1.6 Å Structure of Pil_{Bac1}: Insights into Long-Range Electron Transfer in Bacteria and a Sulfur-SAD Success Story

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Introduction: Bacterial nanowires are natural cables

- The metal-reducing bacteria *Shewanella oneidensis* and *Geobacter sulfurreducens* can use metals like Fe(III), Mn(IV) and U(IV) as electron acceptors: They literally *breathe* metals. BUT: most metals are only poorly soluble (Fe(III), Mn(IV)) and often highly toxic (U(IV), ^{99}Te(VII), Hg(II)) ⟶ they cannot enter the cell!

⟶ The bacteria need to **transfer the electrons from the inside of the cell to the metals at the outside**. For this, they produce nanowires[1,2] (Fig. 1)! These are long, extracellular filaments that act like natural cables!

- Data from Geobacter show that **type IV pili**, which are commonly expressed in many diverse bacteria, are part of nanowires[2,3].
  QUESTION: How Can Type IV Pili Form Nanowires in *Shewanella Oneidensis*?

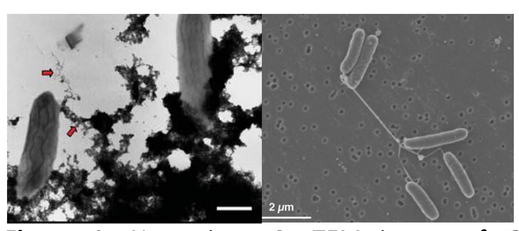
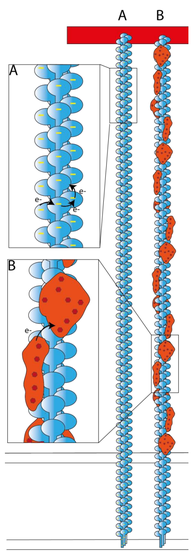


Figure 1: Nanowires. **A**: TEM image of *G. sulfurreducens* nanowires[2]. Arrows indicate pili. **B**: AFM image of *S. oneidensis* nanowires[1].

Electron transfer theories



Basis: **Type IV pili** form nanowires!

A: Metallic-like theory[4,5]: **Aromatic residues** in a type IV pilus are so **closely positioned** to each other, that the electrons can be delocalized and transferred like in a metal lattice (Fig. 2A).

B: Multi-step hopping theory[6]: Type IV pili form a non-conductive backbone to which **multi-heme cytochromes attach**. Electrons can then hop from one heme to the other along the full nanowire (Fig. 2B).

Figure 2: **A**: Metallic-like theory. **B**: Multi-step hopping theory.

Structure of Pil_{Bac1}

- Sequence analysis and MS data indicate that the type IV pilus (T4P) protein Pil_{Bac1} is a **putative nanowire** subunit.
- We determined its **1.6 Å structure** (Fig. 3A) by S-SAD (see right): ⟶ common T4P fold.
  BUT: straight α-helix.
  unusual position of disulfide bridge.
- Model of a complete Pil_{Bac1} pilus (Fig. 4)
  - based on T4P from *N. gonorrhoeae* (PDB: 2HIL).
  - NT α-helix buried in the core with β-sheet to the outside.
- Distribution of aromatic residues (Fig. 3B,C)
  ⟶ Indeed clusters of **closely spaced aromatic side chains**!
  ⟶ BUT THESE CLUSTERS ARE TOO FAR FROM EACH OTHER (**11 Å !**) THAT ELECTRONS COULD MOVE BETWEEN THEM!

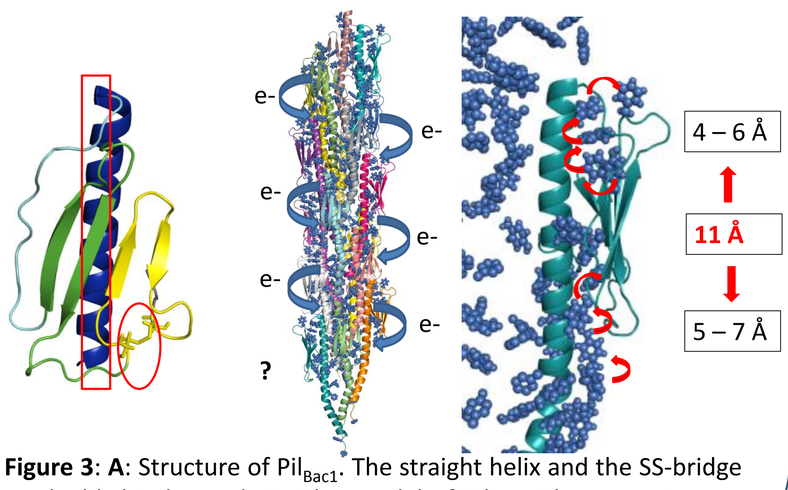


Figure 3: **A**: Structure of Pil_{Bac1}. The straight helix and the SS-bridge are highlighted in red. **B**: Pilus model of Pil_{Bac1}. The aromatics are shown as spheres. **C**: Zoomed in view on the aromatics in the pilus and the distances between them.

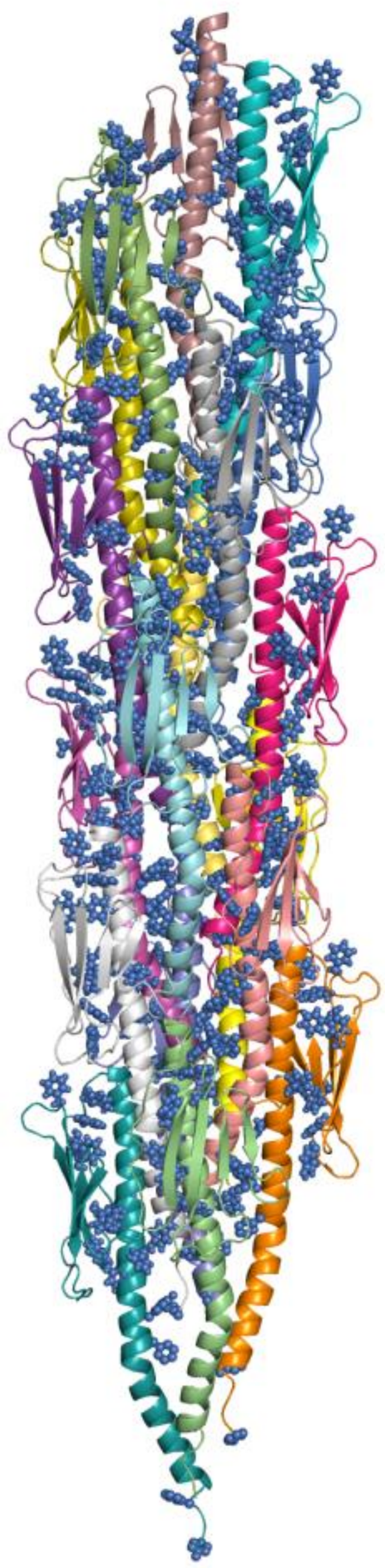


Figure 4: Model of a PilBac1 pilus based on a pilus from *N. gonorrhoeae* (PDB:2HIL). Aromatic residues are shown in spheres.

Sulfur-SAD Phasing

Data collection and processing
- 3 data sets of 360° on 3 different volumes of 1 single crystal
- At a wavelength of 1.8 Å
- **Very low Bijvoet ratio ΔF/F**: 0.9%
- Very low **Multiplicity**: 5.5

→ Not very promising so far

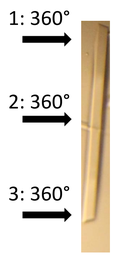


Figure 5: Data collection on 1 crystal.

Table 1: Data processing statistics: 3 data sets were processed with XDS[7] and merged (1-3). Data sets that were successful are highlighted in green.

	1	2	3	1-3 (merged)
Multiplicity	6.1 (4.8)	6.1 (4.9)	5.5 (4.3)	17.5 (13.9)
Completeness (%)	96.0 (93.4)	96.8 (94.6)	96.3 (94.6)	97.1 (94.9)
R_{merge} (%)	3.3 (8.1)	3.4 (8.6)	3.0 (7.4)	3.7 (8.8)
$R_{r.i.m.}$ (%)	3.6 (9.1)	3.7 (9.6)	3.3 (8.5)	3.8 (9.2)
Wilson B-factor	28.6	27.8	28.0	27.8
Mean $I/\sigma I$	34.1 (13.5)	33.1 (12.9)	34.6 (13.2)	51.7 (21.2)
CC½	0.99 (0.99)	0.99 (0.99)	0.99 (0.99)	1.0 (0.99)

6 Sites found by Autosol[8]
- 2 Cysteine bridges (Fig. 6A,B)
- 1 SO_4^{2-} ion (Fig. 6C)
- and a metal ion (Fig. 6D).

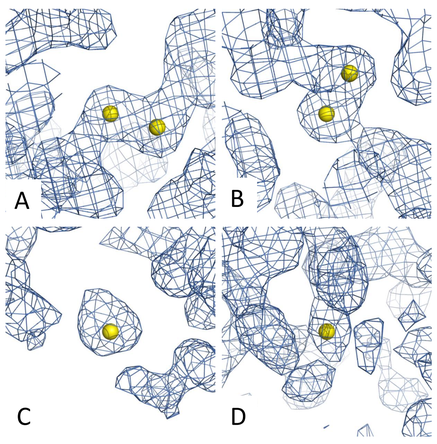


Figure 6: Sites found by Autosol. $2F_o$-F_c map at 1.5σ.

Density modification
- Large solvent channels (Fig. 7)
- Only 2 mol/ASU, **against Matthew's coefficient** analysis!

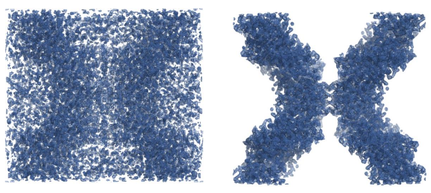


Figure 7: Overall electron density map. **A**: Before, **B**: After density modification for 1-3 at 1.5σ.

Model building by Autobuild[9]
- >90% built (Fig. 8).

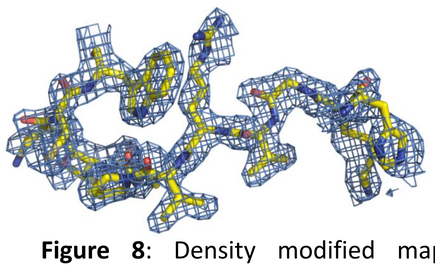


Figure 8: Density modified map comprising residues 76-87 (HKGVRVPATCNWV) at 1.5σ.

Validation

Anomalous difference maps
- Sites 1,2 and 3,4: Cys bridges (Fig. 9A,B).
- Site 5: SO_4^{2-} (Fig. 9C)
- Site 6: **Na^+ ion**! Despite low anomalous signal at 1.8 Å (Fig. 9D)!

Isomorphous difference maps
- Strong radiation damage at Cys bridges; after 180°: already oxidized (Fig. 10)!

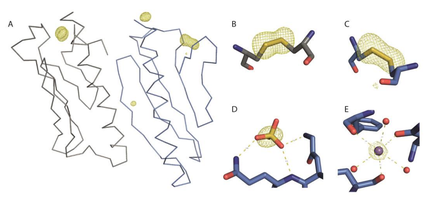


Figure 9: Anomalous difference map at 4σ. **A**: Overall molecule. **B**, **C**: Cys bridges. **D**: Sulfate ion. **E**: Na^+ ion.

S-SAD CONCLUSION
- The predicted limitations of S-SAD (**low anom. signal, low multiplicity**) were challenged.
- A well-diffracting crystal lead to excellent phases and defined maps in which even a sodium ion could be identified!

⟶ An overview over 100 S-SAD statistics is given in detail in [10].

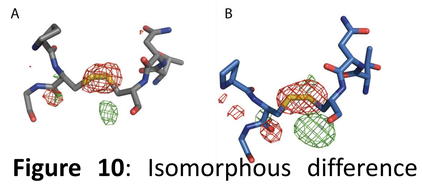


Figure 10: Isomorphous difference map between the first and second 180° of data set 3 around the Cys bridges at 4σ.

Conclusion/Outlook

- In fact, clusters of closely spaced aromatic residues can be found in a Pil_{Bac1} pilus model, but these will be too far away from each other to allow for electron flow along the pilus. Therefore, the **Pil_{Bac1} pilus will not be conductive on its own** – additional factors like cytochromes will be necessary. This is in favor with the multi-step hopping theory!
- Currently, **EM studies on nanowires** are ongoing for further reconstitution. Here, we see nanowires covered with protein complexes, probably cytochromes.

References: 1: Gorby et al. (2006). *PNAS*. **103**(30), 11358–33. **2**: Reguera et al (2005). *Nature* **435** (7045), 1098-01. **3**: Reguera et al (2006). *Applied and Environmental Microbiology* **72**(11),7345–48. **4**: Bond et al (2012). *Chemsuschem*. **5**(6), 1099-05. **5**: Strycharz-Glaven et al (2011). *En. And Environ. Sci*. **4**(11), 4366-79. **6**: Malvankar et al (2011). *Nature Nanotech.* **6**(9), 573-9. **7**: Kabsch (2010). *Acta cryst D.* **66**, 125-32. **8**: Terwilliger et al (2009). *Acta Cryst. D* **65**, 582-01. **9**: Terwilliger et al (2008). *Acta Cryst. D.* **64**, 61-9. **10**: Gorgel et al. (2015). *Acta Cryst. D.* **24** (71), 1095-01.