Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information:

Geobacter sulfurreducens Pili Support Ohmic Electronic Conduction in Aqueous Solution

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SUPPORTING INFORMATION MATERIALS AND METHODS

Generation of cleavable pilin fusion standard: Coding sequence for mature *G. sulfurreducens* PilA was amplified from genomic DNA by PCR using the following primers: CGCGGTACCGAGAACCTGTACTTCCAAGGTTTCACCCTTATCGAG (forward) and CGCGCGGCCGCTTAACTTTCGGGCGGATAGG (reverse). The PCR product was gel purified and cloned into the pMocr plasmid (University of Michigan HTP Expression Lab) [1] using the KpnI and NotI restriction sites. The resultant pMocr-Gs*pilA* plasmid has a gene encoding a polyhistidine-tagged N-terminal Mocr solubility domain which is cleavable by an engineered tobacco etch virus (TEV) protease site immediately preceding the C-terminal mature pilin. As this product was found to not cleave efficiently, a second construct encoding 5 glycines immediately downstream from the TEV cleavage site was created using the QuikChange II protocol (Agilent) with pMocr-Gs*pilA* as the template and the following primers: CAAGGCGGTGGCGGTGGCGGTTTCACCCTTATCGAG and ACCGCCACCGCCACCGCCTTGGAAGTACAGGTTCTC. Plasmids were maintained in *E. clonii* 10G (Lucigen).

The Mocr-gly_5-PilA fusion was expressed from Rosetta2 (Novagen) cells grown at 37° C in terrific broth (Amresco) supplemented with 4% glycerol. Upon reaching an OD_{600} of 0.6, cells were transferred to 20° C, induced with 0.25 mM IPTG, stirred vigorously for 20 hours, and harvested by centrifugation at 8,000g. The resultant pellet was resuspended in 10 ml lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM imidazole, 2 mM β-mercaptoethanol, 5% glycerol) supplemented with 1 mg lysozyme and submitted to lysis by sonication. Cell lysate was

clarified by centrifugation at 15,000g for 45 min; the soluble fraction was combined with 1 ml Ni-NTA slurry pre-equilibrated in lysis buffer and batch bound overnight at 4° C. This mixture was passed through a gravity flow column; retained resin was washed with lysis buffer in 10 ml stepwise increments containing 10, 20 and 40 mM imidazole. Bound protein was eluted with 5 ml lysis buffer containing 300 mM imidazole. Eluate was concentrated using Corning Spin-X 10 kDa MWCO filters and buffer-exchanged into 25 mM Tris-HCl pH 7.5, 50 mM NaCl, 20% glycerol for storage at -80° C. Purified fusion protein was processed using Promega Pro-TEV Plus following the manufacturer's instructions.

Analysis of purified pili: For MALDI-TOF analysis, purified pili were denatured with 10% OG overnight and then diluted with ultrapure water to a final OG concentration of 2% prior to combining with the matrix solution of α-Cyano-4-hydroxycinnamic acid dissolved in a 2:1 solution of ultrapure water: acetonitrile and 0.2% trifluoroacetic acid. The final sample for MALDI was a 12:7:5 ratio of matrix to ultrapure water to diluted and denatured protein. Mass spectra were collected in positive ion mode.

For SDS-PAGE analysis, unless otherwise noted in the text, all samples were prepared by boiling in 1x SDS loading dye for 20 min. OG-treated samples were incubated in OG overnight prior to SDS preparation. Samples were run on a Tris-HEPES gel with 16% polyacrylamide at a constant voltage of 110 mV for 60 min. Gels were silver stained following overnight fixation.

For LC-MS/MS identification, OG-denatured gsPilA was subject to digestion by trypsin enzyme (Fig. S1). Proteomic analysis of the cleaved peptides was performed by the Proteomics Mass Spectrometry Facility at UC Irvine. Results gave complete coverage of the C-terminal

domain, whereas the hydrophobicity of the N-terminal domain may have interfered with the sequencing procedure.

Atomic force microscopy imaging: Pili on devices were imaged using atomic force microscopy (Asylum MF3PD) operating in tapping mode. The measurements were performed with aluminum-coated silicon probes (Asylum Research AC160TS-R3) with a tip radius of 9nm and a resonant frequency of 300kHz. Scans were rastered at either 0.5Hz or 1.0 Hz. Statistical pili lengths were determined by measuring fiber lengths with ImageJ (Fig. S3). Lengths were determined from a total of 14 AFM images.

Fabrication of interdigitated electrode devices for solid state and electrochemical measurements: The electrodes are comprised of 200 parallel 5 μm wide x 2 mm long gold electrode bands patterned onto a Pyrex substrate, with a 5 μm intra-band spacing. For percolation network analysis, 5 μm wide electrodes with intra-band spacings of 5, 7.5, 11.66, 20, and 35 μm; 6.25 μm wide electrodes with an intra-band spacing of 6.25 μm; and 10 μm wide electrodes with intra-band spacings of 10, 15, 23, and 30 μm were used. Different electrode widths were used to maintain a fixed total device size of 2mm x 2mm, and differences in widths were accounted for by normalizing electrode spacings to reduced channel dimensions.

Devices were photolithographically patterned onto silicon oxide or Pyrex wafers using a chrome mask with arrays of the interdigitated electrode pattern. Gold was deposited onto the patterned design with a titanium adhesion layer. The total thickness of the metallic contacts was 50 nm. After liftoff, the wafers were coated with photoresist and diced into individual devices. The photoresist was removed and each device was tested for shorts prior to depositing material.

Device fabrication was performed by the foundry services at the University of California, in Irvine and at the University of California, in San Diego.

For solution-gated measurements, 22 gauge solid core insulated wire leads were connected to the source and drain with conductive silver epoxy (MG Materials 8331-14g) after film deposition. After drying, exposed electrode and lead connections were sealed with waterproof silicone sealant (DAP All-Purpose Adhesive Sealant).

Preparation of control channels for conductivity measurements: Aβ fibers were self-assembled from synthesized peptides [2,3]. Lyophilized peptides were incubated in 150 mM phosphate buffered saline at 37° C for two weeks to form fibers. Fiber formation was verified using atomic force microscopy (Fig. S8**)**. *P. aeruginosa* PAO1 strains were cultured for pili purification as described in ref [4] and verified by AFM (Fig. S2). PEDOT:PSS (Heraeus Clevios PH1000) films were spun cast and baked overnight at 70° C prior to electrode assembly. PVF (Sigma Aldrich) was dried overnight under vacuum prior to dispersal in dichloromethane and were drop cast onto devices prior to electrode assembly. Due to the volatility of the dichloromethane, drop casting PVF was observed to be the most efficient method to obtain uniform films.

G. sulfurreducens **biofilm electrode fabrication:** Electrode fabrication for biofilm growth was similar to the procedure detailed in ref.[5]. To construct the electrodes, glass slides (2.5 cm x 3 cm) were cleaned ultrasonically using successive rinses of acetone, isopropanol, and ultrapure water, following blow drying with nitrogen. A 25-μm-diameter tungsten wire (Sigma Aldrich) was placed on the glass substrate as a deposition mask to achieve an insulating gap in the electrodes.

An 8 nm chromium adhesion layer and a 40 nm gold film were thermally evaporated onto the substrates to produce gold split electrodes with a 25 ± 2 μm non-conductive spacing. Uniformity of the gap was confirmed through optical microscopy and electrical insulation between the two sides of the gap was confirmed through resistance measurements. A single 22 gauge core insulated wire lead was connected to each electrode using conductive silver epoxy. After drying, the lead connections were sealed with waterproof silicone sealant.

***G. sulfurreducens* biofilm growth:** *G. sulfurreducens* biofilm were grown in a standard microbial fuel cell (MFC) configuration purchased from Adams and Chittenden (MFC 100.25.3). The anode and cathode glass compartments are separated by a Nafion membrane (Fuel Cell Store, Nafion 117), held in place by an O-ring and clamp. Wires were inserted through the rubber septum seals to connect electrodes to external connections. A split electrode with two externally-connected wires functioned as the anode and a carbon cloth electrode functioned as the cathode. Prior to inoculation, the fully assembled MFC was autoclaved. The anode chamber was inoculated with a log-phase culture of *Geobacter sulfurreducens* strain (PCA wild type, DSMZ strain 12127) and maintained under anaerobic conditions (N_2:CO_2, 80:20%) with 100 mL of freshwater medium containing fumarate (40 mM) and acetate (10 mM) using previously described methods [6]. Freshwater medium contained 2.5 g/L sodium bicarbonate, 0.1 g/L potassium chloride, 0.25 g/L ammonium chloride, and 0.6 g/L sodium phosphate dibasic, 10 mL vitamin mixture, and 10 mL mineral mixture [7]. 10 mM cysteine was added as an oxygen scavenger [6]. The cathode consisted of 20mM $K_3[Fe(CN)_6]$ in 100 mL of autoclave-sterilized potassium phosphate buffer: 0.31 g/L ammonium chloride, 2.69 g/L sodium phosphate monobasic, 4.33 g/L sodium phosphate dibasic, 0.13 g/L potassium phosphate. The biofilm was

grown using established methods [6] by poising both the electrodes as anodes at +0.300 V vs. Ag/AgCl. After three days, biofilm were switched to fumerate-free freshwater medium. Biofilm were maintained until there was an approximately 0 mV potential difference between both sides of the split electrode, corresponding to biofilm spanning the electrode separation. Bipotentiostat electrochemical gating of *G. sulfurreducens* biofilm was performed in the MFC cell configuration.

SUPPORTING INFORMATION FIGURES

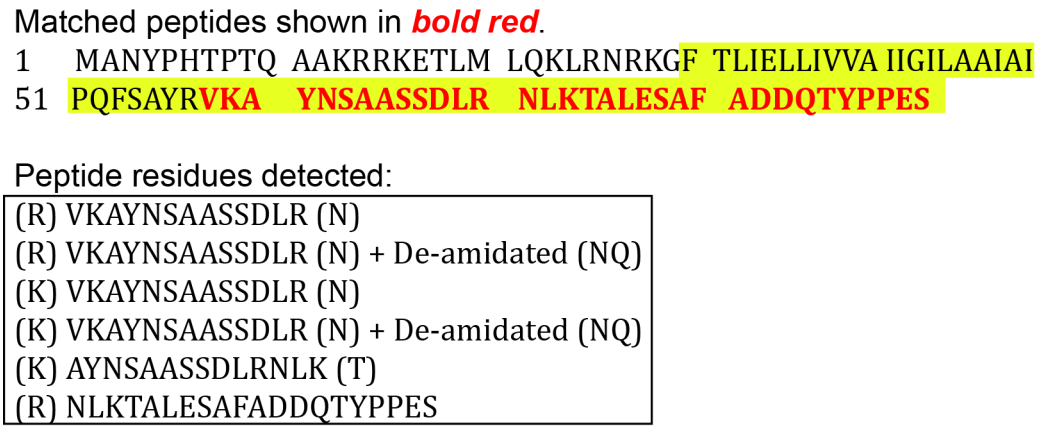

Fig. S1. LC-MS/MS identification of mature, isolated gsPilA (sequence highlighted in yellow). The amino acids noted in parenthesis indicate the cleavage sites of the digestion enzyme trypsin, which cleaves after the R and K residues. The error probability in sequence identification ranged from 10^{-6} to 10^{-7}.

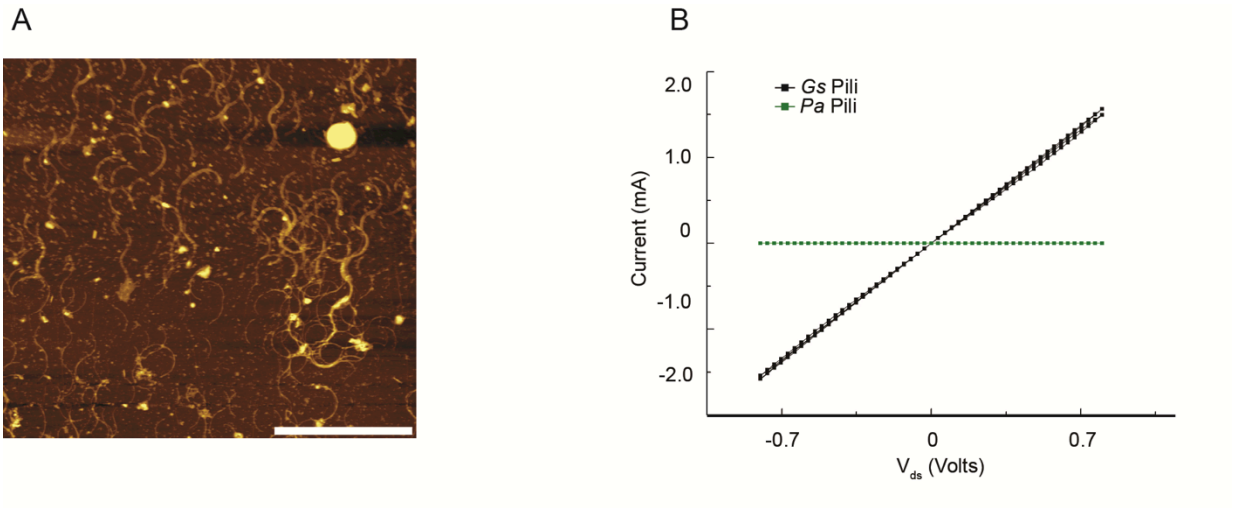

Fig. S2. *(A)* Representative AFM micrograph of purified PAO1 pili fibers (scale bar = 5 μm). *(B)* I-Vs of films of *G. sulfurreducens* and *P. aeruginosa* PAO1 pili fibers on interdigated electrodes. *G. sulfurreducens* pili films are five orders of magnitude more conductive than PAO1 pili fibers.

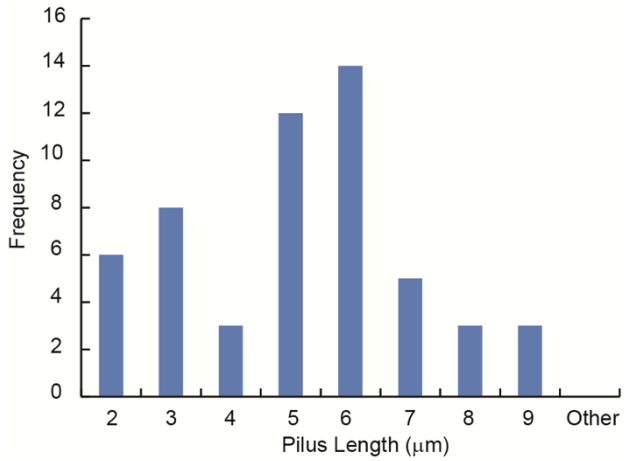

Fig. S3. Distribution of individual purified pilus lengths across 14 AFM images. The average pilus length is 4.57 ± 0.26μm.

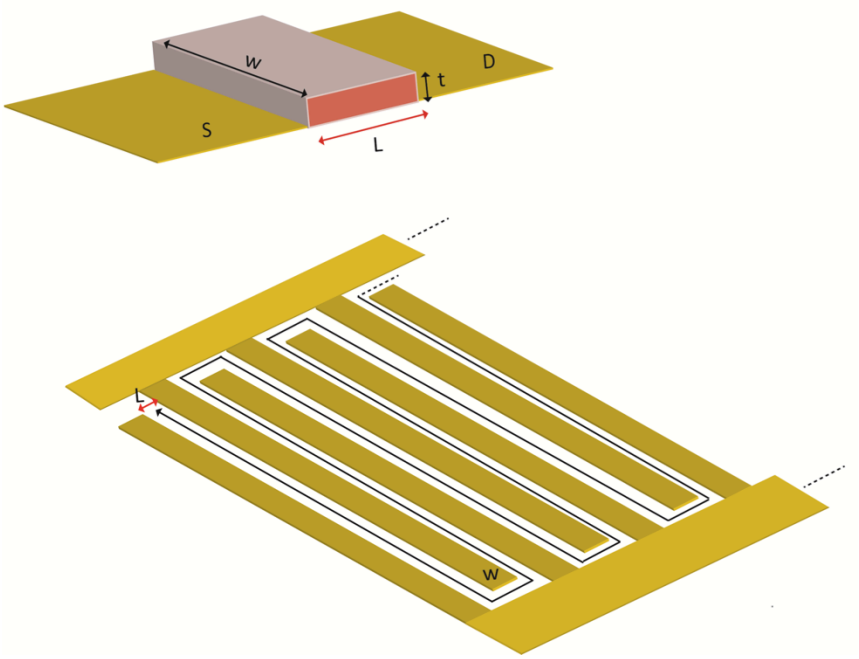

Fig. S4. Illustration of reduced channel dimension on interdigitated electrode (*L/w*). All devices had a fixed total area, while the spacing between electrodes (*L*) differed, resulting in different channel widths *(w*). Electrode spacing is thus normalized to the ratio *L/w*.

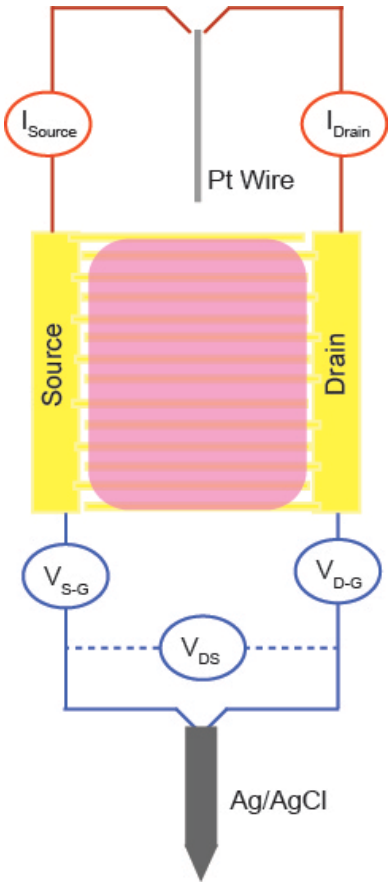

Fig. S5. Schematic of the electrochemical circuit for biopotentiostat cyclic voltammetry. The pink shaded region is the device area covered by conducting channels of biofilms, pili, or control materials.

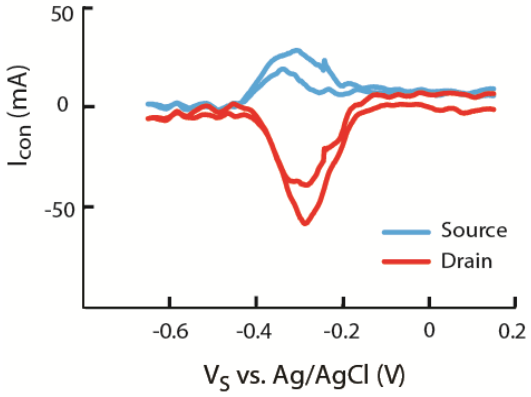

Fig. S6. Electrochemical gating measurements of a live *G. sulfurreducens* biofilm. Source and drain current responses show symmetric current entering the source and leaving the drain at V_{DS} = 10 mV. Electrode spacing = 25 μm.

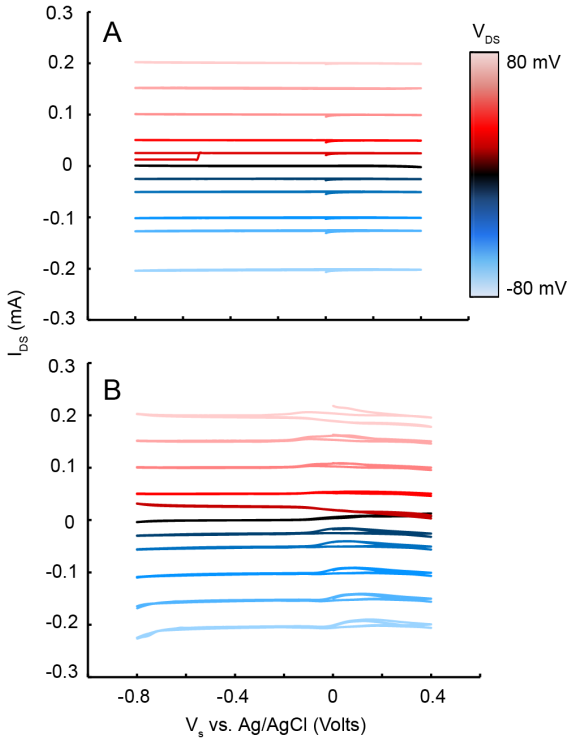

Fig. S7. Electrochemical transfer characteristics of a purified pili film, at different V_{DS} values according to the color scale at the right, in 0.1 M phosphate citrate buffer at (*A*) pH 7.0 and (*B*) pH 4.0.

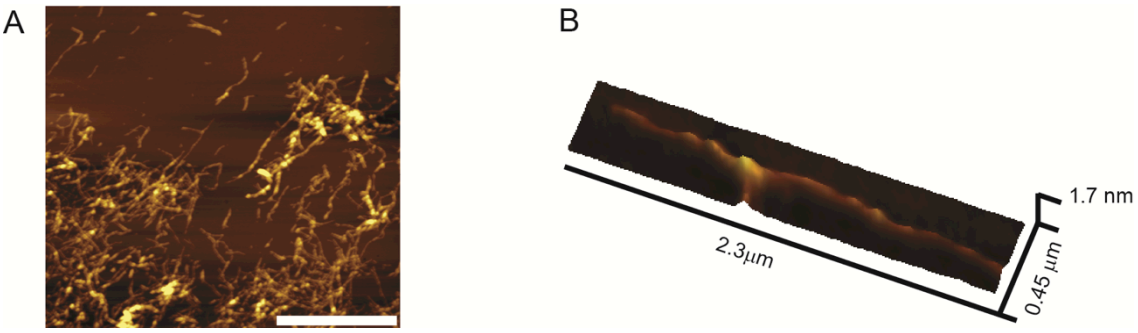

Fig. S8. (*A*) Representative AFM micrograph of Aβ fibers. Scale bar is 5 μm. (*B*) Perspective view of a single Aβ fiber from AFM imaging.

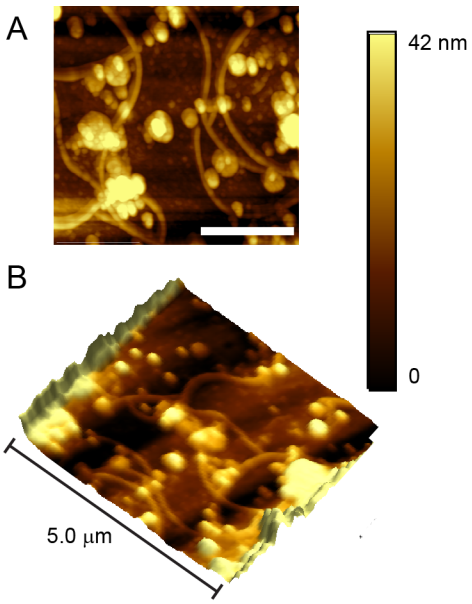

Fig. S9. (*A*) AFM micrograph of purified pili fibers bridging the gap on interdigitated electrode devices. Scale bar is 1 μm. (*B*) Perspective view of pili fibers bridging the gap between electrodes, as indicated by the tall features at the top left and bottom right sides of micrograph.

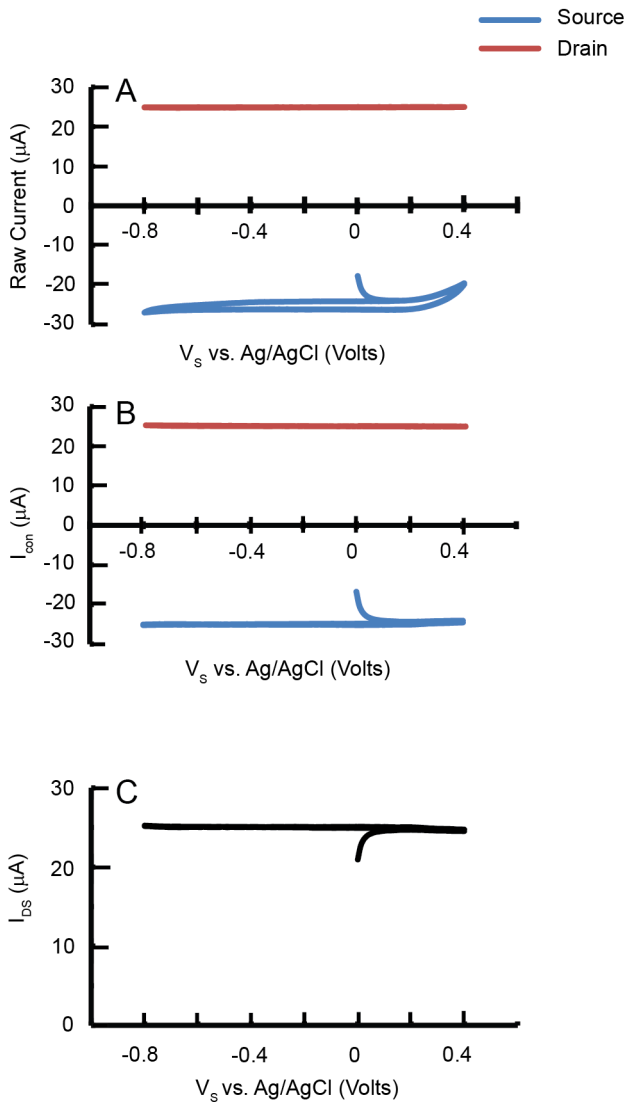

Fig. S10. Gated bipotentiostat cyclic voltammograms of purified pili films: (*A*) crude source and drain currents at V_{DS} = 10 mV, (*B*) conducting source and drain currents with background subtraction (background currents obtained from V_{DS} = 0 mV), and (*C*) source-drain current I_{DS} (drain current minus source current). Measurements were obtained in 0.1 M phosphate citrate buffer.

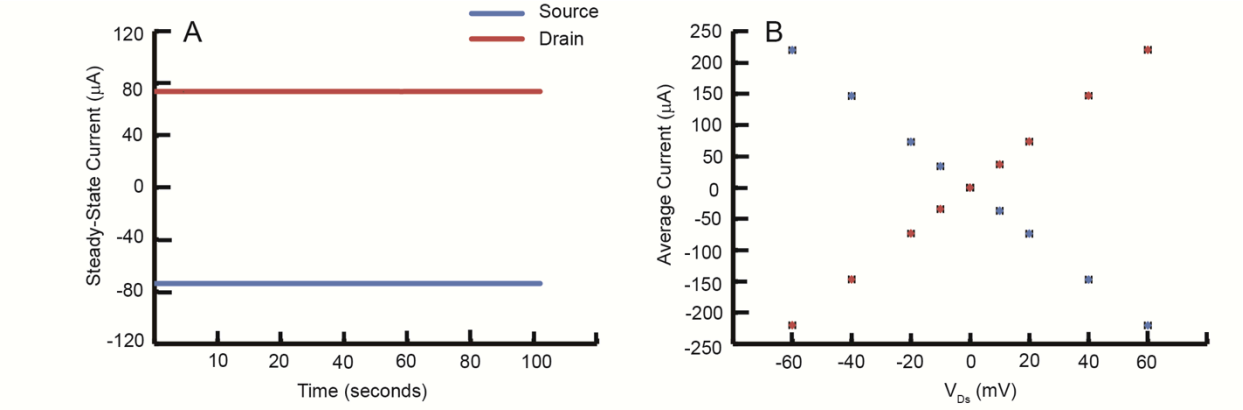

Fig. S11. (*A*) Steady-state source and drain currents of a purified pili film obtained at room temperature at V_{DS} = 10 mV. (*B*) Average steady-state source and drain currents of pili film plotted as a function of V_{DS}. Measurements were obtained taken in 0.1 M phosphate citrate buffer pH 7.0.

SUPPORTING INFORMATION REFERENCES

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