

An *Escherichia coli* Chassis for Production of Electrically Conductive Protein Nanowires

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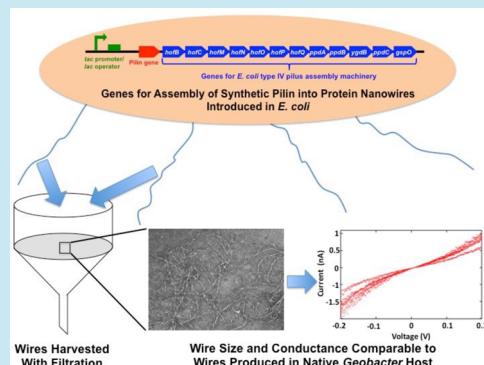
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ABSTRACT: *Geobacter sulfurreducens*' pilin-based electrically conductive protein nanowires (e-PNs) are a revolutionary electronic material. They offer novel options for electronic sensing applications and have the remarkable ability to harvest electrical energy from atmospheric humidity. However, technical constraints limit mass cultivation and genetic manipulation of *G. sulfurreducens*. Therefore, we designed a strain of *Escherichia coli* to express e-PNs by introducing a plasmid that contained an inducible operon with *E. coli* genes for type IV pilus biogenesis machinery and a synthetic gene designed to yield a peptide monomer that could be assembled into e-PNs. The e-PNs expressed in *E. coli* and harvested with a simple filtration method had the same diameter (3 nm) and conductance as e-PNs expressed in *G. sulfurreducens*. These results, coupled with the robustness of *E. coli* for mass cultivation and the extensive *E. coli* toolbox for genetic manipulation, greatly expand the opportunities for large-scale fabrication of novel e-PNs.

KEYWORDS: protein nanowire, e-pili, bioelectronic materials, e-biologics, *Geobacter*, sustainable electronics



Electrically conductive protein nanowires (e-PNs) show promise as revolutionary, sustainably produced, and robust electronic materials.^{1–8} They can readily be adapted for a multitude of sensing applications.^{1–7} Devices comprising thin layers of e-PNs function as “humidity-powered electrical generators”, continuously producing electricity when exposed to atmospheric humidity.⁸ However, the implementation of e-PNs in electronic devices has been limited by a lack of methods for mass production. *In vitro* assembly of peptides into conductive nanofilaments is feasible,^{9,10} but the filaments tend to agglomerate into gels at high peptide concentrations, limiting the possibilities for large-scale fabrication. Furthermore, synthesis of the peptide monomers required for *in vitro* assembly is expensive, reducing e-PN affordability.

In vivo assembly of e-PNs with microorganisms has several advantages over *in vitro* synthesis. Benefits include much lower cost and greater flexibility in e-PN design options with a production platform fueled with inexpensive, renewable feedstocks. A diversity of bacteria and archaea assemble peptides that show homology to bacterial type IV pilins into e-PNs,^{11–15} but the e-PNs of *Geobacter sulfurreducens* have been most intensively investigated.^{1,16} *G. sulfurreducens* e-PNs can be fabricated *in vivo* with acetate as the carbon and energy source. Once the cells are grown, the e-PNs can be harvested and retain their conductive properties.^{12,17–19}

The exquisite machinery that bacteria possess to assemble pilin proteins into filaments²⁰ confers great control over e-PN

production, yielding a highly uniform product. The microbial assembly process also offers substantial opportunities for producing diverse new types of e-PNs. For example, the conductivity of e-PNs produced with *G. sulfurreducens* has been tuned over a million-fold with simple modifications to the *G. sulfurreducens* pilin gene to either increase or decrease the abundance of aromatic amino acids.^{1,16} Pilin genes can be designed to encode additional peptides at the carboxyl end of the pilin, yielding e-PNs that retain their conductivity and display the added peptides on the outer surface of the wires.⁷ Peptide ligands can be introduced to confer specific sensing functions to e-PN devices with a flexibility in sensor design that is not feasible with other materials such as carbon nanotubes or silicon nanowires.⁷ Peptides displayed on the outer surface of e-PNs might also be designed to promote binding of e-PNs to surfaces to facilitate wire alignment or to function as chemical linkers with polymers for the fabrication of composite materials.⁷ Synthetic gene circuits introduced to control the expression of multiple e-PN monomer genes within a single cell make it possible to further tune e-PN function by

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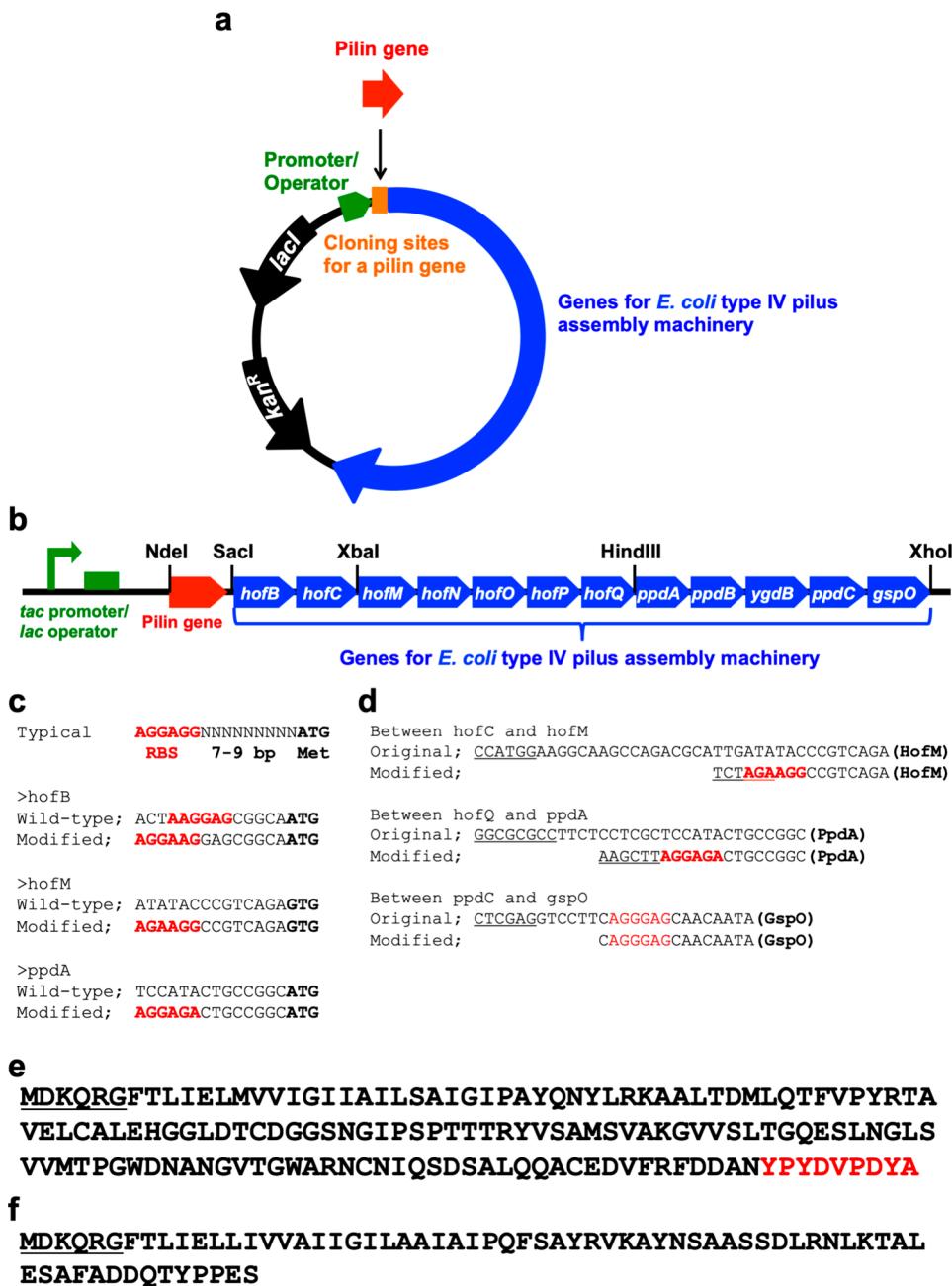


Figure 1. Construction of the type IV pilus assembly system in *E. coli* and synthetic peptide monomer for e-PN assembly. (a) Expression vector for genes for type IV pilus assembly in *E. coli* (*lacI*, Lac repressor gene; *kan*^R, kanamycin resistance gene). (b) Gene organization for *E. coli* type IV pilus assembly with cloning sites designated. (c) Ribosome binding sites (designated in red) that were changed from those in previous studies.²⁶ (d) Intergenic regions in the synthetic operon that were changed from those in previous studies²⁶ (ribosome binding sites in red, restriction enzyme sites underlined). (e) Amino acid sequence of the HA-tagged PpdD (signal sequence underlined, HA tag sequence in red). (f) Amino acid sequence of the synthetic peptide monomer designed for assembly in e-PNs, which comprises a portion of the *G. sulfurreducens* pilin, PilA, and the *E. coli* PpdD signal sequence (underlined).

producing heterogeneous wires comprising multiple types of e-PN monomers, with the stoichiometry of each monomer type precisely controlled.⁷ These design options would be difficult to replicate with *in vitro* assembly of e-PNs or fabrication of nanowires from nonbiological materials.

Geobacter-fabricated e-PNs have several other advantages over traditional nonbiological nanowire materials. Production of the e-PNs requires 100-fold less energy than is required for the fabrication of silicon nanowires or carbon nanotubes.² No toxic chemicals are required for e-PN fabrication, and the final product is environmentally benign and recyclable.² Never-

theless, e-PNs are remarkably robust, maintaining function even under harsh CMOS-compatible fabrication conditions.⁶ Proof-of-concept studies have demonstrated the dynamic sensing response of *Geobacter*-fabricated e-PNs; the ability of these e-PNs to function as the conductive component in flexible electronics; and, in the appropriate electrode/e-PN configurations, generation of electricity by thin films of *Geobacter* e-PNs from the humidity naturally present in air.^{6–8,18}

Barriers to large-scale e-PN production have been a limitation to realizing the potential of *Geobacter* e-PNs for

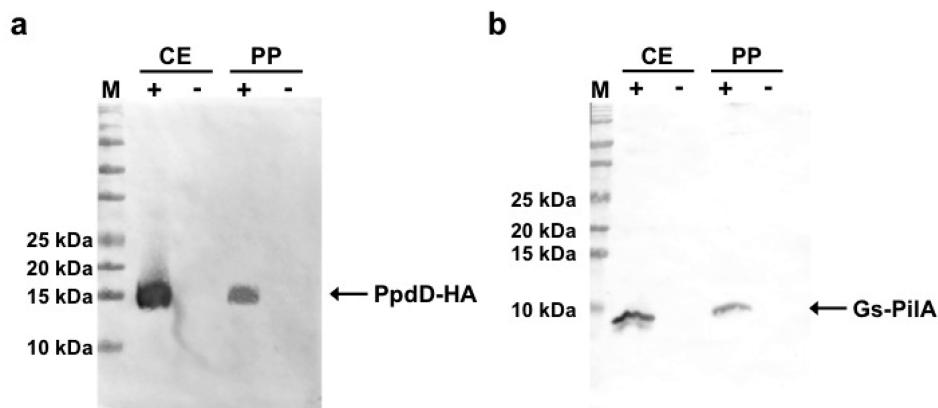


Figure 2. Expression of pili and e-PNs in *E. coli*. (a) Results with the strain of *E. coli* with genes for pilus assembly and the gene for PpdD-HA. (b) Results from *E. coli* strain GPN, which contained genes for pilus assembly and the gene for a synthetic pilin monomer designed to yield e-PNs assembled from a modified *G. sulfurreducens* PilA monomer. Shown are Western blot analyses of denatured proteins separated on an SDS PAGE gel and analyzed with antibody for (a) the HA tag on PpdD-HA pilin or (b) *G. sulfurreducens* PilA. Strains with the pilin genes are designated with (+). Control strains without the pilin genes are designated (−). Samples from whole-cell extracts (CE) and the pili preparations (PP) were examined. Lanes designated M show molecular weight standard markers.

these and other possible applications. *G. sulfurreducens* must be grown anaerobically to produce e-PNs, adding technical complexity and costs. A strain of *Pseudomonas aeruginosa* that was grown aerobically heterologously expressed the *G. sulfurreducens* pilin gene with the assembly of e-PNs with properties similar to the e-PNs expressed in *G. sulfurreducens*.²¹ However, *P. aeruginosa* is a pathogenic microorganism and thus is not ideal for large-scale commercial e-PN production. Furthermore, the expression of the e-PNs in *P. aeruginosa* remained under the control of the native regulatory system, limiting options for controlling the timing and extent of e-PN expression.²¹

We thought that *Escherichia coli* might be an ideal chassis for e-PN fabrication. *E. coli* is a common platform for the commercial-scale production of organic commodities.^{22,23} The substantial *E. coli* genetic toolbox, including the possibility of introducing unnatural amino acids,^{24,25} could provide broad options for designing e-PNs with different properties and functions. Nonpathogenic strains of *E. coli* typically do not express type IV pili. However, the introduction of an artificial operon of pilus assembly protein genes from pathogenic *E. coli* into nonpathogenic *E. coli* yielded a nonpathogenic strain that expressed the same type IV pili that pathogenic *E. coli* express.²⁶ This finding, and the fact that bacteria will often assemble heterologous pilins into pili,^{12,13,21,27–29} suggested that it might be possible to develop a nonpathogenic strain of *E. coli* that would express *Geobacter* e-PNs.

Another limitation to large-scale *in vivo* production of e-PNs concerns the methods for collecting e-PNs once they have been sheared from cells. Previously described methods have included multiple laborious steps, often with strategies such as ultracentrifugation and/or salt precipitation procedures that would be difficult to scale economically.^{12,17}

Here we report the construction of a strain of *E. coli* amended with genes for the expression of type IV pili assembly machinery and a synthetic gene designed to yield e-PNs comparable to those produced by *G. sulfurreducens*. This new *E. coli* strain produces e-PNs with characteristics similar to the e-PNs expressed by *G. sulfurreducens*. Simple aerobic growth of *E. coli*, coupled with a new simplified method for harvesting e-

PNs, suggests that large-scale production of e-PNs will be feasible.

RESULTS AND DISCUSSION

We engineered a standard lab strain of *E. coli* to produce e-PNs by introducing the genes known from previous studies²⁶ to be required for type IV pili biogenesis. We modified the previous design of *E. coli*,²⁶ which was constructed to produce pili from the *E. coli* pilin protein PpdD, as follows: (1) the gene for pilin monomer is within a separate cloning site to make it convenient to exchange the gene for the pilin of interest (Figure 1a); (2) the gene clusters were constructed with common and less expensive restriction enzymes to aid in modularity (Figure 1b); (3) ribosome binding sites for *hofB*, *hofM*, and *ppdA* were changed to improve the translation efficiency (Figure 1c); (4) a gene cluster containing *ppdA*, *ppdB*, *ygdB*, and *ppdC* and *gspO* were connected by two-step PCR instead of using a restriction enzyme to delete unnecessary sequence (Figure 1b,d); (5) intergenic regions were shortened to delete unnecessary sequence (Figure 1d); (6) the *tac* promoter,³⁰ one of the strongest promoters in *E. coli*, was incorporated to enhance transcription of the genes for the assembly of type IV pili (Figure 1b); and (7) a gene for the LacI repressor was included in the expression vector to repress the genes when desired, such as during cloning (Figure 1a). We also deleted the gene *fimA* with previously described genetic methods^{31,32} to prevent the formation of type I pili.

The initial strain was constructed with a pilin gene that added the HA tag (YPYDVPDYA) at the carboxyl terminal end of the *E. coli* pilin protein PpdD (Figure 1e). With this modification, the tagged pilin protein (PpdD-HA) could readily be detected with the commercially available antibody for the HA tag. PpdD-HA was detected in the cell extract from the strain containing the genes for PpdD-HA pilus assembly but not in extracts from a control strain that lacked the gene for PpdD-HA (Figure 2a). When pili were sheared from the cells, PpdD-HA was detected in the sheared fraction from the strain containing the genes for PpdD-HA pilus assembly but not from the control strain without the PpdD-HA gene (Figure 2a). These results confirmed that the modified expression

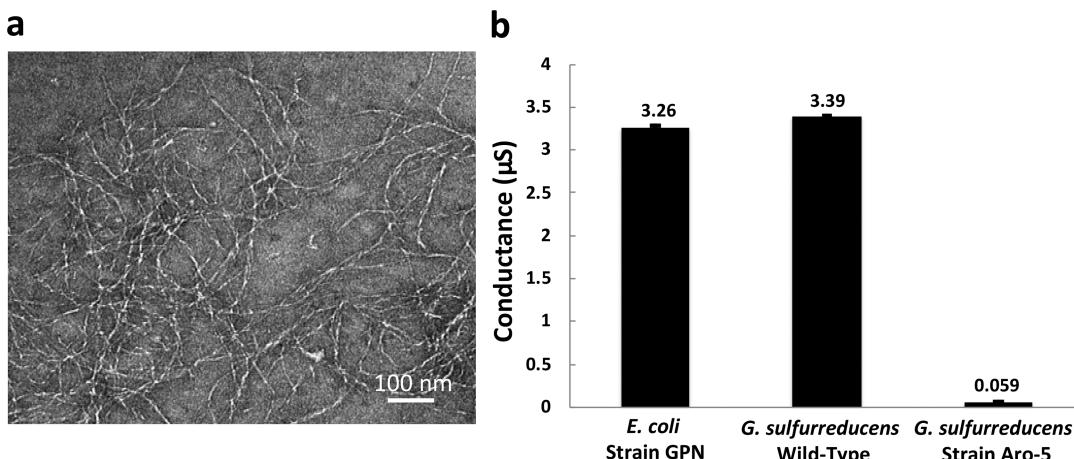


Figure 3. Characterization of the e-PNs expressed in *E. coli* strain GPN. (a) Transmission electron micrograph. (b) Conductance of films of e-PNs expressed in *E. coli* strain GPN compared with e-PNs from wild-type *G. sulfurreducens* and the Aro-5 strain of *G. sulfurreducens*. The results are the means and standard deviations of triplicate measurements on each nanoelectrode array for at least three independent nanoelectrode arrays. Results for wild-type *G. sulfurreducens* and the Aro-5 strain were published previously.¹³

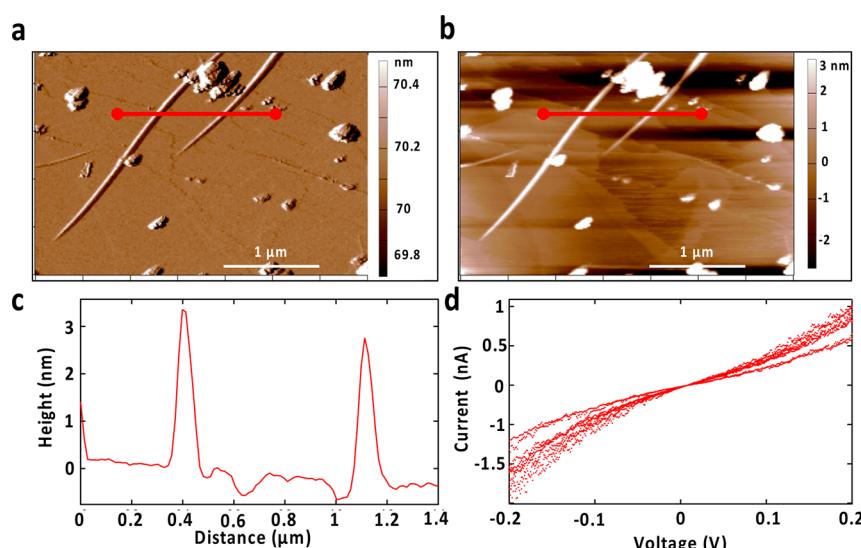


Figure 4. Characterization of individual e-PNs expressed in *E. coli* strain GPN. (a) Amplitude image of two e-PNs in amplitude modulation mode. (b) Height image of each e-PN. (c) Cross-section line trace showing the height of two individual e-PNs, designated in (a) and (b) by the red line, demonstrating a height (diameter) of ~ 3 nm. (d) Current–voltage response of nine individual measurements (three measurements on each of three e-PNs).

system for type IV pilus assembly was effective for pili production.

To express e-PNs in *E. coli* similar to those expressed in *G. sulfurreducens*, we designed a gene to yield a synthetic peptide monomer for assembly into e-PNs. The peptide was similar to the *G. sulfurreducens* pilin monomer, PilA, with the exception that the signal sequence was replaced with the *E. coli* PpdD signal sequence to facilitate e-PN assembly in *E. coli* (Figure 1f). The gene for the synthetic e-PN monomer was cloned into the location designated “pilin gene” (Figure 1a). The strain with the synthetic gene for the e-PN monomer was designated *E. coli* strain GPN (*Geobacter* protein nanowire). The e-PN monomer was detected in whole-cell extracts of strain GPN with PilA antibody but not in the control strain that lacked the gene for the e-PN monomer (Figure 2b).

e-PNs were harvested from strain GPN with physical shearing from the cells, as in previous studies of e-PNs expressed in *G. sulfurreducens*.¹⁷ In those previous studies, the

cells were separated from the sheared e-PNs by centrifugation, and then the e-PNs in the supernatant were collected with ultracentrifugation or ammonium sulfate precipitation.¹⁷ These methods of e-PN collection are labor-intensive and would be difficult to adapt to large-scale production. Therefore, the e-PN supernatant preparation was treated with Triton X-100 detergent to solubilize any remaining cell debris, and then the e-PNs were collected on a filter with a 100 kDa molecular weight cutoff. This method is simpler and faster than previously described¹⁷ e-PN purification methods.

The e-PNs harvested from *E. coli* strain GPN were ca. 3 nm in diameter and several micrometers in length (Figure 3a), a morphology similar to the e-PNs expressed in *G. sulfurreducens*. No filaments were observed in similar preparations when the gene for the e-PN monomer was omitted from *E. coli* strain GPN. Denaturation of the e-PNs from *E. coli* strain GPN yielded a monomer that reacted with PilA antibody, whereas the monomer was not detected in preparations from the

control strain without the gene for the e-PN monomer (Figure 2b).

The conductance of thin films of the e-PNs from *E. coli* strain GPN, determined with a nanoelectrode array as previously described,¹³ was $3.26 \pm 0.35 \mu\text{S}$, similar to the conductance of $3.39 \pm 0.04 \mu\text{S}$ for e-PNs harvested from *G. sulfurreducens* (Figure 3b). The conductance of these e-PNs was much higher than the conductance of wires harvested from the Aro-5 strain of *G. sulfurreducens* (Figure 3b), which expresses a synthetic pilin gene designed to yield protein nanowires with low conductivity.^{18,27}

The conductance of individual e-PNs was evaluated on highly oriented pyrolytic graphite with atomic force microscopy employing a conductive tip, as previously described.¹⁵ The diameter of the e-PNs was $3.00 \pm 0.04 \text{ nm}$ ($n = 18$; six measurements on three independent pili), the same as e-PNs expressed with *G. sulfurreducens* (Figure 4). The e-PNs were conductive with an Ohmic-like current–voltage response (Figure 4d). The conductance of the individual e-PNs, $4.3 \pm 0.8 \text{ nS}$ ($n = 9$), compared well with the previously reported¹⁵ conductance of $4.5 \pm 0.3 \text{ nS}$ for individual e-PNs expressed in *G. sulfurreducens*.

CONCLUSIONS

The fabrication of e-PNs with *E. coli* described here has substantial advantages over expression in *G. sulfurreducens*. Special equipment and expertise are required to anaerobically culture *G. sulfurreducens*, whereas *E. coli* can be simply grown under ambient aerobic atmospheric conditions. Thus, when coupled with our finding that the e-PNs can be collected with a simple filtration method, expression of e-PNs in *E. coli* offers the potential for large-scale e-PN production.

Furthermore, e-PN expression in *E. coli* enables much greater flexibility for the design of a wider diversity of e-PNs than would currently be possible with *G. sulfurreducens*. Tools for the genetic manipulation of *G. sulfurreducens* are limited, and only the most simple synthetic gene circuits have been adapted for this organism.³³ The much broader range of strategies for introducing genes and controlling their expression in *E. coli*^{22,23} is expected to facilitate the design and expression of e-PNs with unique properties and functionalities that could not readily be fabricated with *G. sulfurreducens*. Most notably, *E. coli* is an excellent platform for modifying proteins with unnatural amino acids that can confer diverse new functionalities to proteins.^{24,25} The development of new e-PNs in the *E. coli* chassis for enhanced sensor, electronics, and energy-harvesting applications^{1–8} is underway.

MATERIALS AND METHODS

E. coli Strain and Culture Conditions. *E. coli* NEB 10-beta (New England Biolabs) was grown at 37°C in LB medium supplemented with appropriate antibiotics as necessary for plasmid preparation, as previously described.³⁴ The gene for FimA, the primary monomer for type I pili, was deleted as previously described^{31,32} to construct *E. coli* Δ fimA (kanamycin-sensitive). The strains expressing the modified *E. coli* pilin or the synthetic peptide for assembly into e-PNs were built in this strain.

Construction of the Expression Vector for Type IV Pilus Assembly. An expression vector for type IV pilus assembly was constructed as described previously²⁶ with several modifications (Figure 1). To construct the basic

expression vector for type IV pilus assembly, the T7 promoter in the plasmid vector pET24b (Novagen) was replaced with the *tac* promoter.³⁰ The DNA fragment containing the *tac* promoter and *lac* operator was amplified by PCR with the primer pair Ptac-F/Olac-R (Table S1) and pCD341³⁵ as the template. The PCR product was digested with *Bgl*II and *Xba*I and replaced the *Bgl*II–*Xba*I region containing T7 promoter and *lac* operator in pET24b. The resultant plasmid was designated p24Ptac.

Next, genes for *E. coli* type IV pilus assembly without the major pilin gene were cloned in p24Ptac. The genes include *hofB* (ATPase), *hofC* (platform protein), *hofM* (assembly protein), *hofN* (assembly protein), *hofO* (assembly protein), *hofP* (assembly protein), *hofQ* (secretin), *ppdA* (minor pilin), *ppdB* (minor pilin), *ygdB* (minor pilin), *ppdC* (minor pilin), and *gspO* (prepilin peptidase).²⁶ The DNA fragment containing *ppdA*, *ppdB*, *ygdB*, *ppdC*, and *gspO* was prepared by two-step PCR. Fragments containing *ppdA*, *ppdB*, *ygdB*, and *ppdC* or *gspO* were amplified by PCR with primer pairs, *ppdA*-F/*ppdC*-R and *gspO*-F/*gspO*-R (Table S1), respectively. The fragment containing *ppdA*, *ppdB*, *ygdB*, *ppdC*, and *gspO* was amplified by PCR with these PCR products as the template and the primer pair *ppdA*-F/*gspO*-R. The PCR product was digested with *Hind*III and *Xba*I and cloned in pBluescript II SK (Stratagene). The fragment containing *hofM*, *hofN*, *hofO*, *hofP*, and *hofQ* was amplified by PCR with the primer pair *hofM*-F/*hofQ*-R (Table S1). The PCR product was digested with *Xba*I and *Hind*III and cloned in the plasmid containing *ppdA*, *ppdB*, *ygdB*, *ppdC*, and *gspO*. The fragment containing *hofB* and *hofC* was amplified by PCR with the primer pair *hofB*-F/*hofC*-R (Table S1). The PCR product was digested with *Sac*I and *Xba*I and cloned in the plasmid containing *hofM*, *hofN*, *hofO*, *hofP*, *hofQ*, *ppdA*, *ppdB*, *ygdB*, *ppdC*, and *gspO*. The fragment containing *hofB*, *hofC*, *hofM*, *hofN*, *hofO*, *hofP*, *hofQ*, *ppdA*, *ppdB*, *ygdB*, *ppdC*, and *gspO* was prepared by digesting the plasmid containing *hofB*, *hofC*, *hofM*, *hofN*, *hofO*, *hofP*, *hofQ*, *ppdA*, *ppdB*, *ygdB*, *ppdC*, and *gspO* with *Sac*I and *Xba*I and cloned in p24Ptac (Figure 1). The resultant plasmid was designated T4PAS/p24Ptac.

Expression and Harvesting of Pili Composed of the *E. coli* pilin PpdD. The fragment containing a gene for PpdD with the HA tag (Figure 1e) was amplified with the primer pair *ppdD*-F/*ppdD*-HA-R (Table S1), digested with *Nde*I and *Sac*I, and cloned in T4PAS/p24Ptac. The resultant plasmid was termed *ppdD*-HA/T4PAS/p24Ptac.

For initial studies with the *E. coli* strain expressing the *E. coli* pilin PpdD, the plasmids *ppdD*-HA/T4PAS/p24Ptac or T4PAS/p24Ptac were transformed into *E. coli* Δ fimA. A single colony from an LB agar plate containing kanamycin³⁴ was inoculated in TB medium (Novagen) supplemented with 1% glycerol and kanamycin and incubated at 30°C for 24 h to the stationary phase. Pili were sheared from the cells and precipitated with TCA as described previously.²⁶

Expression and Harvesting of e-PNs. A fragment encoding a gene for a synthetic pilin monomer, which was similar to the PilA monomer of *G. sulfurreducens* but included the signal sequence of PpdD instead of the original PilA signal sequence (Figure 1f), was amplified with the primer pair EPS-GspilA-F/GspilA-R (Table S1). The amplified fragment was digested with *Nde*I and *Sac*I and cloned in T4PAS/p24Ptac. The resultant plasmid, designated GspilA/T4PAS/p24Ptac, was transformed into *E. coli* Δ fimA. The resultant strain, designated *E. coli* strain GPN (*Geobacter* protein nanowires)

was grown on 10 cm diameter culture plates of standard LB medium³⁴ amended with kanamycin and solidified with agar. After overnight growth at 30 °C, cells were scraped from the surface and suspended in 6 mL of M9 medium.³⁴ Twenty plates of M9 medium supplemented with 0.5% glycerol, 0.5 mM IPTG, and kanamycin were spread-plated with 300 μ L of the suspended cells. The plates were incubated at 30 °C for 48 h. The cells were harvested from the plates with 1 mL of M9 medium (500 μ L to scrape, 500 μ L to wash) for each plate. The 20 mL suspension of cell scrapings was centrifuged at 4000 rpm for 15 min at 4 °C to pellet the cells. The supernatant was discarded, and the cells were resuspended in 30 mL of 150 mM ethanolamine buffer (pH 10.5) and poured into a Waring blender. The tubes were washed three times with 20 mL of the ethanolamine buffer, which was also added to the blender. The 90 mL suspension was blended for 2 min on low speed to shear the e-PNs from the cells. The contents of the blender were transferred to a centrifuge bottle along with a wash of the blender with 10 mL of ethanolamine buffer. The blended material was centrifuged at 5000g for 30 min at 4 °C to remove the cells. The supernatant containing the e-PNs was collected. Triton X-100 detergent was added at a final concentration of 6 mM to solubilize any remaining cellular debris. The mixture was shaken at 100 rpm at room temperature for 45 min and then added to a stirring filtration unit that had a 100 kDa molecular weight cutoff membrane filter made from poly(ether sulfone) (Omega membrane 100 K 76 mm, Pall Corporation) to collect the e-PNs on the filter. Additional ethanolamine buffer was added to dilute the sample to yield a final Triton X-100 concentration of 2 mM. The sample was filtered under nitrogen gas (69 kPa). The sample on the filter was washed four times with 100 mL of water. The e-PNs were collected from the filter by scraping the surface into 500 μ L of water. The scraping procedure was repeated two more times to yield a suspension of e-PNs in 1.5 mL of water.

Western Blot Analysis. The presence of pilin monomers in whole-cell extracts and pili preparations was evaluated with Western blot analysis. Whole-cell extracts were prepared with B-PER Complete Bacterial Protein Extraction Reagent (Thermo Fisher Scientific). Western blot analysis was conducted as described previously.⁷ PpdD-HA pilin was detected with an anti-HA antibody (HA Tag Polyclonal Antibody, Invitrogen). The *G. sulfurreducens* pilin monomer, PilA, was detected with an anti-PilA antibody.⁷

Protein Nanowire Conductance. The conductance of e-PNs expressed in *E. coli* from the *G. sulfurreducens* pilin was analyzed as previously described.^{13,15} For four-probe measurements of thin-film conductance, the e-PN preparation in water was adjusted to 500 μ g of protein/ μ L. As previously described,¹³ a 2 μ L aliquot of the e-PN preparation was drop-cast onto the center of three different gold electrode nanoarrays (Figure S1) and allowed to dry for 1 h at 24 °C, after which another 2 μ L was drop-cast and left to dry overnight at 24 °C. Each of the three electrode nanoarrays was analyzed with a Keithley 4200 semiconductor characterization system set up with four probes to conduct a current–voltage (I – V) curve using a $\pm 30 \times 10^{-8}$ V sweep with a 5 s delay and a 250 s hold time. The voltage was injected at the source and removed at ground, and the current between the two inner electrodes was measured (Figure S1). The analysis of each of the three nanoarrays was repeated in triplicate. Thin-film conductance was calculated by extracting the slope of the linear

fit of the current–voltage response for each of the three measurements on the three electrodes using the formula $G = I/V$, where G is the conductance, I is the current, and V is the voltage.

In order to evaluate the conductance of individual e-PNs, a 100 μ L aliquot of a culture of *E. coli* expressing the *G. sulfurreducens* pilin was drop-cast onto highly oriented pyrolytic graphite and allowed to sit for 10 min. Then the excess liquid was wicked away with a Kimwipe, and an equal volume of deionized water was added to wash off excess salts, etc. The sample was blotted dry and then dried for 12 h at 24 °C. The prepared amples were loaded into an Oxford Instruments Cypher ES Environmental AFM and equilibrated for at least 2 h. The AFM was operated in ORCA electrical mode with a Pt/Ir-coated Arrow-ContPT tip with a force constant of 0.2 N/m (NanoWorld AG, Neuchâtel, Switzerland). e-PNs were identified in amplitude mapping mode (AM-AFM). Point-mode current–voltage spectroscopy was carried out by switching to contact mode and gently touching the conductive tip, which acted as a translatable top electrode, to the top of the e-PN with a force of 1 nN. A voltage sweep of ± 0.6 V set at 0.99 Hz was applied to three independent points on each of three individual e-PNs. The conductance was calculated, as above, from the slope of the linear fit of the current–voltage response between –0.2 and 0.2 V.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssynbio.9b00506>.

Primers used in this study and a description of the four-probe conductance measurements ([PDF](#))

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Author Contributions

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Notes

The authors declare no competing financial interest.

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