The Archaellum of *Methanospirillum hungatei* Is Electrically Conductive

David J. F. Walker,a,b Eric Martz,a Dawn E. Holmes,a,c Zimu Zhou,d Stephen S. Nonnenmann,b,d Derek R. Lovleya,b

aDepartment of Microbiology, University of Massachusetts—Amherst, Amherst, Massachusetts, USA
bInstitute for Applied Life Sciences, University of Massachusetts—Amherst, Amherst, Massachusetts, USA
cDepartment of Physical and Biological Science, Western New England University, Springfield, Massachusetts, USA
dDepartment of Mechanical and Industrial Engineering, University of Massachusetts—Amherst, Amherst, Massachusetts, USA

**ABSTRACT** Microbially produced electrically conductive protein filaments are of interest because they can function as conduits for long-range biological electron transfer. They also show promise as sustainably produced electronic materials. Until now, microbially produced conductive protein filaments have been reported only for bacteria. We report here that the archaellum of *Methanospirillum hungatei* is electrically conductive. This is the first demonstration that electrically conductive protein filaments have evolved in *Archaea*. Furthermore, the structure of the *M. hungatei* archaellum was previously determined (N. Poweleit, P. Ge, H. N. Nguyen, R. R. O. Loo, et al., Nat Microbiol 2:16222, 2016, https://doi.org/10.1038/nmicrobiol.2016.222). Thus, the archaellum of *M. hungatei* is the first microbially produced electrically conductive protein filament for which a structure is known. We analyzed the previously published structure and identified a core of tightly packed phenylalanines that is one likely route for electron conductance. The availability of the *M. hungatei* archaellum structure is expected to substantially advance mechanistic evaluation of long-range electron transport in microbially produced electrically conductive filaments and to aid in the design of “green” electronic materials that can be microbially produced with renewable feedstocks.

**IMPORTANCE** Microbially produced electrically conductive protein filaments are a revolutionary, sustainably produced, electronic material with broad potential applications. The design of new protein nanowires based on the known *M. hungatei* archaellum structure could be a major advance over the current empirical design of synthetic protein nanowires from electrically conductive bacterial pili. An understanding of the diversity of outer-surface protein structures capable of electron transfer is important for developing models for microbial electrical communication with other cells and minerals in natural anaerobic environments. Extracellular electron exchange is also essential in engineered environments such as bioelectrochemical devices and anaerobic digesters converting wastes to methane. The finding that the archaellum of *M. hungatei* is electrically conductive suggests that some archaea might be able to make long-range electrical connections with their external environment.

**KEYWORDS** protein nanowire, conductive pili, electromicrobiology

Electrically conductive pili (e-pili) expressed by microbes in the domain *Bacteria* play an important role in extracellular electron exchange between cells and their extracellular environment (1, 2). e-Pili are found in diverse bacteria (1, 3, 4) but have been studied most extensively in *Geobacter sulfurreducens* and related *Geobacter* species in which e-pili are essential for long-range electron transport to Fe(III) oxide minerals, interspecies electron transfer, and electron conduction through biofilms (1).
e-Pili enable unprecedented long-range (micrometer) electron conduction along the length of a protein filament, which not only has important biological implications but also suggests diverse applications for these “protein nanowires” as a sustainably produced electronic material (1, 5–7). There is substantial debate over the potential mechanisms of long-range electron transport in e-pili (1, 6, 7). Although it has been possible to determine the structure of some pili with cryo-electron microscopy (cryo-EM) (8), an experimentally determined structure of G. sulfurreducens e-pili that could help clarify electron transport mechanisms is not available. However, from the known importance of aromatic amino acids for the conductivity of e-pili (1), synthetic electrically conductive protein nanowires have been designed that are either microbially produced (9) or assembled in vitro (10).

The finding that e-pili have independently evolved multiple times in Bacteria (3) raised the question of whether conductive protein filaments have ever evolved in Archaea. Diverse Archaea exchange electrons with their extracellular environment, reducing extracellular electron acceptors or engaging in direct interspecies electron transfer (DIET) with bacteria (2, 11). The alpha-helix filament structure of archaella, as well as the mechanisms for assembly and export, resembles that of type IV pili (8, 12, 13). However, detailed analysis of the Methanospirillum hungatei archaellum also revealed important differences from previously described structures of bacterial pili, such as a lack of an inner channel and a distinct tertiary structure and subunit packing arrangement (13).

**The Methanospirillum hungatei archaellum is electrically conductive.** We chose the methanogen Methanospirillum hungatei for the initial search for an electrically conductive archaellum (e-archaellum) because M. hungatei is capable of reducing extracellular electron acceptors (14), archaellum expression is readily induced in M. hungatei (15), and a cryo-EM (3.4-Å) structure of the archaellum is available (13).

Initial screening of the relative conductivity of diverse bacterial pili is typically determined with conductive atomic force microscopy in which samples are deposited on a conductive surface and a conductive tip serves as a translatable top electrode (16–19). Therefore, 100 µl of a culture of M. hungatei grown in low-phosphate medium to induce archaellum expression (15) was drop-cast onto highly oriented pyrolytic graphite (HOPG), washed, dried, and then equilibrated at 40% relative humidity for conductivity measurements. This process was designed to mimic physiologically relevant conditions by avoiding chemical alteration of the archaellum structure and determining conductivity of hydrated archaella.

Cells with a polar archaellum with the expected height of 10 nm (13) were readily detected with topographic imaging in contact mode (Fig. 1a, b, and d). Conductive imaging demonstrated that the archaellum was electrically conductive (Fig. 1c to e; see also Fig. S1 and S2 in the supplemental material). Point-mode current-voltage (I-V) spectroscopy revealed a linear-like response with currents that were higher than at the same voltage with G. sulfurreducens e-pili prepared in the same manner (Fig. 1e). The pili of G. sulfurreducens strain Aro-5, which produces pili specifically designed for low conductivity (20, 21), exhibited very low currents at the same voltages (Fig. 1e). Conductance estimated from the linear portion of the I-V curves yielded conductance estimates of 16.9 ± 3.9 nS (mean ± standard deviation; n = 9; three independent points on three separate archaella; 8,000 points of measurement taken for each experimental I-V curve comprised of quadruplicate 0.6-V-bias sweeps) for the archaella, 4.5 ± 0.3 nS for the wild-type G. sulfurreducens pili, and only 0.004 ± 0.002 nS for the Aro-5 pili. The estimated conductance of the wild-type G. sulfurreducens pili was similar to the values found in previous studies that employed a comparable measurement technique (16).

These results demonstrated that the M. hungatei archaellum is conductive and suggest that a search for electrically conductive protein filaments in other Archaea as well as the Eukarya is warranted. It has been proposed that electrically conductive filaments of anaerobic methane-oxidizing archaea may be conduits for extracellular...
transfer to electron-accepting partners (22). Other possible benefits of archaellum conductivity might include facilitating attachment by dissipating charge barriers between cells and surfaces or electrical signaling between cells. Expression of synthetic, poorly conductive pili has played an important role in elucidating the function of e-pili in Geobacter species (1). Similar functional studies of M. hungatei will require the development of genetic tools for this microbe.

The M. hungatei archaellum contains a core of closely packed phenylalanines. The cryo-EM structure of the M. hungatei e-archaellum (Fig. 2a), previously reported by Poweleit et al. (13), provides a much needed first opportunity to directly evaluate possible routes for long-range electron transport along a biologically produced protein filament. Aromatic rings of phenylalanine, tyrosine, and tryptophan are grouped into three well-separated regions: an outer sleeve (Fig. 2b and Fig. S5a), a middle sleeve (Fig. 2b and Fig. S5b), and a core (Fig. 2c). It was previously noted that the N-terminal phenylalanine residues in the archaellin subunits (Phe1) interact to “create a spokes effect via a π-stacking sandwich” that plays a key role in stabilizing the structure (13). Additional analysis of the distribution of aromatic amino acids (Fig. 2b and c and

FIG 1 Electrical conductivity of the Methanospirillum hungatei archaellum determined with atomic force microscopy. (a) Contact topographic imaging of M. hungatei showing the polar archaellum protruding from the cell. The white box designates the region chosen for additional analysis. (b) Higher-resolution topographic image of the archaellum from the region shown in a white box in panel a. The red line indicates the position for the topographic height and current cross-sectional line profile analysis. (c) Local current image of the individual archaellum with an applied bias of 300 mV. (d) Topographic height and current response from the cross-section designated in panel b. (e) Point-mode current response (I-V) spectroscopy of the individual archaellum (blue). The applied force was 1 nN (see Fig. S3 in the supplemental material). Similar I-V analyses of the wild-type e-pili of G. sulfurreducens (black) and the poorly conductive pili of G. sulfurreducens strain Aro-5 (green) are shown for comparison. A HOPG control is shown in Fig. S4. The M. hungatei archaellum conductivity measurement shown is representative of three independent measurements on three archaella (see Fig. S1 and S2 for additional examples).
Fig. S5) further revealed that the aromatic rings of Phe1 and Phe13 in the core of the structure are packed almost as close as is physically possible (distances between ring centers of Phe1 and Phe13 of 4.5 and 5.1 Å), with angled T-shaped geometric orientations, which previous studies have suggested may enable \(\pi\)-\(\pi\) interactions (23). Furthermore, recent experimental evidence has indicated that, even in the absence of \(\pi\)-\(\pi\) stacking, phenylalanines within the hydrophobic core of an amino acid \(\alpha\)-helical structure can facilitate long-range electron transport (10, 24). Therefore, our working hypothesis is that the Phe1-Phe13 core is at least one of the features contributing to the e-archaellum conductivity. Other aromatic amino acids of note include Phe20 (Fig. 2b), which is positioned close to the Phe1-Phe13 core, as well as outer and middle sleeves of aromatics that are well separated from each other and from the Phe1,13,20 core (Fig. 2b). Unlike the core, the outer and middle aromatic sleeves lack any closely spaced continuous chain of aromatics extending the length of the filament (Fig. S5).

Fig. 2 A core chain of tightly packed aromatic rings is evident in the distribution of aromatic amino acids in the structure of the *M. hungatei* archaellum determined previously by Poweleit et al. (PDB accession no. or code 5TFY and EMDB code 8405 [13]). (a) The atomic model 5TFY is an assembly of 26 archaellin protein chains (all atoms shown space filling at van der Waals radii, each chain a distinct color, axis vertical). The cryo-EM map (EMDB code 8405), not shown, spans a larger number of chains, and a complete archaellum consists of ~61,500 archaellin chains (13). (b) In cross section (axis perpendicular to the image), aromatic rings form three well-separated groups: a core (Phe1 blue, Phe13 cyan, Phe20 dim yellow), a middle sleeve, and an outer sleeve (Phe and Tyr yellow; Trp orange). (c) Tightly packed core of alternating Phe1 (blue) and Phe13 (cyan) rings (axis horizontal). Ring center distances are 4.5 and 5.1 Å. Phe20, shown in dim yellow in panel b, is not shown in panel c due to wider spacing and positioning peripheral to the core chain of Phe1 and Phe13. Protein main chain traces are shown in green in panels b and c. Images and measurements were made with Jmol.Org.

Microbially produced protein nanowires show substantial promise as a sustainable “green” electronic material with possibilities for functionalization and biocompatibility not available with other nanowire materials (1, 5–7). e-Archaella offer a unique opportunity to directly examine how synthetic designs to tune conductivity and/or add functionality influences protein nanowire structure, enabling a less empirical approach to the design of protein nanowire electronics.
**Methods.** *M. hungatei* was grown as previously described (1) in low-phosphate medium to induce archaellum expression. An aliquot (100 μl) of the culture was drop-cast onto highly oriented pyrolytic graphite (HOPG). Cells were allowed to attach to the HOPG for 10 min, and then the liquid was removed with a pipette tip. The surface was washed twice with 100 μl of deionized water, the surface was blotted dry at the edge with a Kimwipe, and the sample was placed in a desiccator overnight. All samples were equilibrated with atmospheric humidity (40%) inside the atomic force microscope (AFM) chamber for at least 2 h at 26.1°C at 1.1 mbarg. Conductive atomic force microscopy was performed using an Oxford Instruments/Asylum Research Cypher ES atomic force microscope. All topographic and current imaging was performed with a Pt/Ir-coated Arrow-ContPT tip with a 0.36701-N/m spring constant (NanoWorld AG, Neuchâtel, Switzerland). Topographic imaging was performed at a force of 0.1 nN. The conductive tip was attached to an ORCA dual-gain transimpedance amplifier and held at ground to serve as a translatable top electrode. A 300-mV bias was applied to the HOPG, and the locally detected current response of the archaellum was identified. Point-mode current-voltage (I-V) spectroscopy was performed by applying the conducting AFM tip at a force of 1 nN to the top of the archaellum and performing a voltage sweep at a frequency of 0.99 Hz. Continual force and current responses were collected for each I-V curve (Fig. S3) to ensure good consistent contact with the sample and avoid archaellum damage. The HOPG was periodically touched between samples to ensure the correct I-V response and tip quality (Fig. S4). Conductance was calculated from the linear portion of each I-V curve (−0.2 V to 0.2 V) as previously described (2). Average conductance and standard deviation were calculated for each of the three independent points on the three independent archaella.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.00579-19.

**FIG S1**, PDF file, 1.2 MB.

**FIG S2**, PDF file, 0.5 MB.

**FIG S3**, PDF file, 0.8 MB.

**FIG S4**, PDF file, 0.8 MB.

**FIG S5**, PDF file, 0.7 MB.

**ACKNOWLEDGMENTS**

We thank Trevor Woodard for growing the *Methanospirillum hungatei* cultures. This research was supported by Office of Naval Research grant N00014-16-1-2526. D.J.F.W., D.E.H., and D.R.L. conceived of the project. D.J.F.W. performed the atomic force microscopy measurements with assistance and guidance from Z.Z. and S.S.N. E.M. performed the analysis of the structural model. D.J.F.W. and D.R.L. wrote the initial draft of the manuscript with comments and revisions contributed by all authors.

**REFERENCES**

1. Lovley DR. 2017. Electrically conductive pili: biological function and potential applications in electronics. Curr Opin Electrochem 4:190–198. https://doi.org/10.1016/j.coelec.2017.08.015.

2. Lovley DR. 2017. Syntrophy goes electric: direct interspecies electron transfer. Annu Rev Microbiol 71:643–664. https://doi.org/10.1146/annurev-micro-030117-020420.

3. Walker DJF, Adhikari RY, Holmes DE, Ward JE, Woodard TL, Nevin KP, Lovley DR. 2018. Electrically conductive pili from genes of phylogenetically diverse microorganisms. ISME J 12:48–58. https://doi.org/10.1038/ismej.2017.141.

4. Walker DJF, Nevin KP, Nonnenmann SS, Holmes DE, Woodard TL, Ward JE, Rotaru A-E, McInerney MJ, Lovley DR. 2018. Syntrophus conductive pili demonstrate that common hydrogen-donating syntrophs can have a direct electron transfer option. bioRxiv https://www.biorxiv.org/content/early/2018/11/28/479683.

5. Lovley DR. 2017. e-Biologics: fabrication of sustainable electronics with “green” biological materials. mBio 8:e00695-17. https://doi.org/10.1128/mBio.00695-17.

6. Ing NL, El-Naggar MY, Hochbaum AI. 2018. Going the distance: long-range conductivity in protein and peptide bioelectronic materials. J Phys Chem B 122:10403–10423. https://doi.org/10.1021/acs.jpcb.8b07431.

7. Creasey RCG, Mostert AB, Nguyen TAH, Virdis B, Freguia S, Laycock B. 2018. Microbial nanowires — electron transport and the role of synthetic analogues. Acta Biomater 69:1–30. https://doi.org/10.1016/j.actbio.2018.01.007.

8. Egelman EH. 2017. Cryo-EM of bacterial pili and archaeal flagellar filaments. Curr Opin Struct Biol 46:31–37. https://doi.org/10.1016/j.sbi.2017.05.012.

9. Donval Courchesne N-M, DeBenedictis EP, Tresback J, Kim JJ, Duraj-Thatte A, Zanuy D, Keten S, Joshi NS. 2018. Biomimetic engineering of conductive curli protein films. Nanotechnology 29:454002. https://doi.org/10.1088/1361-6528/aadd3a.
10. Ing NL, Spencer RK, Luong SH, Nguyen HD, Hochbaum AI. 2018. Electronic conductivity in biomimetic α-helical peptide nanofibers and gels. ACS Nano 12:2652–2661. https://doi.org/10.1021/acsnano.7b08756.

11. Lovley DR, Holmes DE, Nevin KP. 2004. Dissimilatory Fe(III) and Mn(IV) reduction. Adv Microb Physiol 49:219–286. https://doi.org/10.1016/S0065-2911(04)49005-5.

12. Albers S-V, Jarrell KF. 2018. The archaellum: an update on the unique archaeal motility structure. Trends Microbiol 26:351–362. https://doi.org/10.1016/j.tim.2018.01.004.

13. Poweleit N, Ge P, Nguyen HN, Loo RRO, Gunsalus RP, Zhou ZH. 2016. CryoEM structure of the Methanospirillum hungatei archaellum reveals structural features distinct from the bacterial flagellum and type IV pilus. Nat Microbiol 2:16222. https://doi.org/10.1038/nmicrobiol.2016.222.

14. Cervantes FJ, de Bok FAM, Duong-Dac T, Stams AJM, Lettinga G, Field JA. 2002. Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms. Environ Microbiol 4:51–57. https://doi.org/10.1046/j.1462-2920.2002.00258.x.

15. Faguy DM, Koval SF, Jarrell KF. 1993. Effect of changes in mineral composition and growth temperature on filament length and flagellation in the Archaeon Methanospirillum hungatei. Arch Microbiol 159:512–520. https://doi.org/10.1007/BF00249028.

16. Reguera G, McCarthy KD, Mehta T, Nicol JS, Tuominen MT, Lovley DR. 2005. Extracellular electron transfer via microbial nanowires. Nature 435:1098–1101. https://doi.org/10.1038/nature03661.

17. Steidl RJ, Lampa-Pastirk S, Reguera G. 2016. Mechanistic stratification in electroactive biofilms of Geobacter sulfurreducens mediated by pili nanowires. Nat Commun 7:12217. https://doi.org/10.1038/ncomms12217.

18. Sure S, Ackland ML, Torrieri AJ, Adholeya A, Kochar M. 2016. Microbial nanowires: an electrifying tale. Microbiology 162:2017–2028. https://doi.org/10.1099/mic.0.000382.

19. Liu X, Wang S, Xu A, Zhang L, Liu H, Ma LZ. 2018. Biological synthesis of high-conductive pili in aerobic bacterium Pseudomonas aeruginosa. Appl Microbiol Biotechnol 103:1535–1544. https://doi.org/10.1007/s00253-018-9484-5.

20. Vargas M, Malvankar NS, Tremblay P-L, Leang C, Smith JA, Patel P, Snoeyenbos-West O, Nevin KP, Lovley DR. 2013. Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in Geobacter sulfurreducens. mBio 4:e00105-13. https://doi.org/10.1128/mBio.00105-13.

21. Adhikari RY, Malvankar NS, Tuominen MT, Lovley DR. 2016. Conductivity of individual Geobacter pili. RSC Adv 6:8354–8357. https://doi.org/10.1039/C5RA28092C.

22. Kuikenberg V, Riedel D, Gruber-Vodicka HR, Buttigieg PL, Tegetmeyer HE, Boetius A, Wegener G. 2018. Gene expression and ultrastructure of meso- and thermophilic methanotrophic consortia. Environ Microbiol 20:1651–1666. https://doi.org/10.1111/1462-2920.14077.

23. Sainokrot MO, Valeev EF, Sherrill CD. 2002. Estimates of the ab initio limit for π–π interactions: the benzene dimer. J Am Chem Soc 124:10887–10893. https://doi.org/10.1021/ja025896h.

24. Nathanael JG, Gamon LF, Cordes M, Rablen PR, Bally T, Fromm KM, Giese B, Wille U. 2018. Amide neighbouring-group effects in peptides: phenylalanine as relay amino acid in long-distance electron transfer. Chembiochem 19:922–926. https://doi.org/10.1002/cbic.201800098.

25. Malvankar NS, Yalcin SE, Tuominen MT, Lovley DR. 2014. Visualization of charge propagation along individual pili proteins using ambient electrostatic force microscopy. Nat Nanotechnol 9:1012–1017. https://doi.org/10.1038/nnano.2014.236.

26. Malvankar NS, Vargas M, Nevin KP, Tremblay P-L, Evans-Lutterodt K, Nykypanchuk D, Martz E, Tuominen MT, Lovley DR. 2015. Structural basis for metallic-like conductivity in microbial nanowires. mBio 6:e00084-15. https://doi.org/10.1128/mBio.00084-15.