Supplementary Information for:

# Conductivity of individual Geobacter pili

R. Y. Adhikari<sup>a</sup>, N. S. Malvankar<sup>a,b,§</sup>, M. T. Tuominen<sup>a</sup> and D. R. Lovley<sup>b\*</sup>

- <sup>a.</sup> Department of Physics
- <sup>b.</sup> Department of Microbiology University of Massachusetts, Amherst, Massachusetts, 01003, USA
- <sup>§</sup> Present Address: Department of Molecular Biophysics and Biochemistry, Microbial Sciences Institute, Yale University, Connecticut, 06520, USA

#### **Corresponding Author:**

Derek R. Lovley, E-mail address: dlovley@microbio.umass.edu

## **Detailed Experimental Methods:**

**Device Fabrication:** Copolymer methyl methacrylate (MMA –E10; MicroChem) was spin-coated onto the silicon dioxide surface at 4000 rpm and baked at 180 °C for 5 minutes. This was followed by spin-coating a higher molecular weight polymethyl methacrylate (PMMA –A2) at the same speed followed by baking. Electrode patterns were written with a JEOL JSM-7001F ebeam writer and developed with a 1:3 solution of isopropanol and methyl isobutyl ketone. Ebeam evaporation was used to deposit 10 nm chromium at the bottom of the electrode pattern and 20 nm gold on the top. The dimensions of the electrodes were verified with a Dektak profilometer. Each electrode cluster contained fourteen electrodes (Figure 1b). Each chip consisted of 3x3 array of the fourteen-electrode clusters.

**Pili Sample Preparation:** Pili were harvested from the wild-type and the Aro-5 strain<sup>1</sup> of *G*. sulfurreducens as previously described<sup>2</sup>. Pili were separated from cells by vortexing and resuspended in 150 mM ethanolamine buffer(pH 10.5). Cells were removed by centrifugation, then the pili were concentrated and washed by ultracentrifugation at 100,000*g* or using ammonium sulphate precipitation, and re-suspended in ethanolamine buffer. Presence of pili in the solution was confirmed with transmission electron microscopy.

Buffer containing purified pili (0.2  $\mu$ l) was drop casted on a chip with fabricated devices. After waiting for 15 minutes to allow pili to settle, the residual solution was withdrawn with a micro pipette. Then, a deionized water drop covering the device area was placed on top of the electrode array in order to dissolve any salts. The excess water was withdrawn after 1 min. The water-rinse process was repeated two more times in order to ensure that electrolytes from the growth medium were removed. The samples were gently air dried at room temperature (22°C), which leaves a water layer associated with the pili.<sup>3</sup> Aqueous HCl was used to change pH of the media surrounding pili. Pili on the electrode array were located with atomic force microscopy (Asylum Research).

**Low Current Measurements:** The corresponding electrode pads were wire bonded with aluminum wire and connected to a printed circuit board. The chip containing electrodes was placed in a double-shielded box (Fig. S1). The outer metallic box, which served as a Faraday's cage to protect the signal from external electrostatic interference, was connected to the outer grounded shield with a triaxial cable. The inner metallic box was connected to an inner low impedance guard to prevent leakage current through the circuit due to device circuitry and cabling.<sup>4</sup> The central connecter was connected to circuit box placed inside the guarding box using wire soldering and gold-plated pins.

Measurements were performed with a Keithley 4200 Semiconductor Characterization System (SCS). Two Source Measure Units (SMUs) of the SCS were equipped with preamplifiers 4100-PA providing the system with capability to measure current signals with resolution of up to 100 aA. These SMUs were connected to the two terminals of the double-shielded box with low noise triaxial cable. Current values were recorded by grounding one of the terminals while the other terminal was set to potentials ranging from -0.5V to 0.5V (Fig. S2). The current value for each applied potential was extracted by averaging the measured steady state current over time.



#### Shielding system for low current measurement:



**Fig. S1**: Double shielding system for low current measurements. A) Image of the shielding system. B) Sketch of the shielding system. Outer box is connected to ground of triaxial cable and inner metal box is connected to low impedance inner guard. C) Circuit configuration of the system. In addition to acting like a Faraday cage, the system is designed to help prevent leakage current during high resistance measurements.

### **Steady State Current Measurements:**

Constant voltage was applied and current response over time was recorded until the steady state was observed. During the steady state, current is due to contribution from electronic transport while ionic conduction and leakage current of the system becomes negligible. The value of current at a given voltage for wild type pilus sample at pH 7 (Fig. 2) was extracted from the steady-state value of the current shown below.



**Fig. S2:** Steady state current measurement of the wild type pilus response in Fig. 2. Current values (red) reach steady state over time which can be fitted with exponential offset function (black) to obtain the value of the steady state current.

## Table S1. Experimental conductance and conductivity values.

Sample	Conductance (S)	Conductivity (mS/cm)	рН
<i>Geobacter</i> <i>sulfurreducens</i> Wild Type	5.26 x 10 <sup>-14</sup> ± 3.55 x 10 <sup>-14</sup>	0.037 ± 0.015	10.5
	7.22 x $10^{-11} \pm 2.76 \times 10^{-11}$	51 ± 19	7
	$2.65 \times 10^{-10} \pm 8.28 \times 10^{-11}$	188 ± 34	2
Geobacter sulfurreducens Aro-5 mutant	1.16 x 10 <sup>-14</sup> ± 4.80x 10 <sup>-15</sup>	0.0082 ± 0.001	10.5
	5.43 x 10 <sup>-14</sup> ± 2.73 x 10 <sup>-14</sup>	0.04 ± 0.001	7
	1.41 x 10 <sup>-12</sup> ± 8.23 x 10 <sup>-13</sup>	1 ± 0.03	2

**Table S1**: Experimental values of conductance and corresponding conductivity of varioussamples. Calculations were performed using equation (1).

#### References

- 1. M. Vargas, N. S. Malvankar, P. L. Tremblay, C. Leang, J. A. Smith, P. Patel, O. Snoeyenbos-West, K. P. Nevin and D. R. Lovley, in *mBio*, 2013, vol. 4, pp. e00105-00113.
- 2. N. S. Malvankar, M. Vargas, K. P. Nevin, A. E. Franks, C. Leang, B. C. Kim, K. Inoue, T. Mester, S. F. Covalla, J. P. Johnson, V. M. Rotello, M. T. Tuominen and D. R. Lovley, *Nature nanotechnology*, 2011, **6**, 573-579.
- 3. N. S. Malvankar, M. T. Tuominen and D. R. Lovley, *Energy & Environmental Science*, 2012, **5**, 8651-8659.
- 4. in *Keithley Application Note Series*, ed. I. Keithley Instruments, 2008.