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Assessing Possible Mechanisms of Micrometer-Scale Electron Transfer in Heme-Free *Geobacter sulfurreducens* Pili

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Abstract

The electrically conductive pili of *Geobacter sulfurreducens* are of both fundamental and practical interest. They facilitate extracellular and interspecies electron transfer (ET) and also provide an electrical interface between living and nonliving systems. We examine the possible mechanisms of *G. sulfurreducens* electron transfer in regimes ranging from incoherent to coherent transport. For plausible ET parameters, electron transfer in *G. sulfurreducens* bacterial nanowires mediated only by the protein is predicted to be dominated by incoherent hopping between phenylalanine (Phe) and tyrosine (Tyr) residues that are 3 to 4 Å apart, where Phe residues in the hopping pathways may create delocalized “islands.” This mechanism could be accessible in the presence of strong oxidants that are capable of oxidizing Phe and Tyr residues. We also examine the physical requirements needed to sustain biological respiration via nanowires. We find that the hopping regimes with ET rates on the order of 10^8 s^{-1} between Phe islands and Tyr residues, and conductivities on the order of mS/cm, can support ET fluxes that are compatible with cellular respiration rates, although sustaining this delocalization in the heterogeneous protein environment may be challenging. Computed values of fully coherent electron fluxes through the pili are orders of magnitude too low to support microbial respiration. We suggest experimental probes of the transport mechanism based on mutant studies to examine the roles of aromatic amino acids and yet to be identified redox cofactors.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.9b01086.

Details of the effective hopping rate analysis within one repeating unit, block diagonalization, reorganization energy calculation, temperature dependence in the incoherent regime, conductance dependence in the coherent regime, and delocalization metrics in the mixed coherent–incoherent regime (PDF)

The authors declare no competing financial interest.

INTRODUCTION

Extracellular electron transfer (EET) is of great importance in anaerobic bacterial metabolism, pathogenesis associated with biofilm-mediated diseases, microbial fuel cells, interspecies ET, and biological–abiological electrical interfaces.^{1–3} Anaerobic bacteria have thrived on earth for millions of years, employing a “rock-breathing” respiratory strategy.⁴ The bacteria use metal oxides as terminal electron acceptors.⁴ Dissimilatory metal-reducing bacteria (DMRBs) have a significant influence on the earth’s geochemistry, as the bacteria form syntrophic consortia with methanogenic archaea to couple sulfate reduction with methane oxidation.^{5–8}

Prior to the discovery of the metal-reducing *Shewanella* and *Geobacter* genera in late 1980s, much of the attention in biological ET focused on nanometer-scale photosynthetic and mitochondrial reactions.^{9–11} The conventional paradigms of biological ET are challenged by these bacteria, which transport electrons over micrometer distances through long, filamentous appendages (also called bacterial nanowires).¹² *Shewanella oneidensis* bacterial nanowires are outer-membrane extensions with reported conductivities of 1 S/cm and measured lengths of up to 9 μm .¹³ *Geobacter sulfurreducens* bacterial nanowires are composed of type IVa pili, with reported conductivities of 50 mS/cm in vitro.¹⁴ Although experiments have found that these bacterial nanowires are electrically conductive, the underpinning ET mechanisms remain poorly understood. *Shewanella* nanowires are particularly well studied.^{15,16} Both decaheme cytochromes and riboflavin are believed to contribute to EET¹⁷ (see Figure 1a). Cyclic voltammetry of *G. sulfurreducens* biofilms indicate redox activity of both cytochromes and type IV pili.¹⁸ Cytochromes are believed to be loosely associated with the pili (the cytochromes are believed to be separated by hundreds of nanometers).^{19,20} This large spacing between the cytochromes in *G. sulfurreducens* pili is expected to disfavor long-distance multistep hopping transport that could support the electron flux of 10^6 s^{-1} needed for typical cellular metabolism,²¹ suggesting the need for an alternative charge transport mechanism in *G. sulfurreducens* pili if these appendages are to carry the respiratory electron flux.

G. sulfurreducens pili are the subject of intensive study.^{22–25} Lovley et al. found that charges injected into *G. sulfurreducens* pili can propagate along the length of the pili with temperature and pH dependences similar to those found in carbon nanotubes and organic conductors, suggesting at least a partially metallic transport mechanism²⁶ (see Figure 1b). To explore the transport mechanism in detail, Lovley et al. replaced aromatic amino acids in

the pili with alanines, which decreased the conductivity. This observation suggested either a structural or electronic role for aromatic residues.^{27,28} Other studies challenged this interpretation, supporting a mechanism of thermally activated multistep hopping in *G. sulfurreducens* pili.^{29,30} Atomistic modeling and analysis of pili is challenging because of the lack of structural data. Although the *G. sulfurreducens* pilin structure is known (Protein Data Bank (PDB) ID 2M7G),³¹ the structure of pilin assemblies is unknown. Tretiak et al. used homology models based on *Neisseria gonorrhoeae* type IV pili (PDB ID 2HIL) as templates to model *G. sulfurreducens* pili.³² They identified a proposed helical ET pathway among stacked aromatic residues and calculated the conductivity through the chain using an incoherent transport model. The computed conductivities were orders of magnitude smaller than the experimental values, presumably because of the large distances (~10 Å) between the aromatic amino acids.

Structures derived from homology models are very sensitive to the homology template. *N. gonorrhoeae* has ~40% sequence homology with *G. sulfurreducens*. A *G. sulfurreducens* pilin structure was modeled recently by Lovley et al. using a template with ~50% homology.³³ An energy-minimized model structure suggests closely packed aromatic residues.³⁴ These new structures are believed to offer an improved description of the *G. sulfurreducens* pili. We used these two new structural models to explore *G. sulfurreducens* transport mechanisms. The results point to a mixed ET mechanism in *G. sulfurreducens* pili, with charge delocalized in the phenylalanine dimers and incoherent hopping between these dimers and the adjacent tyrosines (4 to 5 Å away). The Phe-Phe-Tyr repeats form a periodic chain with a periodicity of about ~10 Å, similar in dimensions to delocalized G-blocks studied recently in DNA.³⁵ Incoherent transport among delocalized “islands” is found in our analysis to explain the experimentally measured conductivity data.

METHODS AND COMPUTATIONAL DETAILS

Pili Structural Models.

We model charge flow through the two recently modeled pili structures described above.^{33,34} The structures were obtained using homology models based on a type IV pilin template from *Pseudomonas aeruginosa*³³ and are constrained by docking-energy-minimized structure analysis.³⁴ Previous theoretical studies of *G. sulfurreducens* pili relied largely on structures derived from homology models of *N. gonorrhoeae* type IV pilin (~40% sequence homology),³² while the type IV pilin of *P. aeruginosa* shares ~50% homology.³³ The *P. aeruginosa*-based structure (denoted GS_{PA}) has a larger number of closely packed aromatic residues that are more likely to support coherent transport.³³ Both *N. gonorrhoeae* and *P. aeruginosa* have longer sequences of amino acids than *G. sulfurreducens* near the C-terminal regions that may cause the pilus to be loosely packed with an inner pore. The truncated pilin of *G. sulfurreducens* may be more tightly packed compared with both the *N. gonorrhoeae* and *P. aeruginosa* pili.³⁶ To study pili that lack the inner pore, a new model was built using energy-minimization methods to model a new *G. sulfurreducens* pilus that does not contain the pore but still has closely packed aromatic residues in the inner region (denoted GS_{ARC}).³⁴ We performed theoretical analysis of electron transport using these two new structural models. In our study, we assumed that the interfacial electron transfer between pili and

electrode does not limit the measured currents, and it was not included explicitly in our models.

Incoherent Hopping Model.

Charge hopping is a well-known mechanism of long-distance biological charge transport when the hopping sites interact weakly.^{37,38} Each hopping step is described using nonadiabatic ET theory, and the Marcus expression is often a good approximation for the Franck–Condon factor:³⁹

$$k_{\text{et}} = \frac{2\pi}{\hbar} |H_{\text{AB}}|^2 \frac{1}{\sqrt{4\pi\lambda kT}} e^{-(\Delta G^\circ + \lambda)^2/4\lambda kT} \quad (1)$$

The key rate parameters are the electronic coupling (H_{AB}), the reorganization energy (λ), and the reaction free energy (ΔG°). We use density functional theory with the B3LYP functional^{40,41} and the 6-31+G** basis set⁴² to evaluate H_{AB} , λ , and ΔG° . Previous experimental studies found that the charge carriers in *G. sulfurreducens* pili are holes,²⁶ so we explore hole transfer (HT) between aromatic residues. We first assume that HT occurs between nearest-neighbor amino acid side chains with distances ranging from 3 to 4 Å. This approximation neglects hopping among delocalized “islands” formed by multiple amino acids and places a lower bound on the HT rate. Nearest-neighbor electronic couplings are calculated using the block diagonalization method with the Kohn–Sham matrix.⁴³ The reaction free energy is estimated from the site energy (HOMO energy) gap between the aromatic side-chain pairs, where the HOMO energy of a site is obtained using the block diagonalization method (see section S2 in the Supporting Information (SI) for details). The inner-sphere reorganization energy is calculated using Nelsen’s four-point method by evaluating the energy difference between charged and neutral states (see Tables S1 and S2).⁴⁴ The outer-sphere reorganization energy is calculated with the Marcus two-sphere model. Since the ET pathway lies inside the pilus structure, the effective dielectric constant is likely to be low, and the associated outer-sphere reorganization energy is expected to be less than or equal to the inner-sphere reorganization energy. We first approximate the total reorganization energy as the inner-sphere reorganization energy. Both the reaction free energy and the reorganization energy are also treated as free variables to explore the upper bound of the conductivity.

The extracellular ET chain in *G. sulfurreducens* is composed of repeating units of three amino acids. In a previous study, the charge transport kinetics through the pilus was described using a steady-state approximation with a nearest-neighbor hopping model.⁴⁵ The carrier diffusion constant and conductivity were found to be sensitive to the choice of the repeating unit (see SI section S1 for further discussion). This is the case because the assumptions used to estimate the effective rate of ET between repeating units ignore the last reverse rate, and this approximation breaks down when the energy landscape of the donor–acceptor pair varies (i.e., when the last reverse ET step is not uphill and the corresponding reverse rate is not negligible). We compute the diffusion coefficient for a periodic one-dimensional (1D) hopping chain with the internal structure for each repeating unit indicated in Figure 2.⁴⁶ The diffusion constant is derived by assuming an infinitely long periodic chain

with cyclic boundary conditions. The carrier diffusion coefficient (D) and conductivity (σ) are independent of the choice of repeating unit. A periodic 1D hopping chain has its carrier velocity and diffusion coefficient set by the nearest-neighbor forward and backward hopping rates ($W_{n+1,n}$ and $W_{n,n+1}$, respectively).⁴⁶

The diffusion constant is⁴⁶

$$D = \frac{\Delta x^2}{\left(\sum_{n=1}^N r_n\right)^2} \left(A \sum_{n=1}^N u_n \sum_{i=1}^N i r_{n+i} + N \sum_{n=1}^N W_{n+1,n} u_n r_n \right) - A \Delta x^2 \frac{N+2}{2} \quad (2)$$

where Δx is the average nearest-neighbor distance between hopping sites and A , u_n , and r_n are given by

$$\begin{aligned} A &= \frac{N}{\sum_{n=1}^N r_n} \left[1 - \prod_{n=1}^N \left(\frac{W_{n,n+1}}{W_{n+1,n}} \right) \right] \\ u_n &= \frac{1}{W_{n+1,n}} \left[1 + \sum_{i=1}^{N-1} \prod_{j=1}^i \left(\frac{W_{n-j,n+1-j}}{W_{n+1-j,n-j}} \right) \right] \\ r_n &= \frac{1}{W_{n+1,n}} \left[1 + \sum_{i=1}^{N-1} \prod_{j=1}^i \left(\frac{W_{n+j-1,n+j}}{W_{n+j+1,n+j}} \right) \right] \end{aligned} \quad (3)$$

The rate ratios in eq 3 can be simplified because the forward and backward rates between nearest-neighbor sites satisfy detailed balance. The products in eq 3 include N sites in each repeating unit and are equal to 1 as a result of periodicity, based on detailed balance, and A is zero:

$$\begin{aligned} A &= \frac{N}{\sum_{n=1}^N r_n} \left[1 - \prod_{n=1}^N \left(\frac{W_{n,n+1}}{W_{n+1,n}} \right) \right] \\ &= \frac{N}{\sum_{n=1}^N r_n} \left[1 - \prod_{n=1}^N e^{(E_{n+1} - E_n)/kT} \right] \\ &= \frac{N}{\sum_{n=1}^N r_n} \left[1 - e^{\sum_{n=1}^N (E_{n+1} - E_n)/kT} \right] \\ &= \frac{N}{\sum_{n=1}^N r_n} (1 - 1) \\ &= 0 \end{aligned} \quad (4)$$

Thus, the diffusion coefficient is

$$D = \frac{N\Delta x^2}{\left(\sum_{n=1}^N r_n\right)^2} \sum_{n=1}^N W_{n+1,n} u_n r_n \quad (5)$$

When all of the hopping rates and distances are equal, the familiar expression $D = W x^2$ results. For a carrier density ρ , the charge mobility (μ) and electrical conductivity (σ) are

$$\mu = \frac{eD}{kT} \text{ and } \sigma = |e|\rho\mu \quad (6)$$

Coherent Electron Transfer.

In the coherent regime, we use a nonequilibrium Green's function (NEGF) strategy⁴⁷ to calculate the conductance of a single *G. sulfurreducens* pilus. NEGF methods are typically used to calculate currents and conductances of nanostructures that are in contact with electrodes. The transmission coefficient $T(\epsilon)$ for an electron of energy ϵ flowing from D to A is

$$T(\epsilon) = \text{Tr}[\Gamma_L \mathcal{G}(\epsilon) \Gamma_R \mathcal{G}^\dagger(\epsilon)] \quad (7)$$

where Γ_L and Γ_R are broadening functions for the left (L) and right (R) electrodes, respectively, and the Green's function is

$$G(\epsilon) = \frac{1}{(\epsilon \mathbf{I} - \mathbf{H} - \Sigma_L - \Sigma_R)} \quad (8)$$

in which $\mathbf{H}$ is the Hamiltonian matrix of the molecular system, $\mathbf{I}$ is the identity matrix, and the self-energy matrices Σ_L and Σ_R describe molecular eigenstate broadenings and shifts induced by coupling of the left and right electrodes to the molecular structure. The current as a function of bias voltage V is computed using the Landauer expression:

$$I(\epsilon) = \frac{e}{h} \int T(\epsilon) [f_L(\epsilon) - f_R(\epsilon)] d\epsilon \quad (9)$$

where f_L and f_R are the Fermi functions of the left and right electrodes. The zero-voltage conductance (G) is

$$G = \frac{dI}{dV} \Big|_{V=0} = -\frac{e^2}{h} \int T(\epsilon) \frac{\partial f(\epsilon)}{\partial \epsilon} d\epsilon \quad (10)$$

The conductance is the quotient of the current and the applied bias voltage

$$G = \frac{I(V)}{V} \quad (11)$$

and the conductivity σ is

$$\sigma = G \frac{l}{A} \quad (12)$$

where l is the length of the pilus and A is its cross-sectional area.

Mixed Coherent–Incoherent Transport.

Mixed coherent–incoherent transport has been studied for both exciton and electron transport.^{48–50} For 1D quantum transport, coherent evolution of the density produces $\langle R^2 \rangle \propto t^2$ while diffusive spreading of the density gives $\langle R^2 \rangle \propto t$,⁵¹ where

$$R^2(t) = \langle \psi(t) | r^2 | \psi(t) \rangle - \langle \psi(t) | r | \psi(t) \rangle^2 \quad (13)$$

and $|\psi(t)\rangle$ is the time-dependent wave function. Previous studies⁵² found that on long time scales, environment-induced decoherence changes the carrier distribution, $\langle R^2 \rangle$, from the ballistic to the diffusive limit, where the variance of the carrier density grows linearly with time.^{51,52} The carrier diffusion coefficient D is computed from the mean-square displacement (MSD) for an infinite 1D chain,

$$2Dt = \lim_{t \rightarrow \infty} R^2(t) \quad (14)$$

and the MSD in a discrete system is

$$R^2(t) = \sum_n r_n^2 \rho_{nn}(t) - \left(\sum_n r_n \rho_{nn}(t) \right)^2 \quad (15)$$

where $\rho_{nn}(t)$ is the time-dependent electron density on site n . We used a tight-binding description of the nanowire electronic Hamiltonian,

$$H = \sum_n \epsilon_n |n\rangle \langle n| + V_{n,n+1} |n\rangle \langle n+1| + \text{h.c.} \quad (16)$$

with one effective orbital per aromatic residue from the defined dominant helical pathway (see Figure 3). $V_{n,n+1}$ is the nearest-neighbor electronic coupling, and ϵ_n is the energy of site

n. The mixed coherent–incoherent time evolution of the system is described by the quantum Liouville equation with the Lindblad decoherence model:⁵³

$$\frac{\partial}{\partial t}\rho(t) = -\frac{i}{\hbar}[H, \rho(t)] + L(\rho(t)) \quad (17)$$

where $\rho(t) = |\psi(t)\rangle\langle\psi(t)|$ is the time-dependent density matrix and $L(\rho)$ is the Lindblad operator, which describes the evolution of the density matrix with simulated site energy fluctuations for open quantum systems:

$$L(\rho(t)) = \sum_{i < j}^N \gamma_{i,j} \left(A_{i,j} \rho A_{i,j}^\dagger - \frac{1}{2} [A_{i,j}^\dagger A_{i,j}, \rho]_+ \right) \quad (18)$$

in which $\gamma_{i,j}$ is the electronic dephasing rate and $[\cdot]_+$ is an anticommutator. We consider only pure dephasing (arising mainly from the site-energy fluctuations) in the mixed coherent–incoherent regime. The time scale of the coupling fluctuations is orders of magnitude longer than the time scale of the site-energy fluctuations, so we neglect the contributions of the coupling fluctuations to the dephasing. For states *i* and *j*,

$$A_{i,j} = \frac{1}{\sqrt{N}} \begin{pmatrix} 1_{ii} & 0 \\ 0 & -1_{jj} \end{pmatrix} \quad (19)$$

The pure dephasing part of the Lindblad operator is

$$\frac{\partial}{\partial t} \begin{pmatrix} \rho_{ii} & \rho_{ij} \\ \rho_{ji} & \rho_{jj} \end{pmatrix} = \gamma_{i,j} \begin{pmatrix} 0 & -\rho_{ij} \\ -\rho_{ji} & 0 \end{pmatrix} \quad (20)$$

For an electron that is initially localized in the middle of a 1D chain, $\psi(t=0) = (0, 0, \dots, 0, 1, 0, 0, \dots, 0)^T$. The position of the middle of the chain is set to be zero, and the wave function spreads with time in both the negative and positive directions. A 1D chain of 10 repeating units was chosen on the basis of MSD convergence tests. We found that the computed MSD reaches a converged value and loses its linear dependence on time if the chain length is short, since the finite-length chain prohibits carrier spreading beyond the chain ends. In our calculation, the chain has 10 repeating units (31 sites) to avoid MSD convergence problems, and we compute the time-dependent evolution of the wave function for this chain (see Figure 3).

RESULTS AND DISCUSSION

Electron Transfer Pathways.

We analyzed the dominant ET pathways on the basis of hopping distances (see Figure 4a,b) and computed ET parameters (H_{AB} , λ , and G°) for the GS_{PA} and GS_{ARC} structures (see

Table 1). Lovley et al. proposed a mechanism of coherent electron transfer mediated by π -stacked aromatic residues inside the pilus assembly based on the measured temperature-dependent conductivity.^{27,28} In the *G. sulfurreducens* pilin, six aromatic residues are present in the PilA subunit: three phenylalanines at positions 1, 24, and 51 and three tyrosines at positions 27, 32, and 57. The suggested hole transfer pathway, based on the spatial proximity of aromatic residues, has three of the six aromatic residues clustered in the inner space of the *G. sulfurreducens* pilus within 5 Å, establishing the functional HT pathway Phe-1, Phe-24, and Tyr-27 with interaromatic distances ranging from 4 to 7 Å in both the GS_{PA} and GS_{ARC} models (see Figure 4c,d). These three aromatic residues from each pilin monomer form a closely packed periodic helical pathway for electron transport, with the nearest-neighbor pairs in either a parallel-displaced or a T-shaped geometry. These aromatic residues were proposed to serve as possible HT intermediates.²⁹ The HT pathways formed by other aromatic chains are disfavored by their larger interaromatic distances (the other aromatic residues are located in the pilus outer space). The following calculations are based on the HT pathways formed by Phe-1, Phe-24, and Tyr-27.

We performed electronic structure calculations on these two model structures to estimate electronic couplings, reaction free energies, and reorganization energies. We included aromatic side chains of the residues up to the β carbon atom and capped them with hydrogen atoms. The Phe side chain is represented by a toluene molecule, and the Tyr side chain is represented by 4-methylphenol. The electronic couplings between nearest-neighbor aromatics do not exceed tens of millielectronvolts (see Table 1), consistent with results found at similar distances in previous studies of the *G. sulfurreducens* pili system³² (see Table 1). The electronic couplings computed in the previous studies of Tretiak et al.³² are as small as 10^{-5} meV because of the larger distances (~ 10 Å) between aromatics in the earlier modeled structure. The closely packed aromatics in GS_{PA} and GS_{ARC} produce larger electronic couplings. Electronic couplings between π -stacked aromatics in van der Waals contact are typically tens to hundreds of millielectronvolts.^{54–56}

Incoherent Transport.

We now examine nearest-neighbor hopping transport through the aromatic pathway identified above. Although the helically organized aromatics in GS_{PA} are well-separated in distance, steric clashes remain among the other residues in the homology-modeled structure. For GS_{ARC} , the constraints on the model building for closely packed aromatics may reduce the reliability of other features of the modeled structure. Deviations of both modeled structures from the actual structure will change the chemical environment of the dominant ET pathway. Such changes will cause the free energies, couplings, and reorganization energies to change from the modeled values, but we assume that the closely packed aromatics in the inner space of the pili are rigid enough to sustain their relative orientations and distances near those of the modeled structures and that the electronic couplings are not likely to change very much. We computed conductivities for energy landscapes with modified reaction free energies and reorganization energies in order to determine the upper limit of the carrier diffusion constant in the purely incoherent regime. In this regime, effects arising from the electrodes are not taken into explicit consideration in order to approximate

the case where the Fermi levels of the electrodes and ET chains are degenerate, defining the upper bound of the conductivity.

Homology-Modeled Structures.—Our DFT calculations of the truncated aromatic groups found that the HOMO energy of Phe-24 is 0.24 eV below that of Phe-1 because of differences in the protein environments. We examined the dependence of the diffusion coefficient on the Phe-24 HOMO energy and reorganization energy. Both of these energies were varied to find the upper limit of the carrier diffusion coefficient. The computed diffusion coefficient can be as large as 10^{-9} to 10^{-8} cm²/s (eq 5) when the reorganization energy varies between 0.2 and 0.4 eV and the Phe-24 HOMO energy varies between 0.0 and 0.3 eV. The Phe-1 HOMO energy sets the zero of the energy scale (see Figure 5). Therefore, the upper bound of the conductivity is on the order of μ S/cm when the HT Franck–condon factor is maximized.

In a second study, we approximated the reorganization energy for the nearest-neighbor HT between aromatic residue pairs to be equal to the inner-sphere reorganization energies computed with the DFT calculations. We allowed both the Phe-24 and Tyr-27 HOMO energies to be free variables (with the Phe-1 HOMO energy set to 0.0 eV as a reference) in order to model the different chemical environments of the amino acids and to examine the HT reaction free energy dependence. The upper bound of the calculated diffusion coefficient ranges from 10^{-10} to 10^{-9} cm²/s, and the corresponding conductivity is on the order of μ S/cm (see Figure 6). The larger value occurs when the HOMO energy of Phe-24 is near 0.0 eV and the HOMO energy of Tyr-27 is near 0.3 eV. Both studies indicate that the computed conductivity of *G. sulfurreducens* pili can only reach the μ S/cm range, and the computed conductivity is 3 to 4 orders of magnitude smaller than the experimental value (51 mS/cm at pH 7¹⁴) in the nearest-neighbor hopping regime.

Energy-Minimized Structure.—The structural differences between the energy-minimized *G. sulfurreducens* pilus structure and the homology model structure do not change the HT pathways but do change the interaromatic distances and electronic couplings. Two of the three nearest-neighbor couplings increase by factors of 4–5 in the energy-minimized structure compared with the prior homology-modeled structure, while the smallest coupling between Phe24 and Tyr27 remains nearly unchanged (see Table 1). We examined the dependence of the HT rate on G° and λ in the energy-minimized GS_{ARC} structure, as was done in the homology-modeled structures. When the Phe-24 HOMO and reorganization energies are varied in the ranges shown in Figure 7, the upper limit of the diffusion coefficient is 3 orders of magnitude smaller than the value computed for the homology-modeled structure. This decrease in the diffusion coefficient arises from the 0.16 eV energy gap of the initial Tyr-27 HOMO energy relative to the Tyr-27 energy in the homology-modeled structure (see Figure 7). However, when both the Phe-24 and Tyr-27 HOMO energies are varied and the reorganization energy is in the range of 0.2–0.9 eV (obtained from the DFT calculations), the upper bound for the computed diffusion coefficient is 10^{-10} to 10^{-9} cm²/s (where the HOMO energy of Phe-24 is $\sim$ 0.0 eV and the HOMO energy of Tyr-27 is $\sim$ 0.3 eV). These diffusion coefficients correspond to conductivities on the order of μ S/cm (see Figure 8). This result indicates that although the

energy-minimized structure has enhanced electronic couplings, the smallest coupling values cause the diffusion coefficient to be comparable to that of the homology-modeled structure.

Physical Requirements for Cellular Respiration.—We define the effective HT rate $k_{\text{eff}} = D/z^2$ as the inverse of the time required to transverse one repeating unit of the pilus structure, where z is the size of one repeating unit (~ 10 Å) and k_{eff} is 10^5 – 10^6 s $^{-1}$. This effective HT rate is consistent with the reported hopping flux in solvated multiheme cytochromes⁵⁷ and could support the flow of electrons needed to sustain regular cellular respiration in *Geobacter*.²⁹ The average hole transport rate derived experimentally from the linear resistance–distance correlation of a 1 μ m long pilus at a 100 meV bias voltage is on the order 10^8 – 10^9 s $^{-1}$, 2 orders of magnitude larger than the cellular respiration rate.²⁹ Our derived HT rates are 2–3 orders of magnitude smaller than the experimentally measured HT rates, mainly because of the endoergic ET steps from the disordered energy landscapes of the current structures of *G. sulfurreducens* pili.

Temperature Dependence of the Incoherent Hopping Conductivity.—

Experiments by Lovley et al.²⁷ found an inverse temperature–conductivity dependence, similar to the temperature dependence for transport in metals. As a result, coherent ET was proposed as the charge transport mechanism in *G. sulfurreducens* pili.²⁷ However, the observation of an inverse temperature dependence for HT is not sufficient to prove a coherent HT mechanism. For example, in our study the conductivities calculated in the incoherent regime feature an inverse temperature dependence only when the energy landscapes are flat and the reorganization energies are small enough (0.1 eV) to make both the forward and backward ET rates activationless ($G^\ddagger = 0.025$ eV; see Figure S3).

In our multistep hopping model, the predicted temperature dependence of the conductivity can in fact be different from the temperature dependence of each single-step hopping rate (see eqs 1, 5, and 6). We examined the temperature–conductivity dependence of *G. sulfurreducens* pili in the incoherent hopping regime as we varied the Phe-24 HOMO energy and the reorganization energy. We found that the computed diffusion coefficient divided by temperature increases with temperature in the range of 273 to 323 K (see Figures S1 and S2). Tender et al. recently suggested that the temperature dependence of *G. sulfurreducens* biofilm electrical properties can be modified by the hydration state, adding complexity to the relationship between temperature and transport mechanism in *G. sulfurreducens* biofilms.⁵⁸ Theoretical and experimental analyses by other groups also indicate a complex relation between temperature and transport mechanism in *G. sulfurreducens* pili.³⁰

Coherent Transport.

To investigate the coherent ET mechanisms in greater detail, we calculated the conductance as a function of bias voltage using NEGF methods. In the analysis, the temperature was set to 298 K, and the Fermi level of the gold electrode was $E_{\text{Fermi}} = -5.27$ eV, which is about 1.4 eV above the HOMO energy of phenylalanine ($E_{\text{phe}} = -6.67$ eV).⁵⁹ The coupling between the contact electrode and the molecular system is taken to be on the same order of magnitude as the nearest-neighbor coupling, 0.02 eV in this case. In the coherent regime, we used the Fermi level of the gold electrode as the energy reference and placed the HOMO

energies of Phe-1 and Phe-24 in degeneracy, which maximized the charge delocalization. The HOMO energy of Tyr-27 was varied from 0.3 to 0.5 eV above the HOMO energy of Phe for the two structures studied ($E_{\text{Tyr}} = -6.37$ and -6.17 eV).

Homology-Modeled Structure.—We computed the conductances for models of *G. sulfurreducens* pili consisting of 10 to 160 repeating units (10–160 nm). The zero-voltage conductivities computed in the NEGF calculations were 10^{-26} – 10^{-24} S/cm, which are 23 orders of magnitude smaller than the experimentally measured conductivity (51 mS/cm at pH 7¹⁴). With bias voltages from –600 to 600 meV (similar to the experimental values¹⁴), the computed conductivity was on the order of 10^{-26} S/cm, which is also significantly smaller than the experimentally measured conductivity (see Figure 9).

Energy-Minimized Structure.—We computed the conductance and conductivity with the HOMO energy landscape using the energy-minimized structure of GS_{ARC} . With both zero and nonzero bias voltages, the conductivities of the *G. sulfurreducens* pili are on the order of 10^{-26} S/cm for lengths of 10–160 nm, similar to the values computed for the homology-modeled GS_{PA} structure (see Figure 10).

Dependence of the Conductance on the Fermi Energy and Electrode–Molecule Coupling.—To further explore the possibility of purely coherent HT, we investigated the dependence of the conductivity/conductance on the Fermi energy, the electrode–molecule coupling, and the relative energies of the amino acids. We used the same orbital energy configurations as in the previous section (with the HOMO energies of Phe-1 and Phe-24 set to 0.0 eV to maximize delocalization and the HOMO energy of Tyr-27 set to 0.38 eV). We found an increase in the computed conductivity by as much as 4–5 orders of magnitude as the electrode–molecule contact coupling increased from 0.01 to 0.2 eV (see Figure S4). To probe the influence of the nearest-neighbor coupling on the computed conductivity, we increased the nearest-neighbor coupling as much as 10-fold and found as much as a 4 order of magnitude increase in the conductivity (see Figure S5). The most significant conductivity growth was found when the electrode Fermi level was degenerate with the Phe HOMO energy (see Figure S6). The gold electrode Fermi energy inferred from experimental and theoretical studies is in the range of 5.1 to 5.4 eV.⁶⁰ Therefore, the lower limit of the energy gap between the Fermi level and the Phe HOMO is 1.2 eV. The corresponding conductance at this lower limit of the Fermi level is ~20 orders of magnitude smaller than the measured conductance. Therefore, purely coherent transport is unlikely to describe HT in *G. sulfurreducens* pili.

Physical Requirements for Cellular Respiration.—In both of the above studies of purely coherent transport, the conductivity is about 20 orders of magnitude smaller than the measured conductivity (i.e., it is much too small to support respiration), indicating that a purely coherent mechanism for long-range electron transfer in *Geobacter* is unlikely.

Mixed Coherent—Incoherent Transport.

The time scale for fully coherent electron transport over 1 μm in pili systems is about $\tau = 50$ ps, based on a nearest-neighbor coupling of 0.02 eV and a resonant energy structure with

$\tau = \frac{R\hbar}{2rV}$ (where R is the end-to-end distance and r is the nearest-neighbor distance) based on studies of Zhang et al.⁶¹ This time scale is a lower limit to the time scale for coherent ET. Typical decoherence times for biological ET are on the scale of tens of femtoseconds, which is much shorter than the time scales for coherent ET over micrometer distances. Therefore, the interplay between the coherence generated by the electronic interactions and the environment-induced decoherence tunes the ET time scale, creating a mixed coherent–incoherent regime. This mixed ET mechanism is familiar in DNA charge transport, where structural effects can tune the ET mechanism between coherent and incoherent regimes.^{49,50} We assumed that pure dephasing is the dominant cause of decoherence and computed the quantum transport dynamics using the Lindblad model for decoherence. Previous studies of the Fenna–Matthews–Olson (FMO) complex of green sulfur bacteria with a spatially uniform temperature-dependent dephasing rate description⁶² gave dephasing rates from $(18 \text{ fs})^{-1}$ to $(69 \text{ fs})^{-1}$ over a range of temperatures.⁵¹ This time scale is comparable to those found in earlier studies of decoherence time scales in condensed-phase systems.^{63,64} While the dephasing rate in *G. sulfurreducens* pili is unknown, we used approximate values taken from the FMO complex, exploring the dynamics for dephasing times from 10 to 100 fs. We examined the dependence of the transport mechanism and its efficiency on the strength of this environment-induced decoherence. Similar to the incoherent regime, effects arising from electrodes were not taken into consideration in order to approximate the case where the Fermi level of the electrode and the ET chain site energies are degenerate, maximizing the calculated conductivity. The tight-binding Hamiltonian of the system is defined in eq 16, with the HOMO site energy and nearest-neighbor electronic couplings from Table 1.

Homology-Modeled Structure.—The computed hole wave packet width $\langle R^1 \rangle$ as a function of dephasing time is shown in Figure 11. At long times (on the order of 10 ps), the transport reaches the diffusive regime, and $\langle R^2 \rangle$ grows linearly with time. The higher the dephasing rate, the shorter is the time required to reach the diffusive regime and the larger is the diffusion coefficient. When the dephasing rate is $\gamma = (50 \text{ fs})^{-1}$, the computed diffusion coefficient is $5 \times 10^{-5} \text{ cm}^2/\text{s}$, corresponding to a conductivity of 65 mS/cm on the basis of eq 6. This computed conductivity is close to the experimentally measured value of 51 mS/cm at pH 7 and 295 K.¹⁴

Energy-Minimized Structure.—The computed values of $\langle R^2 \rangle$ as a function of time for different dephasing rates are shown in Figure 12. As was found with the homology model, the transport also reaches the diffusive limit at long times, with corresponding conductivities on the order of mS/cm. However, in contrast to the homology-modeled structure, the high dephasing rate does not necessarily produce a large diffusion coefficient in the energy-minimized structure. The largest computed conductivity of 330 mS/cm corresponds to a dephasing rate of $\gamma = (100 \text{ fs})^{-1}$. Fast diffusion for this dephasing rate could result from environmental-noise-assisted quantum transport (ENAQT) produced by the interplay among electronic coupling fluctuations, site energy fluctuations, and interactions with the environment.^{35,49,65–67}

The effects of ENAQT on the intermediate transport regime may originate from several sources: suppression of destructive interference by accessing multiple geometries, electronic

resonance among neighboring sites, or transitions between otherwise stationary eigenstates. Energetic disorder tends to localize wavepackets through destructive interference or Anderson localization effects, thus slowing hole propagation.⁶⁸ Adding decoherence thus diminishes the coherent processes and shortens the time scale required for charge to reach the target. Site-energy fluctuations enhance site-to-site charge transfer rates by bringing neighboring sites into transient resonance. Finally, the system eigenstates are stationary in the absence of decoherence. Incoherent hopping induced by decoherence permits transitions between the eigenstates, producing greater mobility.

Physical Requirements for Cellular Respiration.—In the mixed coherent–incoherent transport regime, the effective electron transfer rate (k_{eff}) in one repeating unit is on the order of 10^9 s^{-1} , which is the same time scale as found for transport along individual *G. sulfurreducens* pili derived from experimentally measured currents.²⁹ This current is sufficient to support extracellular respiration. These results, combined with the previous analysis of purely incoherent and coherent ET, indicate that ET in *G. sulfurreducens* pili is dominated by mixed coherent–incoherent transport. In this mixed regime, electron delocalization among quasi-degenerate phenylalanines may survive long enough to enable hopping among these transient delocalized states. This hopping among delocalized islands can be faster than simple nearest-neighbor hopping.

Role of Coherence in Long-Range HT of *G. sulfurreducens* Pili.—The exponent b of the power law for the mean-square displacement, $\langle R^2 \rangle \propto t^b$, is a reporter of the transport regime, with $b = 1$ corresponding to diffusive transport and $b = 2$ corresponding to ballistic quantum transport.⁵¹ Using the results for GS_{pa} ($\gamma = (20 \text{ fs})^{-1}$), Figure 13 shows the value of b as a function of time for charge transport. At long times (tens of picoseconds), the mixed coherent–incoherent regime produces a transition from the ballistic to the diffusive transport regime. This transition begins at tens of femtoseconds but finishes with a diffusive signature at tens of picoseconds.

The time to reach the diffusive limit ($b = 1$) increases to about 10 ps when the Tyr-27 HOMO energy (above the Phe-1 energy) decreases from 380 meV to resonance with Phe (0 meV) (see Figure 13a). Since energy fluctuations are expected to be on the scale of $\sqrt{\lambda kT}$,⁶¹ these fluctuations are accessible at room temperature. In all cases, the initial rapid change in b with time reflects fast electronic decoherence. The minimum value of b ($b < 1$) corresponds to charge localization in the pilus. As b approaches 1, incoherent hopping dominates hole transport. We also examined the influence of nearest-neighbor couplings on sustaining coherent transport. We increased the nearest-neighbor couplings by factors of 2 and 4, and we found that b decreased to 1 on a time scale on the order of 10 ps, similar to the results of the previous study with calculated nearest-neighbor couplings (see Figure 13b). We also examined the role of static coherence in various dephasing rate regimes. We define a static coherence parameter as follows:

$$\text{static coherence} = \text{Tr}(\rho^2) - \sum_i \rho_{ii}^2 \quad (21)$$

This equation captures the contribution of off-diagonal density matrix elements to the static coherence. In dephasing rate regimes where γ varies from $(10 \text{ fs})^{-1}$ to $(100 \text{ fs})^{-1}$, the static coherence in a 200 ps propagation calculation ranges from $\sim 10^{-4}$ to $\sim 10^{-6}$ depending on the dephasing rate (see Figure 14). These results indicate that fast dephasing (in this case, $(10 \text{ fs})^{-1}$) corresponds to a low static coherence value (10^{-6} in Figure 14a), associated with an ET regime closer to pure nearest-neighbor hopping. However, for slow dephasing (in this case, $(100 \text{ fs})^{-1}$), corresponding to high static coherence (10^{-4} in Figure 14b), a mixed coherent–incoherent regime results. These results indicate that in biological coupling regimes (meV-scale coupling among charge localizing groups), the existence of pure dephasing produces systems that have transport dominated by incoherent hopping. These two studies indicate that in tens to hundreds of femtoseconds the pilus system changes from a quantum walker ($b \approx 2$) to a diffusive walker ($b \approx 1$). In Tender's studies, the average electron transport rate at a voltage of 100 mV is $\sim 9 \times 10^8 \text{ s}^{-1}$.³⁰ This nanosecond transport time scale is longer than the lifetime for coherent transport, confirming our finding that with pure dephasing incoherent hopping dominates the HT mechanism in *G. sulfurreducens* nanowires.

In order to develop a deeper understanding of the contribution of coherence and delocalization to long-range HT in *G. sulfurreducens* pili, we also computed the coherence metric:^{52,69}

$$C(n) = \sum_i |\rho_{i,i+n}| \quad (22)$$

$C(n)$ sums the individual superdiagonals of the density matrix in the site basis, which is a measure of site-to-site coherence. In our calculations, with n ranging from 1 to 3, we computed the coherence within one repeating unit. We found that the nearest-neighbor coherence, $C(1)$, is larger than the second- and third-nearest-neighbor coherences, $C(2)$ and $C(3)$, by a factor of 2 to 5 for various dephasing rates (see SI section S5). This result indicates that the nearest-neighbor coherence produces delocalization between the nearest-neighbor amino acids Phe-1 and Phe-24.

To test our hypothesis of delocalization among nearest-neighbor phenylalanines, we coarse-grained the repeating unit into two sites, one associated with the two Phe's and one with the Tyr. As in the case of purely incoherent hopping, we varied the Tyr HOMO energy and reorganization energy and calculated the corresponding diffusion coefficients. We found that for biologically relevant parameters, compared with the sequential hopping model (see Figure 15b), the diffusion coefficient from the coarse-grained model (see Figure 15a) can be enhanced by ~ 4 orders of magnitude as a result of varying X and G° , indicating that the conductivity can be on the mS/cm scale, which compares favorably to the experimental data.

CONCLUSIONS

Type IV pili are believed to be important contributors to extracellular electron transfer in *G. sulfurreducens*. The long-range HT mechanism in this bacterial nanowire is the subject of

debate, as experiments suggest that *G. sulfurreducens* pili display metallic-like transport characteristics, challenging our understanding of electron transfer in biological systems. We have performed a comprehensive analysis of the HT pathways through aromatic residues based on two new models for the *G. sulfurreducens* pili structures, with mechanisms ranging from purely incoherent transport to purely coherent transport. Neither the purely incoherent nor the purely coherent regime is adequate to describe transport in the *G. sulfurreducens* pili. The computed incoherent conductivity values without delocalization among amino acids are orders of magnitude less than the measured values. In the intermediate regime where both incoherent and coherent mechanisms contribute to transport, the conductivity is computed to be tens of mS/cm, which is similar to the experimental values. However, thermal disorder is expected to make delocalization across these islands difficult to sustain. Our studies find that the likely origin of this intermediate transport mechanism lies in the structure and energetics of the HT pathways. The calculations point to an important role for aromatic residues that form the transport pathways, especially Tyr-27, whose site energy offset from that of Phe residues determines the transport mechanism. Future mutation studies on residue 27 may shed further light on the mechanism. Removal of aromatics (e.g., by alanine mutation) or changes in the aromatic structures (e.g., phenylalanine to tryptophan mutations) will help in understanding the role of sequence in transport. A deeper understanding of how structure influences electron fluxes through bacterial nanowires will likely have implications for biogeochemistry, bioelectrochemistry, microbial pathogenesis, and the development of next-generation bioelectronic systems.

A very recent published paper by Malvankar et al. explores the structure of *G. sulfurreducens* bacterial nanowires composed of micrometer-long assemblies of cytochrome OmcS, with hemes closely spaced (4–6 Å), creating a compelling path for long-range extracellular ET.⁷⁰ The distances between nearest heme cofactors are similar to distances found in the deca-heme cytochrome MtrF of the *S. onedensis* outer membrane, with electronic couplings of 10^{-2} – 10^1 meV.⁷¹ These coupling strengths fall in the coupling regime discussed here, and a mixed coherent–incoherent regime is also possible; the quasidegenerate heme energy landscape will accelerate incoherent transport.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- (1). Malvankar NS; Lovley DR Microbial Nanowires for Bioenergy Applications. Curr. Opin. Biotechnol 2014, 27, 88–95. [PubMed: 24863901]
- (2). Wanger G; Gorby Y; El-Naggar MY; Yuzvinsky TD; Schaudinn c.; Gorur A; Sedghizadeh PP Electrically conductive Bacterial Nanowires in Bisphosphonate-Related Osteonecrosis of the Jaw Biofilms. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod 2013, 115, 71–78.
- (3). Malvankar NS; Lovley DR Microbial Nanowires: a New Paradigm for Biological Electron Transfer and Bioelectronics. ChemSusChem 2012, 5, 1039–1046. [PubMed: 22614997]

- (4). El-Naggar MY; Finkel SE Live Wires. *Scientist* 2013, 27 (May), 38–43.
- (5). Stams AJ; Plugge c. M. Electron Transfer in Syntrophic communities of Anaerobic Bacteria and Archaea. *Nat. Rev. Microbiol* 2009, 7, 568–577. [PubMed: 19609258]
- (6). McGlynn SE; chadwick GL; Kempes c. P.; Orphan VJ Single cell Activity Reveals Direct Electron Transfer in Methano-trophic Consortia. *Nature* 2015, 526, 531–535. [PubMed: 26375009]
- (7). Rotaru A-E; Shrestha PM; Liu F; Markovaite B; chen S; Nevin KP; Lovley DR Direct Interspecies Electron Transfer Between *Geobacter Metallireducens* and *Methanosarcina Barkeri*. *Appl. Environ. Microbiol* 2014, 80, 4599–4605. [PubMed: 24837373]
- (8). Lovley DR Dissimilatory Metal Reduction. *Annu. Rev. Microbiol* 1993, 47, 263–290. [PubMed: 8257100]
- (9). Beratan DN; Betts J; Onuchic J Protein Electron Transfer Rates Set by the Bridging Secondary and Tertiary Structure. *Science* 1991, 252, 1285–1288. [PubMed: 1656523]
- (10). Beratan D; Skourtis S Electron Transfer Mechanisms. *Curr. Opin. Chem. Biol* 1998, 2, 235–243. [PubMed: 9667934]
- (11). Devault D Quantum Mechanical Tunnelling in Biological Systems. *Q. Rev. Biophys* 1980, 13, 387–564. [PubMed: 7015406]
- (12). Boesen T; Nielsen LP Molecular Dissection of Bacterial Nanowires. *mBio* 2013, 4, e00270–13. [PubMed: 23653449]
- (13). Pirbadian S; Barchinger SE; Leung KM; Byun HS; Jangir Y; Bouhenni RA; Reed SB; Romine MF; Saffarini DA; Shi L; et al. *Shewanella Oneidensis* MR-1 Nanowires are Outer Membrane and Periplasmic Extensions of the Extracellular Electron Transport Components. *Proc. Natl. Acad. Sci. U. S. A* 2014, 111, 12883–12888. [PubMed: 25143589]
- (14). Adhikari RY; Malvankar NS; Tuominen MT; Lovley DR Conductivity of Individual *Geobacter* Pili. *RSC Adv.* 2016, 6, 8354–8357.
- (15). Byun HS; Pirbadian S; Nakano A; Shi L; El-Naggar MY Kinetic Monte carlo Simulations and Molecular conductance Measurements of the Bacterial Decaheme cytochrome MtrF. *ChemElectroChem* 2014, 1, 1932–1939.
- (16). Pirbadian S; El-Naggar MY Multistep Hopping and Extracellular charge Transfer in Microbial Redox chains. *phys. Chem. Chem. Phys* 2012, 14, 13802–13808. [PubMed: 22797729]
- (17). Shi L; Richardson DJ; Wang Z; Kerisit SN; Rosso KM; Zachara JM; Fredrickson JK The Roles of Outer Membrane cytochromes of *Shewanella* and *Geobacter* in Extracellular Electron Transfer. *Environ. Microbiol. Rep* 2009, 1, 220–227. [PubMed: 23765850]
- (18). Richter H; Nevin KP; Jia H; Lowy DA; Lovley DR; Tender LM Cyclic Voltammetry of Biofilms of Wild Type and Mutant *Geobacter Sulfurreducens* on Fuel cell Anodes Indicates Possible Roles of OmcB, OmcZ, Type IV Pili, and Protons in Extracellular Electron Transfer. *Energy Environ. Sci* 2009, 2, 506–516.
- (19). Leang C; Qian X; Mester T; Lovley DR Alignment of the c-Type cytochrome OmcS along Pili of *Geobacter Sulfurreducens*. *Appl. Environ. Microbiol* 2010, 76, 4080–4084. [PubMed: 20400557]
- (20). Malvankar NS; Tuominen MT; Lovley DR Lack of Cytochrome Involvement in Long-Range Electron Transport Through Conductive Biofilms and Nanowires of *Geobacter Sulfurreducens*. *Energy Environ. Sci* 2012, 5, 8651–8659.
- (21). Polizzi NF; Skourtis SS; Beratan DN Physical Constraints on Charge Transport Through Bacterial Nanowires. *Faraday Discuss.* 2012, 155, 43–61. [PubMed: 22470966]
- (22). Feliciano GT; da Silva AJ; Reguera G; Artacho E Molecular and Electronic Structure of the Peptide Subunit of *Geobacter Sulfurreducens* Conductive Pili from First Principles. *J. Phys. Chem. A* 2012, 116, 8023–8030. [PubMed: 22779741]
- (23). Feliciano G; Steidl R; Reguera G Structural and Functional Insights into the Conductive Pili of *Geobacter Sulfurreducens* Revealed in Molecular Dynamics Simulations. *Phys. Chem. Chem. Phys* 2015, 17, 22217–22226. [PubMed: 26243427]
- (24). Lebedev N; Mahmud S; Griva I; Blom A; Tender LM On the Electron Transfer Through *Geobacter Sulfurreducens* PilA Protein. *J. Polym. Sci., Part B: Polym. Phys* 2015, 53, 1706–1717.

- (25). Holmes DE; Dang Y; Walker DJF; Lovley DR The Electrically Conductive Pili of *Geobacter* Species are a Recently Evolved Feature for Extracellular Electron Transfer. *Microb. Genomics* 2016, 2, e000072.
- (26). Malvankar NS; Yalcin SE; Tuominen MT; Lovley DR Visualization of Charge Propagation Along Individual Pili Proteins Using Ambient Electrostatic Force Microscopy. *Nat. Nanotechnol* 2014, 9, 1012–1017. [PubMed: 25326694]
- (27). Malvankar NS; Vargas M; Nevin KP; Franks AE; Leang C; Kim B-C; Inoue K; Mester T; Covalla SF; Johnson JP; et al. Tunable Metallic-like Conductivity in Microbial Nanowire Networks. *Nat. Nanotechnol* 2011, 6, 573–579. [PubMed: 21822253]
- (28). Vargas M; Malvankar NS; Tremblay P-L; Leang C; Smith JA; Patel P; Synoeyenbos-West O; Nevin KP; Lovley DR Aromatic Amino Acids Required for Pili Conductivity and Long-Range Extracellular Electron Transport in *Geobacter Sulfurreducens*. *mBio* 2013, 4, e00105–13. [PubMed: 23481602]
- (29). Lampa-Pastirk S; Veazey JP; Walsh KA; Feliciano GT; Steidl RJ; Tessmer SH; Reguera G Thermally Activated Charge Transport in Microbial Protein Nanowires. *Sci. Rep* 2016, 6, 23517. [PubMed: 27009596]
- (30). Yates MD; Golden JP; Roy J; Strycharz-Glaven SM; Tsoi S; Erickson JS; El-Naggar MY; Calabrese Barton S; Tender LM Thermally Activated Long Range Electron Transport in Living Biofilms. *Phys. Chem. Chem. Phys* 2015, 17, 32564–32570. [PubMed: 26611733]
- (31). Reardon PN; Mueller KT Structure of the Type IVa Major Pilin from the Electrically Conductive Bacterial Nanowires of *Geobacter Sulfurreducens*. *J. Biol. Chem* 2013, 288, 29260–29266. [PubMed: 23965997]
- (32). Yan H; Chuang C; Zhugayevych A; Tretiak S; Dahlquist FW; Bazan GC Inter-Aromatic Distances in *Geobacter Sulfurreducens* Pili Relevant to Biofilm Charge Transport. *Adv. Mater* 2015, 27, 1908–1911. [PubMed: 25604785]
- (33). Malvankar NS; Vargas M; Nevin K; Tremblay P-L; Evans-Lutterodt K; Nykypanchuk D; Martz E; Tuominen MT; Lovley DR Structural Basis for Metallic-like Conductivity in Microbial Nanowires. *mBio* 2015, 6, e00084–15. [PubMed: 25736881]
- (34). Xiao K; Malvankar NS; Shu C; Martz E; Lovley DR; Sun X Low Energy Atomic Models Suggesting a Pilus Structure that could Account for Electrical Conductivity of *Geobacter Sulfurreducens* Pili. *Sci. Rep* 2016, 6, 23385. [PubMed: 27001169]
- (35). Liu C; Xiang L; Zhang Y; Zhang P; Beratan DN; Li Y; Tao N Engineering Nanometre-Scale Coherence in Soft Matter. *Nat. Chem* 2016, 8, 941–945. [PubMed: 27657870]
- (36). Tan Y; Adhikari RY; Malvankar NS; Ward JE; Nevin KP; Woodard TL; Smith JA; Snoeyenbos-West OL; Franks AE; Tuominen MT; Lovley DR The Low Conductivity of *Geobacter Uraniireducens* Pili Suggests a Diversity of Extracellular Electron Transfer Mechanisms in the Genus *Geobacter*. *Front. Microbiol* 2016, 7, 980. [PubMed: 27446021]
- (37). Gray HB; Winkler JR Electron Flow Through Proteins. *Chem. Phys. Lett* 2009, 483, 1–9.
- (38). Warren JJ; Ener ME; Vlcek A; Winkler JR; Gray HB Electron Hopping Through Proteins. *Coord. Chem. Rev* 2012, 256, 2478–2487. [PubMed: 23420049]
- (39). Marcus RA; Sutin N Electron Transfers in Chemistry and Biology. *Biochim. Biophys. Acta* 1985, 811, 265–322.
- (40). Becke AD Density-Functional Thermochemistry. III. The Role of Exact Exchange. *J. Chem. Phys* 1993, 98, 5648–5652.
- (41). Lee C; Yang W; Parr RG Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B: Condens. Matter Mater. Phys* 1988, 37, 785.
- (42). Ditchfield R; Hehre WJ; Pople JA Self-Consistent Molecular-Orbital Methods. IX. An Extended Gaussian-Type Basis for Molecular-Orbital Studies of Organic Molecules. *J. Chem. Phys* 1971, 54, 724–728.
- (43). Cave RJ; Newton MD Calculation of Electronic Coupling Matrix Elements for Ground and Excited State Electron Transfer Reactions: Comparison of the Generalized Mulliken–Hush and Block Diagonalization Methods. *J. Chem. Phys* 1997, 106, 9213–9226.
- (44). Nelsen SF; Blackstock SC; Kim Y Estimation of Inner Shell Marcus Terms for Amino Nitrogen Compounds by Molecular Orbital Calculations. *J. Am. Chem. Soc* 1987, 109, 677–682.

- (45). Berlin YA; Ratner MA Intra-Molecular Electron Transfer and Electric Conductance Via Sequential Hopping: Unified Theoretical Description. *Radiat. Phys. Chem* 2005, 74, 124–131.
- (46). Derrida B Velocity and Diffusion Constant of a Periodic OneDimensional Hopping Model. *J. Stat. Phys* 1983, 31, 433–450.
- (47). Ryndyk DA; Gutierrez R; Song B; Cuniberti G Green Function Techniques in the Treatment of Quantum Transport at the Molecular Scale In *Energy Transfer Dynamics in Biomaterial Systems*; Burghardt I, May V, Micha DA, Bittner ER, Eds.; Springer, 2009; pp 213–335.
- (48). Arago J; Troisi A Regimes of Exciton Transport in Molecular Crystals in the Presence of Dynamic Disorder. *Adv. Funct. Mater* 2016, 26, 2316–2325.
- (49). Liu C; Beratan DN; Zhang P Coarse-Grained Theory of Biological Charge Transfer with Spatially and Temporally Correlated Noise. *J. Phys. Chem. B* 2016, 120, 3624–3633. [PubMed: 27008541]
- (50). Renaud N; Berlin YA; Lewis FD; Ratner MA Between Superexchange and Hopping: an Intermediate Charge-Transfer Mechanism in Poly (A)-Poly (T) DNA Hairpins. *J. Am. Chem. Soc* 2013, 135, 3953–3963. [PubMed: 23402652]
- (51). Hoyer S; Sarovar M; Whaley KB Limits of Quantum Speedup in Photosynthetic Light Harvesting. *New J. Phys* 2010, 12, 065041.
- (52). Moix JM; Khasin M; Cao J Coherent Quantum Transport in Disordered Systems: I. The Influence of Dephasing on the Transport Properties and Absorption Spectra on One-Dimensional Systems. *New J. Phys* 2013, 15, 085010.
- (53). Saalfrank P; Baer R; Kosloff R Density Matrix Description of Laser-Induced Hot Electron Mediated Photodesorption of NO from Pt (111). *Chem. Phys. Lett* 1994, 230, 463–472.
- (54). Polizzi NF; Migliore A; Therien MJ; Beratan DN Defusing Redox Bombs? *Proc. Natl. Acad. Sci. U. S. A* 2015, 112, 10821–10822. [PubMed: 26290579]
- (55). Venkatramani R; Keinan S; Balaeff A; Beratan DN Nucleic Acid Charge Transfer: Black, White and Gray. *Coord. Chem. Rev* 2011, 255, 635–648. [PubMed: 21528017]
- (56). Troisi A Charge Dynamics Through Pi-Stacked Arrays of Conjugated Molecules: Effect of Dynamic Disorder in Different Transport/Transfer Regimes. *Mol. Simul* 2006, 32, 707–716.
- (57). Jiang X; Burger B; Gajdos F; Bortolotti C; Futera Z; Breuer M; Blumberger J Kinetics of Trifurcated Electron Flow in the Decaheme Bacterial Proteins MtrC and MtrF. *Proc. Natl. Acad. Sci. U. S. A* 2019, 116, 3425–3430. [PubMed: 30755526]
- (58). Phan H; Yates MD; Kirchhofer ND; Bazan GC; Tender LM; Nguyen T-Q Biofilm as a Redox Conductor: a Systematic Study of the Moisture and Temperature Dependence of Its Electrical Properties. *Phys. Chem. Chem. Phys* 2016, 18, 17815–17821. [PubMed: 27327215]
- (59). Paul A; Bezer S; Venkatramani R; Kocsis L; Wierzbinski E; Balaeff A; Keinan S; Beratan DN; Achim C; Waldeck DH Role of Nucleobase Energetics and Nucleobase Interactions in SingleStranded Peptide Nucleic Acid Charge Transfer. *J. Am. Chem. Soc* 2009, 131, 6498–6507. [PubMed: 19382798]
- (60). CRC Handbook of Chemistry and Physics, 95th ed.; Haynes WM, Ed.; CRC Press, 2014.
- (61). Zhang Y; Liu C; Balaeff A; Skourtis SS; Beratan DN Biological Charge Transfer via Flickering Resonance. *Proc. Natl. Acad. Sci. U. S. A* 2014, 111, 10049–10054. [PubMed: 24965367]
- (62). Rebentrost P; Mohseni M; Kassal I; Lloyd S; Aspuru-Guzik A Environment-Assisted Quantum Transport. *New J. Phys* 2009, 11, 033003.
- (63). Nagy A; Prokhorenko V; Miller RD Do We Live in a Quantum World? Advances in Multidimensional Coherent Spectroscopies Refine our Understanding of Quantum Coherences and Structural Dynamics of Biological Systems. *Curr. Opin. Struct. Biol* 2006, 16, 654–663. [PubMed: 16982186]
- (64). Prezhdo OV; Rossky PJ Relationship Between Quantum Decoherence Times and Solvation Dynamics in Condensed Phase Chemical Systems. *Phys. Rev. Lett* 1998, 81, 5294.
- (65). Walschaers M; Schlawin F; Wellens T; Buchleitner A Quantum Transport on Disordered and Noisy Networks: an Interplay of Structural Complexity and Uncertainty. *Annu. Rev. Condens. Matter Phys* 2016, 7, 223–248.
- (66). Plenio MB; Huelga SF Dephasing-Assisted Transport: Quantum Networks and Biomolecules. *New J. Phys* 2008, 10, 113019.

- (67). Troisi A; Orlandi G Charge-Transport Regime of Crystalline Organic Semiconductors: Diffusion Limited by Thermal Off-Diagonal Electronic Disorder. *Phys. Rev. Lett* 2006, 96, 086601. [PubMed: 16606209]
- (68). Anderson PW Absence of Diffusion in Certain Random Lattices. *Phys. Rev* 1958, 109, 1492.
- (69). Levi F; Mostarda S; Rao F; Mintert F Quantum Mechanics of Excitation Transport in Photosynthetic Complexes: A Key Issues Review. *Rep. Prog. Phys* 2015, 78, 082001. [PubMed: 26194028]
- (70). Wang F; Gu Y; O'Brien JP; Yi SM; Yalcin SE; Srikanth V; Shen C; Vu D; Ing NL; Hochbaum AI; et al. Structure of Microbial Nanowires Reveals Stacked Hemes That Transport Electrons over Micrometers. *Cell* 2019, 177, 361–369. [PubMed: 30951668]
- (71). Breuer M; Rosso KM; Blumberger J Electron Flow in Multiheme Bacterial Cytochromes is a Balancing Act Between Heme Electronic Interaction and Redox Potentials. *Proc. Natl. Acad. Sci. U. S. A* 2014, 111, 611–616. [PubMed: 24385579]

Figure 1.

Schematic representations of electron flow through bacterial nanowires via (a) incoherent electron hopping and (b) metallic-like conduction. In the hopping mechanism, charges move incoherently among adjacent hemes. In the metallic-like mechanism, charge moves in a coherent wavelike manner through the nanowire. Hopping is widely believed to describe transport in *S. oneidensis*, and metallic-like conduction is a candidate mechanism in *G. sulfurreducens*.

Figure 2.

One-dimensional hopping chain with N groups per repeating unit. The ET rates are W_{ij} .⁴⁶

Figure 3. Mixed coherent–incoherent mechanism for the dominant electron transfer route in *G. sulfurreducens* pili, where spreading of the wave function (measured by the mean-square displacement) is computed as a function of time using the Liouville equation with pure dephasing. The effective diffusion coefficient is computed from the time evolution of the particle’s distribution in space.

Figure 4.

(a, b) Helical HT pathways formed by aromatic residues (yellow) in (a) GS_{pa} and (b) GS_{ARC} . (c, d) Geometries of the neighboring aromatic rings that repeat along the axes of (c) GS_{pa} and (d) GS_{ARC} . All center-to-center distances are in Å.

Figure 5. Base-10 logarithm of the diffusion coefficient ($\log_{10}(D/\text{cm}^2/\text{s})$) as a function of the Phe-24 HOMO energy and reorganization energy for the GS_{pa} structure.

Figure 6. Base-10 logarithm of the diffusion coefficient ($\log_{10}(D_{\text{cm}}^2/\text{s})$) as a function of the Phe-24 and Tyr-27 HOMO energies for the GS_{pa} structure.

Figure 7. Base-10 logarithm of the diffusion coefficient ($\log_{10}(D/\text{cm}^2/\text{s})$) as a function of the Phe-24 HOMO energy and the reorganization energy for the GS_{ARC} structure.

Figure 8. Base-10 logarithm of the diffusion coefficient ($\log_{10}(D_{\text{cm}}^2/\text{s})$) as a function of the Phe-24 and Tyr-27 HOMO energies for the GS_{ARC} structure.

Figure 9.
I-V relation for *G. sulfurreducens* pili from NEGF analysis for the GS_{pa} structure.

Figure 10.
I–V relation for *G. sulfurreducens* pili from NEGF analysis for the GS_{ARC} structure.

Figure 11.

Hole wave function width as a function of time for the homology-modeled structure of *G. sulfurreducens* pili. At long times, the diffusive regime is reached, and the diffusion coefficient is given by the slope of the line.

Figure 12. Wave function spreading as a function of time for the energy-minimized structure of *G. sulfurreducens* pili. At long times, the diffusive regime is reached, and the diffusion coefficient is given by the slope of the line.

Figure 13.

Polynomial regression fit of the power-law exponent b for the mean-square displacement $\langle R^2 \rangle \propto t^b$ for transport along GSpa pili with either (a) various Tyr-27 HOMO energies or (b) various coupling factors with the ordinary case.

Figure 14.

(a) Static coherence (eq 21) for a 200 ps calculation on GS_{PA} with a dephasing rate of $(10 \text{ fs})^{-1}$. This rapid dephasing produces low static coherence at long times, resulting in an ET regime similar to nearest-neighbor hopping. (b) Static coherence for a 200 ps calculation on GS_{pa} with a dephasing rate of $(100 \text{ fs})^{-1}$. This slow dephasing produces high static coherence at long times, resulting in mixed coherent–incoherent transport.

Figure 15.

(a) Base-10 logarithm of the diffusion coefficient in a coarse-grained model where the two nearest-neighbor phenylalanines are coarsegrained as one site. The nearest-neighbor Phe–Tyr coupling was set to 20 meV, which is the same magnitude as in the earlier analysis. For biologically relevant windows of energy (the lower left corner of the figures), the diffusion coefficient produces a conductivity on the mS/cm scale, comparable to the experimental data. (b) For comparison, the base-10 logarithm of the diffusion coefficient was computed in the sequential hopping model, where both Phe-1 and Phe-24 have the same HOMO energy while the Tyr-27 HOMO energy and the reorganization energy are varied as shown. The coarse-grained hopping model produces diffusion coefficient enhancements for every combination of λ and G° values compared with the sequential hopping model.

Table 1.

Dominant Physical Charge Transport Pathways in GS_{PA} and GS_{ARC} with Center-to-Center Distances (R)^a and Electronic Couplings (H_{DA})

hole pathway	R of GS_{PA} (Å)	$ H_{DA} $ of GS_{PA} (meV)	R of GS_{ARC} (Å)	$ H_{DA} $ of GS_{ARC} (meV)
Phe-1-Phe-24	7.4 (5.2)	2.4×10^1	4.6 (3.5)	1.1×10^2
Phe-24-Tyr-27	6.5 (4.9)	4.7×10^0	5.5 (3.6)	4.4×10^0
Tyr-27-Phe-1	6.6 (2.9)	1.0×10^1	4.1 (3.5)	5.0×10^1

^aDistances shown in parentheses are the closest distances between heavy atoms on the adjacent aromatic rings.