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), $^{99}$Tl(II), Hg(II)) — they cannot enter the cell! Therefore, the bacteria need to transfer the electrons from the inside of the cell to the metals at the outside. For this, they produce nanowires.\(^2\)\(^3\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\)\(^13\)\(^14\)\(^15\)\(^16\)\(^17\)\(^18\)\(^19\)\(^20\)\(^21\)\(^22\)\(^23\)\(^24\) 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.\(^2\)\(^3\)\(^6\)

**QUESTION:** How Can Type IV Pil Form Nanowires in *Shewanella Oneidensis*?

**Electron transfer theories**

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

- **A: Metallic-like theory\(^1\):** 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\(^2\):** 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).

**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. gonorhoeae* (PDB: 2HIL).
  - NT α-helix buried in the core with β-sheet to the outside.

- Distribution of aromatic residues (Fig. 3B) — 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!

**Conclusion/Outlook**

- In fact, clusters of closely spaced aromatic residues can be found in a Pil$_{Bac1}$ pilus model, but these are 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.

**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 DF/F: 0.9%
- Very low Multiplicity: 5.5
  - Not very promising so far

**6 Sites found by AutoSol**

- 2 Cystine bridges (Fig. 6A,B)
- 1 $SO_2^-$ ion (Fig. 6C)
- and a metal ion (Fig. 6D).

**Density modification**

- Large solvent channels (Fig. 7)
  - Only 2 moieties against Matthew’s coefficient analysis!

**Model building by AutoBuild**

- >90% built (Fig. 8).

**Validation**

**Anomalous difference maps**

- Sites 1, 2 and 3, 4: Cys bridges (Fig. 9A,B).
- Site 5: $SO_2^-$ (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)

**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.

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**References**


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

**Figure 3:** A: Structure of Pil$_{Bac1}$. The straight hela and the SS-bridge are highlighted in red. B: Plus 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 Pil$_{Bac1}$ pilus based on a pilus from *N. gonorhoeae* (PDB:2HIL). Aromatic residues are shown in spheres.

**Figure 5:** Data collection on a crystal.

**Figure 6:** Sites found by Autosol. 2F$_{-}$F$_{c}$ map at 1.5σ.

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

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

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

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