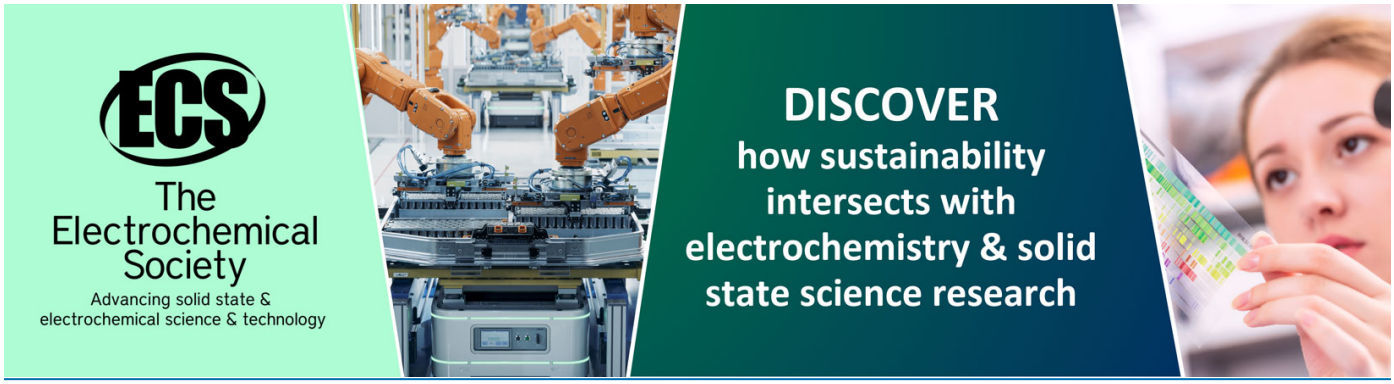
Modeling the Structure of Type IV Pili from Shewanella Oneidensis



Modeling the Structure of Type IV Pili from Shewanella Oneidensis

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Abstract. The metal-reducing bacterium *Shewanella oneidensis* produces conductive nanowires, which are described as extensions of the outer membrane or type IV pili (T4P), of which the structural details remain unknown. To better understand the structure of *S. oneidensis* T4P as a nanowire candidate, and the potential mechanism for electron transport along them, theoretical energy-minimized models of these pili were constructed. The models showed a discontinuous geometry consisting of aromatic rings, with large gaps of ~10 Å, providing a potential pathway for electron hopping, which may account for previously reported conductivity. Moreover, mutations of the pilin subunit based on the models were proposed, which might fill the gaps and possibly increase the conductivity. These models will facilitate further investigation of the nanowires and their applications in bioenergy and bioelectronics.

1. Introduction

The bacterium *Shewanella oneidensis* was known for its capability of dissimilatory reduction of metals, which plays important roles in a globally biogeochemical process driving the cycling of metals such as Fe and Mn, as well as organic matter in soils, sediments, freshwater and marine environments [1]. Is has been reported that *S. oneidensis* employs electrically conductive nanowires in response to electron-acceptor limitation, which offer a pathway for transferring electrons to insoluble external acceptors such as metals in the environment and electrodes in microbial fuel cells (MFC) [2].

Two types of filament have been described for these microbial nanowires from *S. oneidensis*: an extension of the outer membrane and periplasm including the multiheme cytochromes [3] and a type IV pili (T4P) of which the pilin subunit has been structurally determined [4]. Since the nanowires of *S. oneidensis* are often compared to another anaerobe *Geobacter sulfurreducens*, of which the nanowires are determined as T4P and presents metallic-like conductivity attributed to overlapping pi-pi orbitals of aromatic amino acids [5–7], an assembled model of the *S. oneidensis* T4P will be helpful for understanding the differences between the two microbial nanowires and the mechanism for electron transport (ET) along them. Although a homology model of the pili was proposed [4], such a strategy is strongly dependent on the selected template and might result in van der Waals clashes [8].

Here, we present theoretical energy-minimized structure models of the assembled *S. oneidensis* T4P based on our previously reported strategy [9], which has been used to modeling the conductive pili of *Geobacter sulfurreducnes* by employing the Rosetta Symmetric Docking protocol [10, 11] with

a combination of randomized structural parameters and distance constraints [8]. These models are valuable tool to guide further investigation of the microbial nanowires.

2. Methods and Materials

The models of *Shewanella oneidensis* type IV pili were generated with a method previously validated by reconstructing pili of known structure [9], which was based on standard symmetric docking protocols in the Rosetta software suite.

The $Pil_{Bac1}\Delta N$ structure, i.e. the pilin subunit lacking the first 28 residues, was downloaded from Protein Data Bank (PDB ID: 4D40), and a full length structure of the *S. oneidensis* pilin (Figure 1. A) was modeled by employing the coordinates of N-terminalα-helix in PilE from *N. gonorrhoeae* (PDB ID: 2HI2) [12]. SWISS-MODEL [13] and Pymol [14] were used to build and superimpose the N-terminal α-helix. To eliminate steric clashes, the pilin model was relaxed by Rosetta Relax protocol.

A multi-step process was utilized, as suggested previously [9]. In the first step, global low-resolution sampling with side chains in centroid mode was used for searching potential structures. Helical symmetry was enforced during the whole process, with the rotation angle of subunits around the helical axis varying from 0° to 180°, the rise per subunit varying from 5–15 Å, and the radius from the subunit center of mass to the helical axis varying from 15–30 Å. Models from the first step converged into several low-energy clusters of models (Figure 1. B). In the second step, the rotation angle was narrowed down to 80° to 110° according to the distribution of models in the first step, and another round of local sampling was employed. Models was then filtered and clustered, and a full-atomic refinement was applied to the center structure of the largest cluster. During the process of sampling, ambiguous constraints of the charged and aromatic residues were employed, as described in the previous modeling of *Geobacter* pili [8].

Potential pathway of aromatic residues were calculated by RING [15] and molecular images were prepared with PyMOL [14].

3. Results and Discussion

3.1. Initial model output.

5000 low-resolution models were generated from the first step. Since symmetry details, i.e. rotation angle, axial rise and diameter, are correlated with each other, the rotation angle was used as an indication of the symmetry features, and the landscapes of energy score were depicted as a function of the rotation angle (Figure 1. B). One group, with the lowest energy score and the rotation angle located in the common range of T4P (around 100°), was selected for the next step (colored in dark cyan).

In the second step, rotation angle varied in a narrower range and the resulted models were clustered based on Root Mean Square Deviations (RMSD) of their Cα positions, with a cut-off of 2.5 Å. 2000 full-atomic models were then generated by refinement of the center model in the largest cluster. The landscape of interfacial energy score versus the RMSD from the model with the lowest energy shows a trend of convergence (Figure 1. C). And the 50 lowest-energy models were colored in dark cyan and picked for further analysis.

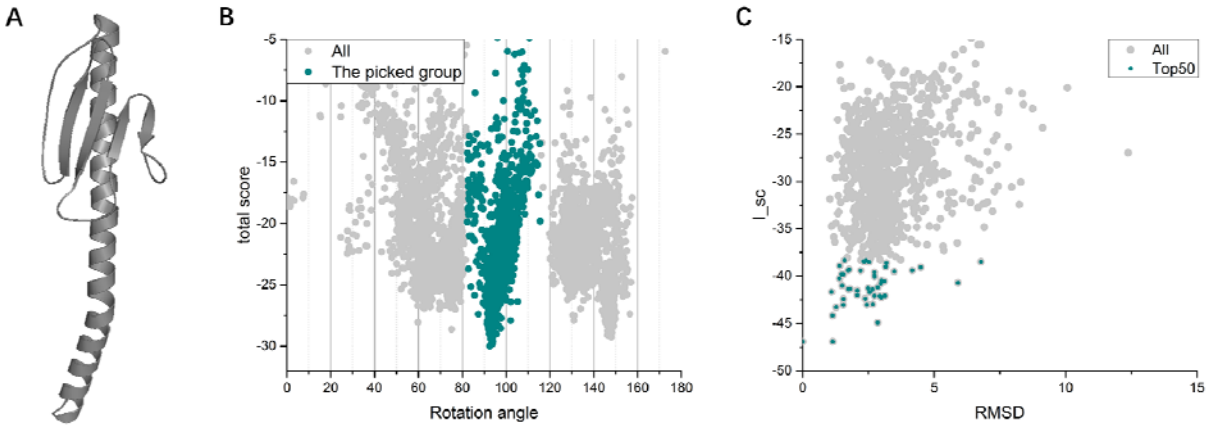

Figure 1. (A) the full-length structure of *S. oneidensis* pili; (B) the landscapes of energy score versus rotation angle of subunits at the end of the first step; (C) the interfacial energy score versus RMSD of the refined models after the second step.

3.2. The Models with the lowest energy.

One model with the lowest interfacial energy score was picked from the 50 models as a representative of these structures. In the model, The subunits align in a right-handed helix, and the rotation angle, the axial rise and the diameter of the assembled structure are ~96°, ~9Å and ~58Å respectively (FigureA and B).

As previously proposed [5–8], nanowires from *G. sulfurreducens* are conductive due to the proximity of aromatic residues in the assembled pili. Accordingly, aromatic amino acids from the assembled T4P models of *S. oneidensis* were analyzed. The possible pi-pi interactions and potential pathways formed by the aromatic residues were generated by the RING-2.0 server[15] with default parameters. The aromatic rings from one chain and the ones from the 4th chain neighboring are close to each other and form a potential chain (FigureC). The proximity of Tyr51 from protomer P and Tyr68 from protomer P+4 results in a continuation of the aromatic chain, which is composed of Tyr69, Phe71, Tyr73, Phe82, Tyr57, Tyr51 and Tyr68 one by one. Most distances between proximal carbon atoms in neighboring rings are less than 4Å, which allow for pi orbital stacking, except the ones between Tyr68 and Tyr69, Tyr69 and Phe71, and Phe71 and Tyr73 (FigureD). The distances between these aromatic pairs are too large for electron delocalization but optimal for charge hopping, especially the latter one which is 10 Å. Such configuration of aromatics is consistent with previously reported conductivity of *S. oneidensis* which is much lower than *G. sulfurreducens* and attributed to a multistep hopping mechanism. Other models from the selected group exhibit similar features of the aromatics.

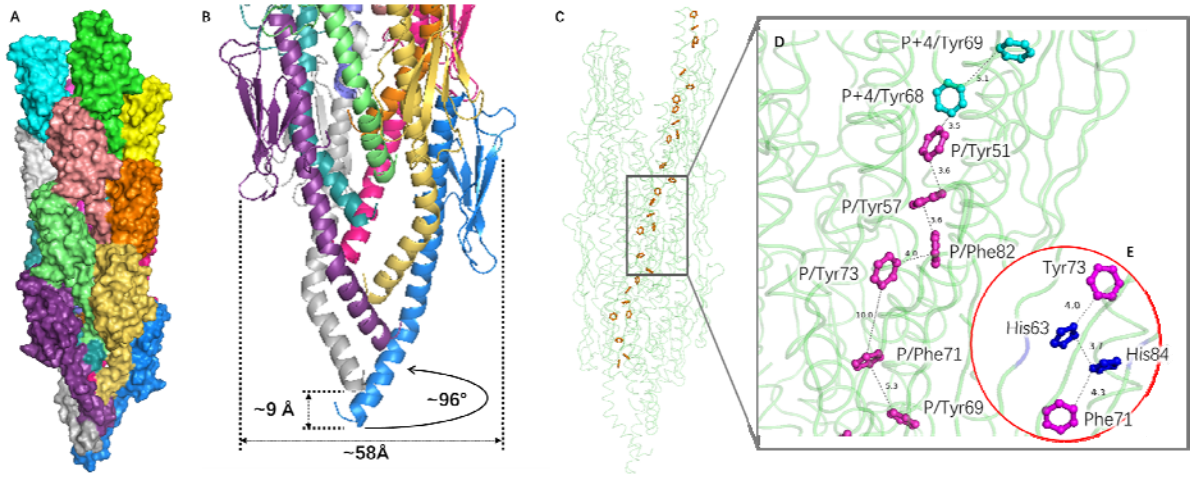

Figure 2. The model with the lowest interfacial *energy*. (A) the filament model containing 15 subunits; (B) the symmetry paramenters, i.e. the rotation angle, axial rise and diameter of the model; (C) a potential pathway formed by aromatic rings; (D) details of the neighbouring aromatic rings; (E) suggested mutations that would fill the gap of the pathway, with mutated residues in blue.

3.3. Mutations that might increase the conductivity.

Because of the gaps in the possible pathway, conductivity would be seriously restrained when electrons go over such potential barriers. Here, we proposed two mutations, which convert Leu63 and Leu84 into Histidine. Such mutations will fill the largest gap in the pathway with aromatic residues, form a possibly more continuous aromatic chain in the assembled pili of *S. oneidensis* (FigureE), and thus increase the conductivity. The mutations could be evaluated by molecular experiments, and provide an approach for further testing of the models.

4. Conclusion

We present here theoretical energy-minimized models of the nanowire candidate from *Shewanella oneidensis*, the type IV pili, by using a method based on symmetric docking. The results of modeling demonstrate that aromatic rings from neighboring chains can form a potential pathway, but with large gaps that cannot allow for electron delocalization. Such results are consistent with previously suggested mechanism of multistep hopping. Moreover, we suggest here a mutant that might fill the gap in the aromatic chain and thus increase the conductivity of the nanowires. Our work provides a new perspective for summarizing the knowledge about the *S. oneidensis* pili, as a nanowire candidate, and will be helpful to further investigation.

Acknowledgements

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