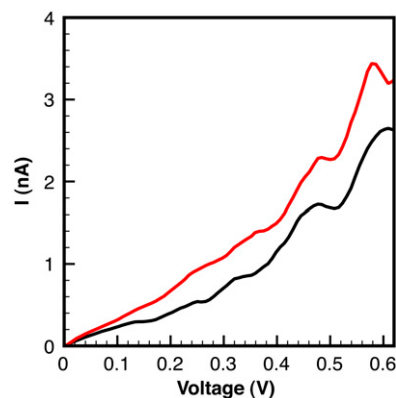


FIGURE 3 Filtered I-V curves from two adjacent points (120 nm apart) on the same nanowire. While the overall resistance can vary slightly due to a number of reasons (changes in tip-sample contact area from point to point, contact resistances between nanowire and electrodes, etc.), subtle irregularities in the I-V behavior are comparable and reflect conductance fluctuations that represent the electronic density of states.

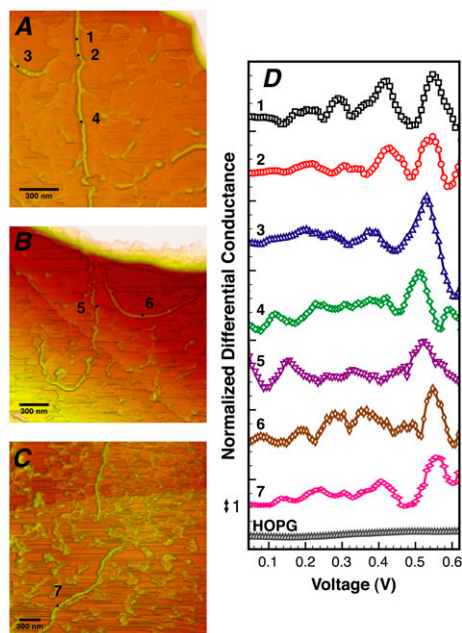


FIGURE 4 (A–C) Contact mode AFM images of a number of distinct bacterial nanowires from wild-type MR-1. (D) The normalized differential conductance $(dI/dV)/(IV)^{-1}$ spectra of a few spots from the three images reveal peaks corresponding to high density of states (DOS) energy levels. The conductance curves are labeled 1–7, indicating the locations, in panels A–C, where the measurements were taken, and are offset for clarity. The conductance spectrum from the bare HOPG surface is featureless, indicating the peaks in 1–7 are due to the nanowires.

molecules found NDC features that were proposed to arise due to the participation of heme electronic energy levels (17). This study suggests that these constituents are distributed along bacterial nanowires and may help pave the way toward understanding the conduction physics in this system. Specifically, the conductance data can be analyzed in the context of three models of electron transport (18): direct tunneling from electrode to electrode not taking into account the molecules in the junction; sequential hopping between molecular localized states; and a molecular band with delocalized energy states over the full junction. The typical wire height (~ 8 nm) and relatively large currents involved in these measurements preclude direct one-step tunneling through the nanowires as featureless dielectrics. Therefore, we conclude that the DOS features observed here reflect the electronic structure of the molecules present on the bacterial nanowires, and propose that transport proceeds by hopping between localized states or using delocalized energy states across the nanowire. The high electron transfer rates (10^{11} /s) observed here arguably make hopping the less likely mechanism (18).

Finally, the nonlinear electrical properties discovered here motivate us to consider these networks for molecular bioelectronics that can be assembled by bottom-up approaches complete with molecular links wiring active molecules, electrodes, sensors, and electronics on a single chip.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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