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A paper published in *Energy and Environmental Science* by Strycharz-Glaven *et al.* suggests that electron transport along the pili and through the conductive biofilms of *Geobacter sulfurreducens* proceeds *via* electron superexchange rather than metallic-like conductivity. Multiple lines of evidence disprove the superexchange hypothesis, but are consistent with metallic-like conductivity.

In their recent article Strycharz-Glaven *et al.*¹ propose a model for electron conduction along pili and through biofilms of *Geobacter sulfurreducens*, and claim that their hypothesis is applicable to our measurements² of metallic-like conductivity through biofilms of *Geobacter sulfurreducens* and networks of *G. sulfurreducens* pili. However, Strycharz-Glaven *et al.* have misinterpreted our data, as well as recent data in other publications, and have not accounted for key experimental results in our paper.²

The metallic-like conductivity that we demonstrated² is significantly different from the “superexchange” model of Strycharz *et al.* “in which electrons are conducted by a succession of electron transfer reactions among redox proteins”. In metallic-like conductivity electrons are delocalized, forming a sea of electrons, and move without any need for thermal activation.³ In contrast, in the superexchange model (sometimes referred to as “electron hopping”)¹ electrons are associated with specific molecules and are transferred along a succession of these molecules that must be close enough (*ca.* 2 nm or less)⁴ to permit this exchange. The two types of conductivity have much different structural requirements for the conductive biological material and have distinct responses to physical/chemical perturbations.

For example, abiobiochemical nanostructured organic metals exhibit an exponential *increase* in conductivity as temperature is

decreased.^{3,5,6} This response is a hallmark of delocalized electrons scattered by high-energy phonons.^{6–8} The conductivity of biofilms of *G. sulfurreducens* and pili networks have a similar temperature response.² In contrast, the superexchange model (eqn (21) of Strycharz-Glaven *et al.*) predicts a *decrease* in conductivity as temperature is decreased. Thus, the starkly contrasting temperature response alone disproves the superexchange hypothesis.

Furthermore, we demonstrated that the conductivity of pili increased two-orders of magnitude with decreasing pH,² consistent with the concept that protons act as a dopant to the pili nanowires and function as a source of carriers, as expected for a metallic-like conduction mechanism.^{7,9,10} Lowering the pH provides more protons, which increases positively charged carriers in pili, commonly referred to as electron holes. This increase in the carrier concentration increases the conductivity of the pili. This pH effect would not be expected if the Strycharz-Glaven superexchange model was correct because redox species and not electron holes are implicated as charge carriers in that model.¹

The superexchange hypothesis for conduction along the pili of *G. sulfurreducens* also has specific biochemical requirements that are not consistent with experimental observations. The superexchange hypothesis requires that cytochromes, or other redox-active proteins, are associated with the pili and that they be packed closely enough for cytochrome-to-cytochrome electron exchange. To make their case, Strycharz-Glaven *et al.* cite previous studies from our laboratory that demonstrated that the *c*-type cytochrome, OmcS, is specifically associated with pili.¹¹

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Broader context

Long-range electron transport along the pili and through the conductive biofilms of *Geobacter* species plays an important role in the biogeochemistry of anaerobic soils and sediments as well as in bioenergy strategies such as the conversion of organic compounds to electricity or methane. Furthermore, the metallic-like conductivity that has been observed in *G. sulfurreducens* pili and biofilms suggests novel possibilities for bioelectronics.

However, that study indicated that the spacing of OmcS along the pili is much too great for electrons to be transported along the pili by cytochrome-to-cytochrome transfer.¹¹ This spacing has subsequently been confirmed with atomic force microscopy (unpublished data). More importantly, the finding that denaturing cytochromes in pili preparations had no impact on conductivity² definitively rules out a role for OmcS, or any other *c*-type cytochrome, in electron conduction along the pili. Thus, a key biochemical requirement for the superexchange hypothesis is not fulfilled. It might be argued that there are redox-active proteins other than cytochromes on the pili that could account for conductivity, but this is ruled out by the temperature and pH dependence of pili conductivity, which as described above is consistent with metallic-like conductivity and inconsistent with the superexchange hypothesis.

Strycharz-Glaven *et al.* hypothesize that earlier measurements of conductivity across the diameter of single pili¹² can be explained in terms of heme spacing in *G. sulfurreducens* cytochromes. This ignores the fact that it was evident from atomic force microscopy in that study that cytochromes were not attached to the pili at the points at which conductance across the pili could be measured.¹² In the instances in which extra proteins were associated with the pili, they acted as insulators.¹² Again, the biochemical evidence is inconsistent with the superexchange hypothesis.

In contrast to the lack of biochemical evidence consistent with the superexchange hypothesis, preliminary structural studies indicated that the pili exhibit features similar to intermolecular π stacking, which promotes the electron delocalization required for metallic-like conductivity and has been previously observed in organic metals and conductive proteins with delocalized electronic states.^{3,5,13}

Strycharz-Glaven *et al.* then extend their superexchange hypothesis from pili to electron transport through *G. sulfurreducens* biofilms. As noted above, the temperature dependence of conductance through the biofilms rules out the superexchange hypothesis.

Other data is also inconsistent with superexchange model for the biofilms. For example, electrochemical gating on biofilms showed a sigmoidal response, which is a characteristic of organic metals.^{8,14,15} In contrast, the superexchange hypothesis predicts a peak in conductivity at the formal potential, the potential at which half of the redox molecules are reduced and half are oxidized (eqn (32) of Strycharz-Glaven *et al.*). No such peak is observed in experiments.² Furthermore, the superexchange hypothesis predicts that there will be no current when the redox molecules are fully oxidized. However, experiments show an increase in current of two orders of magnitude at oxidizing potentials.²

The superexchange hypothesis for biofilms also has specific biochemical requirements that are not fulfilled by experimental observation. Strycharz-Glaven *et al.* suggest that *c*-type cytochromes transfer electrons through the biofilm, but experimental evidence demonstrated that there is no correlation between the conductivity of the biofilms of *G. sulfurreducens* and their cytochrome content.¹⁶ Strains with lower cytochrome abundance produce biofilms that have higher conductivity, not lower conductivity as the superexchange hypothesis predicts. A strain of *G. sulfurreducens* that was unable to produce the most

abundant outer-surface *c*-type cytochromes, including OmcS the only cytochrome known to be associated with pili, produced biofilms with higher conductivity than the wild-type strain.² Furthermore, in a result similar to that described above for pili preparations, denaturing the cytochromes in the biofilm had no impact on conductivity.¹⁶

In several places in their manuscript Strycharz-Glaven *et al.* speculate that cytochromes, which are found in the extracellular matrix of *G. sulfurreducens* under other growth conditions, are spread throughout current-producing biofilms. They suggest that the role of pili is not to conduct electrons, but to provide a wider distribution of cytochromes throughout the biofilm. These conjectures disregard the available data. OmcZ, the only outer-surface cytochrome found to be necessary for high microbial fuel cell current density,¹⁷ is specifically localized at the biofilm–anode interface,¹⁸ not spread in abundance throughout the biofilm. The expression of OmcS, the only cytochrome found to be associated with the pili, is highly down-regulated in high current-density biofilms and a strain that cannot produce OmcS generates high current densities. In contrast, there is a direct correlation between abundance of pili and biofilm conductivity,² consistent with the concept that electron conduction is through the pili network. As previously discussed in detail,^{18,19} a working model which is consistent with all data presently available is that conductive pili facilitate electron flow through the bulk of the biofilm and OmcZ promotes electron transfer from the biofilm to the anode.

In a few instances Strycharz-Glaven *et al.* suggest a similar superexchange mechanism for electron conduction along the conductive filaments that have been reported in *Shewanella oneidensis*. Strycharz-Glaven *et al.* imply that *c*-type cytochromes are aligned along the *S. oneidensis* filaments, but there is no experimental documentation for this claim in the literature. Recently, it has been elegantly demonstrated theoretically that even if cytochromes were aligned along the *S. oneidensis* conductive filaments, the measured conductance cannot be explained with a cytochrome-based electron transport unless physically unrealistic parameter values are incorporated in the model.²⁰ Strycharz-Glaven *et al.* also suggest that *S. oneidensis* produces conductive biofilms, despite a lack of evidence and many studies to the contrary, including their own reported inability to grow substantial current-producing biofilms.¹

Ultimately, we find the comprehensive set of experimental properties to be consistent with the model of a metallic-like polymer in which electron transport is *via* delocalized electronic states and that the data is inconsistent with the model of a redox polymer in which transport occurs *via* superexchange of electrons in localized states. Strycharz-Glaven *et al.* suggest that the conductivity of *G. sulfurreducens* pili and biofilms should be analyzed with their superexchange model because there is no precedent for the reported² metallic-like conductivity, but there is a “long history” of “treating conductive polymers as a sequence of discrete charge carriers... in which current is modeled as electron diffusion”. New ideas based on experimental results require new modeling approaches. There is no evidence that the type of metallic-like conductivity reported in *G. sulfurreducens* pili and biofilms can arise by electron superexchange. A long history of a modeling approach designed to describe significantly different materials is not a suitable justification to apply a model

when it is not consistent with the observed experimental properties.

In summary, the superexchange hypothesis of electron exchange between redox proteins along pili or through biofilms of *G. sulfurreducens* is not substantiated by any experimental evidence, and there is substantial evidence that disproves the hypothesis. In contrast, there is a growing body of evidence that suggests that metallic-like conductivity is the mechanism for long-range electron conduction along the pili of *G. sulfurreducens*.

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