The *C. difficile*'s S-layer structure and assembly

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1. Introduction

C. difficile's cell benefits from an additional layer of protection, the S-layer, a proteinaceous layer that completely covers the cell wall [1]. Working primarily as a molecular sieve, it is also mediates adhesion to host gut and immune response.
SlpA is the *C. difficile*'s S-layer main protein. It comprises two subunits, HMW SLP (SLP_H) and LMW SLP (SLP_L), that interacts via the interacting domain LID/HID [2].
Recently, a new class of antimicrobials based on bacteriocins [3], was able to selectively attack *C. difficile* by targeting SlpA, making it a promising but so far unexploited target in the combat to *C. difficile* infection.

Our aims are the determination of SlpA 3D structure and its assembly into a functional S-layer

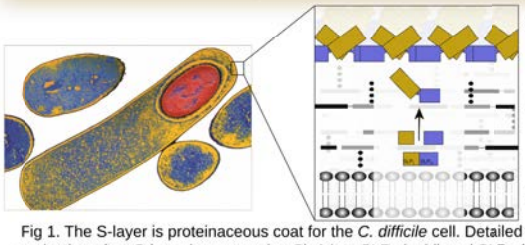

Fig 1. The S-layer is proteinaceous coat for the *C. difficile* cell. Detailed scheme shows the production of an S-layer by processing SlpA into SLP_L (gold) and SLP_H (slate blue) and their arrengement into 2D layer.

2. *In crystallo* SlpA structure and array resembles *in vivo* S-layer

A. The SlpA crystal structure

- SlpA forms 2D plate-like crystals.
- Crystal packing shows stacks of SlpA layers.
- SlpA subunits interact via a double-sandwich folding formed by LID/HID.

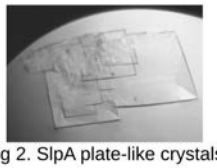

Fig 2. SlpA plate-like crystals

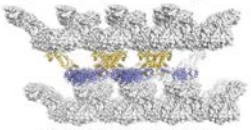

Fig 3. Crystal packing

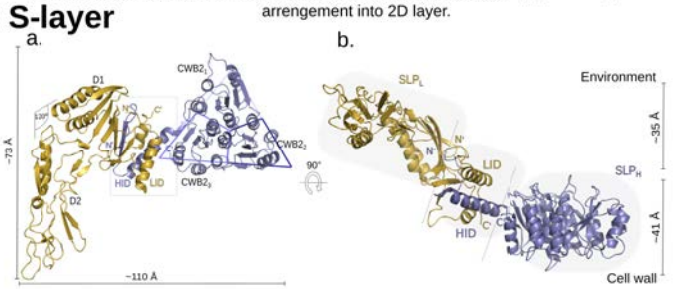

Fig. 4. The SlpA crystal structure. SLP_L (slate blue) interacts with SLP_H (gold) via LID/HID (dashed rectangle). The CWB2 domains (triangle) (a) in SLP_H subunit interacts with the cell wall while SLPL is exposed to the environment (b).

B. The S-layer envelope determined by electron microscopy explains the SlpA 2D layer assembly

- Extracted S-layer forms S-layer ghosts.
- EM-micrograph on S-layer ghosts shows a rugged double-layer.
- EM reconstruction validates crystal SlpA layer.

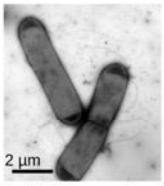

Fig 5. S-layer ghosts

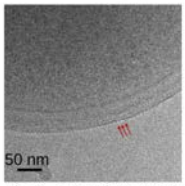

Fig 6. EM-micrograph

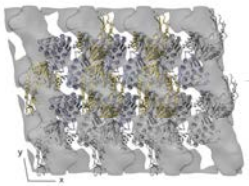

Fig 7. EM reconstruction fit to crystal model. Environment view.

Fig 8. EM reconstruction fit to crystal model. Side view.

3. S-layer relies on electrostatic interactions forming hydrophilic pores

- CWB2 domains form triangular prisms on cell surface.
- Inter-molecule interactions mainly formed via electrostatic forces.
- The SLP_L and interacting domains are responsible for closing the gaps between CWB2 prisms.
- Only 2 hydrophilic pores allow direct contact between cell and environment.

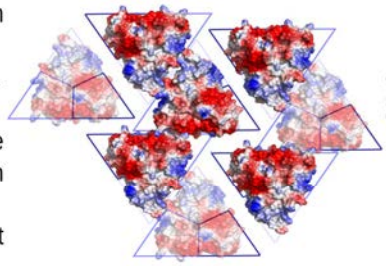

Fig. 9. Electrostatic profile in CWB2 domains, in SLP_H subunit.

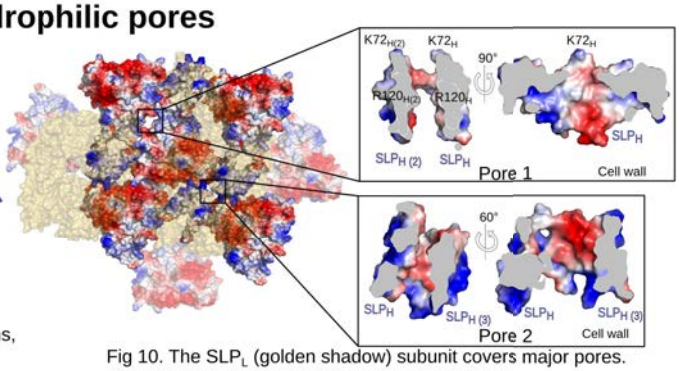

Fig 10. The SLP_L (golden shadow) subunit covers major pores.

4. The overall model of *C. difficile*'s S-layer

- The CWB2 domains bears of most interactions - majority salt bridges mediated by lysines.
- Salt bridges allow a strong and stable maintenance of the S-layer while still allowing some room to flexibility and fluidity.
- The pores are highly hydrophobic and negatively charged - small positively charged molecules would be easier to pass.

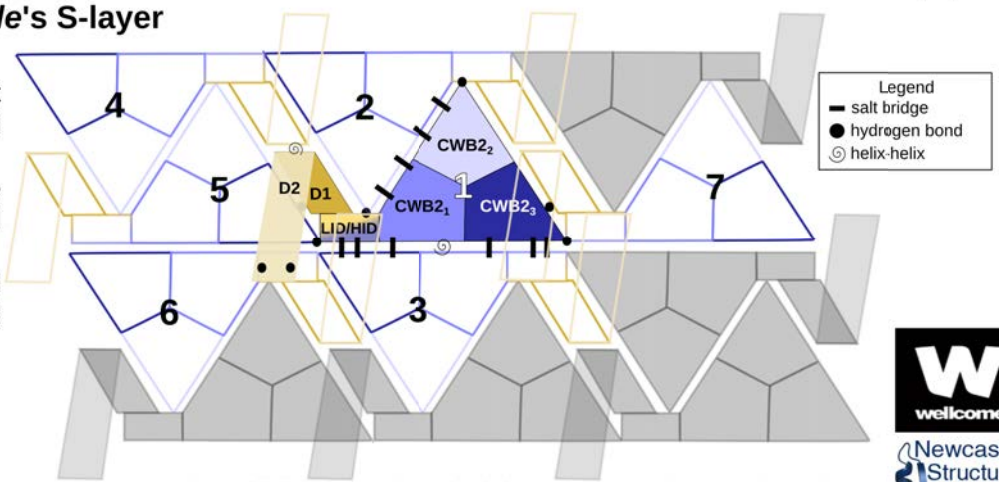

Fig. 11. The *C. difficile* S-layer model and main interactions. Molecule 1 forms interactions with 6 others to maintain layer stability and cover pores. The CWB2 domain in SLP_H mediates the majority of hydrogen bonds (circle) and all salt bridges (bars). Two highly conserved helices interact with itself on two different points in SlpA (whirls).

References

[1]Fagan et al. Nat Rev Microbiol. 2014 Mar;12(3):211-22
[2]Fagan et al. Mol Microbiol. 2009 Mar;71(5):1308-22.
[3]Kirk et al. Sci Transl Med. 2017 September 06; 9(406).