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Conductivity of individual *Geobacter* pili†

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The electrically conductive pili of *Geobacter* species have been proposed to play an important role in long-range electron transfer to Fe(III) oxides and other cells and have potential as a sustainable source of electrically conductive materials. Surprisingly, there have been no previous reports on the actual conductivity of individual pili, probably the most important parameter for evaluating mechanistic models of electron transport and pili function. Therefore, the conductivity of individual pili of *Geobacter sulfureducens* was measured with a low-noise nano-electrode measurement platform along regions of the pili that appeared to be cytochrome-free. Pilus conductivity was highly dependent upon pH with conductivity estimates of 188 ± 34 mS cm^{-1}, 51 ± 19 mS cm^{-1}, and 37 ± 15 μS cm^{-1} at pH 2, 7, and 10.5, respectively. The conductivities of pili from strain Aro-5, which expresses pili in which an alanine was substituted for each of five aromatic amino acids, were significantly lower than the wild-type pili. These results, and the previous finding that stacking of aromatic amino acids increases at low pH, suggest that aromatic amino acids play a key role in pilus conductivity. The conductivity of the *G. sulfurreducens* pili is comparable to conducting organic polymer wires of similar diameter and several bacterial filaments of substantially different composition. These results provide important parameters that should be accommodated in future models of *G. sulfurreducens* pilus conductivity and suggest strategies for enhancing pilus conductivity with genetic manipulation.

Protein-based materials comprised of natural amino acids are attractive candidates for molecular electronics due to their diverse optical, electrical, mechanical, and chemical properties,[1,2] as well as low cost and absence of toxicity.[3] Previous studies suggested that the proteinaceous pili of *Geobacter sulfurreducens* are biologically unique electronic materials because they can conduct electrons over μm distances with metallic-like conductivity.[4,5] However, this concept has been challenged repeatedly on the basis of theoretical modelling or inferences from biofilm behaviour.[6–12] Remarkably, amongst this controversy the most basic data requirement, an estimate of the conductivity of individual pili, has been missing.

Therefore, a low-noise nano-electrode measurement platform was devised to directly measure the conductivity of individual pili (Fig. 1; see ESI† for additional details of construction). Arrays of gold electrodes, 2 μm wide and 10 μm long, separated by non-conducting gaps of 500 nm, were

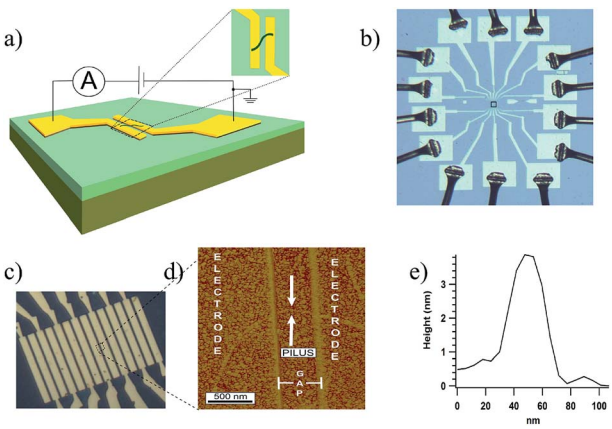

Fig. 1 (a) Schematic of measurement setup. Electrodes were fabricated on top of thermally grown silicon dioxide. (b) Fabricated device with wire bonding. Nine such devices were fabricated in one chip with e-beam lithography. (c) Close-up optical image of the electrodes at the center (indicated by the box in (b)) of each device. Electrodes were 10 microns long, 2 microns wide, 30 nm thick and separated by 500 nm. (d) AFM image of a pilus bridging a pair of electrodes as indicated by arrows. (e) Cross sectional height of the bridging pilus.

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fabricated on n-doped silicon wafers with a 100 nm insulating layer of thermally grown oxide on the surface. The electrodes were connected to 100 μm × 100 μm pads for electrical contacts.

Pili from wild-type *G. sulfurreducens*, and strain Aro-5, a genetically altered strain in which key aromatic residues were replaced by alanine,[13] were prepared as previously described.[14] Buffer containing the pili was drop cast onto the electrode arrays. Excess buffer was removed, the samples rinsed with deionized water, and then gently air-dried (see ESI† for additional experimental details). This leaves pili in a hydrated state.[15] Pili were located with atomic force microscopy. Occasionally, a single pilus bridging two electrodes was located (Fig. 1d). The c-type cytochrome OmcS, binds to the pili.[16–19] Studies with mutant strains demonstrated that OmcS and similar c-type cytochromes on pili can be detected with AFM[19] and the broad spacing between OmcS molecules often found on pili (100 s of nanometers)[16,17] made it possible to conduct all conductivity measurements on pili in which no cytochromes were associated with the section of the pili bridging the gap between the electrodes. Height measurements (Fig. 1e) confirmed that each filament was a pilus, which have a diameter of 3 nm, without additional associated proteins and that the filaments were not flagella, which have a diameter of 12 nm.[14]

The contact pads corresponding to the two electrodes bridged by the individual pili were wire bonded with aluminium wire, connected to a printed circuit board, and the device was placed inside a double-shielded box with the inner box as a guard and the outer box as a ground (Fig. S1†). Additional details of experimental procedures are presented in the experimental section. All the measurements were performed in a temperature (22 °C) and humidity (55%) controlled clean room.

The device was initially evaluated with 150 nm diameter carbon nanotubes, as a positive control. The ohmic response (Fig. 2a) of current–voltage (IV) curve and the conductivity of 6 kS cm^{-1} were consistent with known properties of carbon nanotubes.[20]

At the physiologically relevant pH 7, individual wild-type pili of *G. sulfurreducens*, spanning the non-conducting gap between two electrodes, exhibited linear, ohmic behavior (Fig. 2b). Conductivity values (Table S1†) were calculated from the relation:

$$\sigma = G\left(\frac{l}{\pi r^2}\right) \quad (1)$$

where, G is the conductance value (Table S1†) acquired from the IV curve, l is the electrode gap (500 nm) and r is the radius of the pilus (1.5 nm). The calculated conductivity of the wild-type pili was 51 ± 11 mS cm^{-1} (mean ± standard error of three pili). This is more than 1000-fold higher than the previously reported conductivity of pili networks.[14] The difference can most likely be attributed to pili-to-pili contact resistances within the network that spanned non-conducting gaps of 50 μm, which is much greater than the length of an individual pilus. These results demonstrate that there is substantial conductivity along the length of cytochrome-free regions of the pili, consistent with previous observations of charge propagation in similar regions of individual pili.[18]

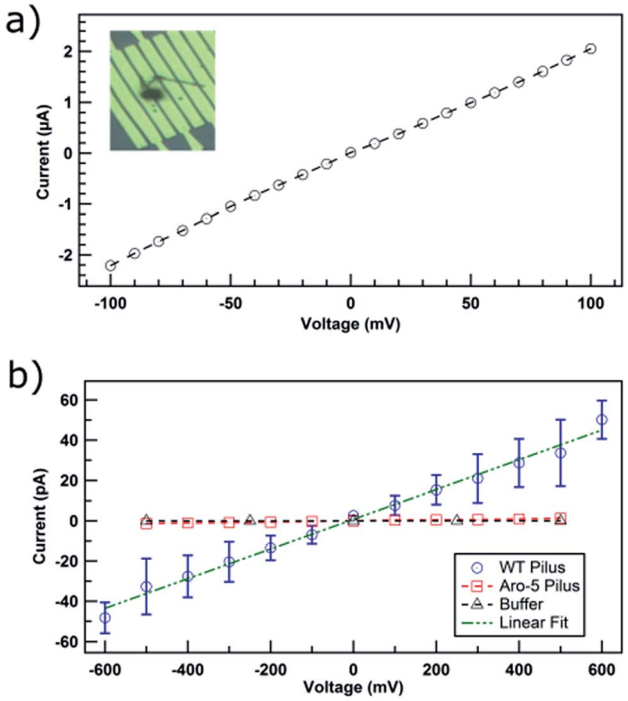

Fig. 2 Current–voltage responses of: (a) carbon nanotube positive control. Inset is an optical image showing where the 150 nm diameter carbon nanotube bridged across electrodes. (b) Individual *Geobacter sulfurreducens* pili at pH 7 bridging two electrodes with the pilus either from wild-type (WT) strain or Aro-5 strain in which key pilus aromatic amino acids are absent. The data represent the mean and standard deviation of three different pili. Standard deviations for the Aro-5 pili are smaller than the symbol representing data points.

The current–voltage response of individual pili from strain Aro-5 was more similar to the response from buffer (Fig. 2b) without pili, yielding a conductivity estimate of 38 ± 1 μS cm^{-1}, three orders of magnitude lower than wild-type pili. This result is consistent the conclusion, based on measurements on pili networks, that the pili from the Aro-5 strain poorly conduct electrons because they lack key aromatic amino acids required for electrical conductivity.[13]

To further analyse the conductive properties of individual pili, conductance was measured at different pH. Increasing the pH to 10.5 dramatically lowered the conductivity of the wild-type pili to 37 ± 15 μS cm^{-1}, whereas decreasing the pH to 2 substantially increased pilus conductivity to 188 ± 33 mS cm^{-1} (Fig. 3). In contrast, the conductivity of the buffer did not change significantly with pH. The change in pilus conductivity with pH is consistent with conformational changes that result in greater π–π stacking of aromatic amino acids at lower pH.[5] This pH response of the individual pilus further suggested that the measured electronic conductivity is an intrinsic property of the pilus.

The pH also influenced the conductivity of the Aro-5 pili, but to a much lower extent than the wild-type (Fig. 3). This suggests

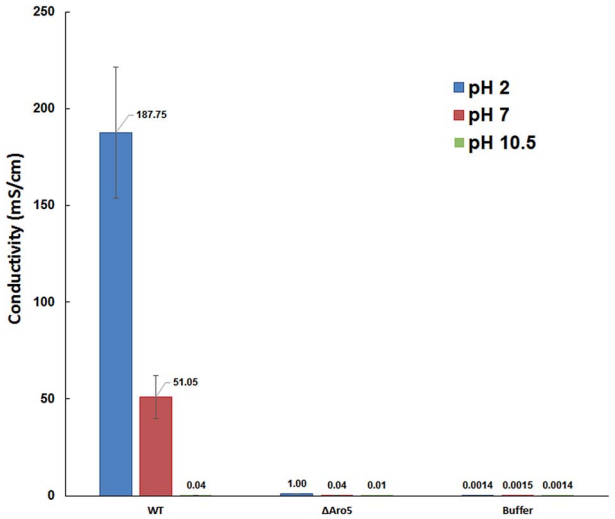

Fig. 3 pH dependent conductivity of wild-type and Aro-5 *Geobacter sulfurreducens* pilus and corresponding buffer. The buffer conductivity did not significantly change with varying pH. Error bars represent the standard error of measurements of three different pili.

that other factors such as charged amino acids may also contribute to conduction through pili, as previously suggested.[5,8]

Regardless of the mechanism of electron conduction, these measurements demonstrate that individual pili are electrically conductive. For comparison with other organic wires of similar diameter, the conductivity of the proton-doped pili is much higher than the conductivity (0.91 mS cm^{-1}) of polypyrrole fibers of 80 nm diameter[21] and compares favourably to the 90–600 mS cm^{-1} conductivity of PEDOT wires with a diameter of less than 10 nm.[22] The *G. sulfurreducens* pili are substantially less conductive than the 1.34 kS cm^{-1} for carbon nanotubes with a diameter of 1.3 nm.[23] Outer-membrane extensions of *Shewanella oneidensis*, which are a complex mixture of lipids, cytochromes, and potentially other proteins, form filaments when fixed with gluturaldehyde and critically point dried[24] with conductivities of 60 mS cm^{-1} to 1 S cm^{-1}.[25] After a similar fixation procedure, the 50 nm diameter filaments of *Rhodopseudmonas palustris*, which are of unknown composition, have conductivities of 35–72 μS cm^{-1}.[26]

Cross-linking proteins with gluturaldehyde has the potential to alter filament structure and thus conductivity. It will be of interest to assess the conductivity of the *S. oneidensis* and *R. palustris* filaments with the method described here. This method may also be useful for further analysis of conductivity along the length of other microbial filaments that are conductive across their diameter,[27–29] often after chemical fixation.

Conclusions

The estimates of individual *G. sulfurreducens* pilus conductivity reported here provide a key piece of data that is needed for assessing the diverse proposed models for the conductivity of *G. sulfurreducens* pili.[6–10,12] Models that include a role of c-type cytochromes in electron conduction along the length of the pili,[7,9,10] must account for conductivity measured here in reaches of the pili that were cytochrome free. The major impact of proton-doping on conductivity should also be accommodated.

The conductivity of *G. sulfurreducens* pili suggests that they may be useful electronic materials with the advantages that they can readily be mass-produced in a sustainable manner and they do not contain toxic components. The pili function in water, are highly chemically stable, and their properties can be readily be genetically modified. The strong dependence of pilus conductivity on pH and their high aspect ratio suggests that *G. sulfurreducens* pili might have applications as highly sensitive pH or other environmental sensors. The finding that there are substantial increases in pilus conductivity associated with proton doping, which increases stacking of aromatic amino acids,[5] suggests that genetic manipulation to further increase interactions of aromatic amino acids may enhance pilus conductivity.

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