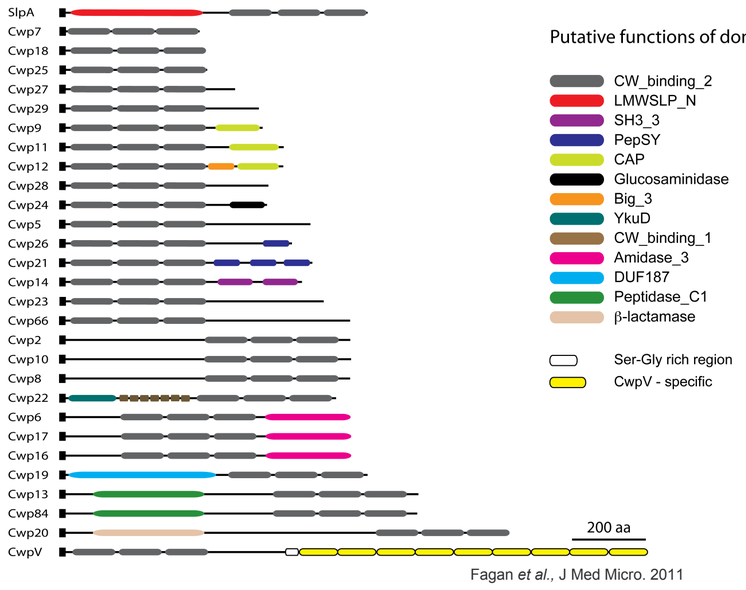
Structural studies of *Clostridium difficile* S-layer

Paula S. Salgado[1]*, R. P. Fagan[1], S. Fletcher[1], J. Marchant[2], P. J. Simpson[2], N. F. Fairweather[1]

[1] MRC Centre for Molecular Bacteriology and Infection, [2] Division of Molecular Biosciences, Imperial College London

*Institute of Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne

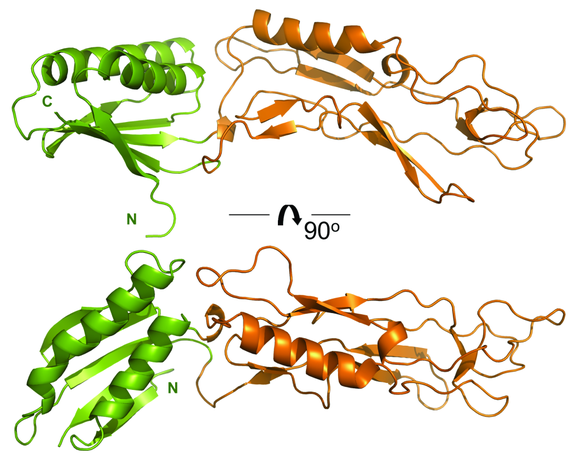


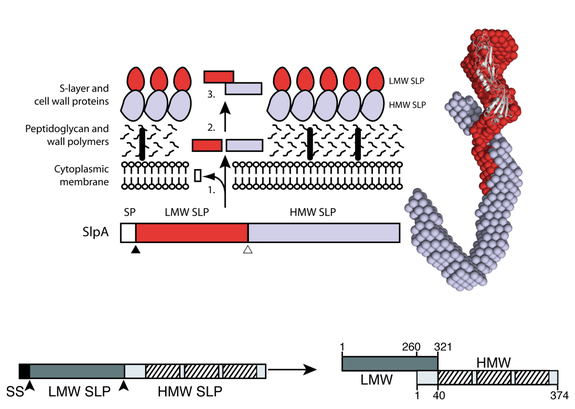
Fagan *et al.*, J Med Micro. 2011

C. difficile S-layer:

- SlpA major constituent
- 29 identified CWPs
- Many of unknown or putatively assigned function

SlpA: Complex of Low (LMW) and High Molecular Weight (HMW)

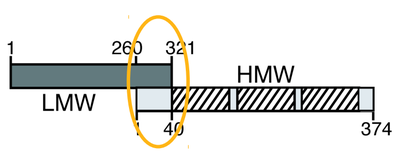


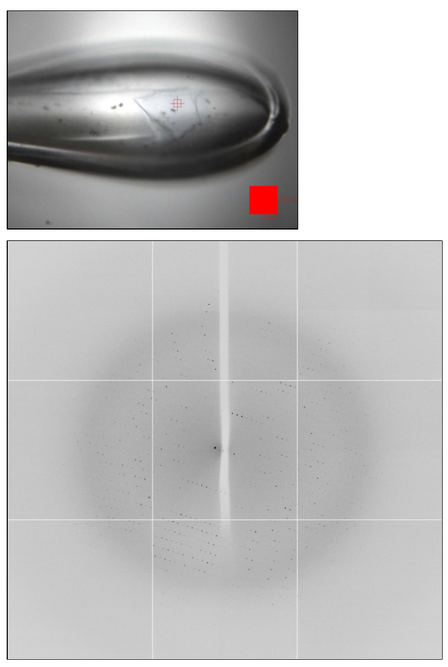


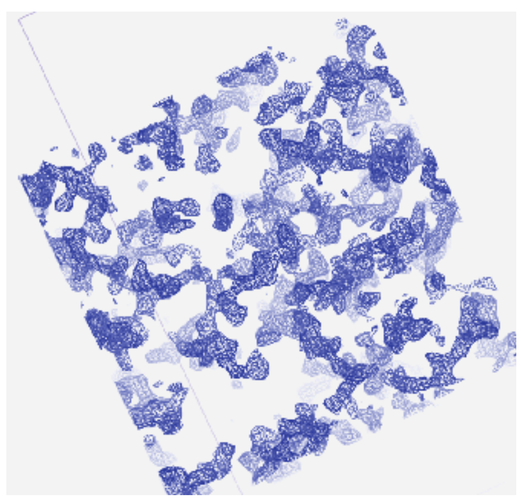
Fagan *et al.*, Mol Microbiol. 2009

LID-HID complex:

- Interacting domains:
  - Residues 260-321 LMW
  - Residues 1-40 HMW

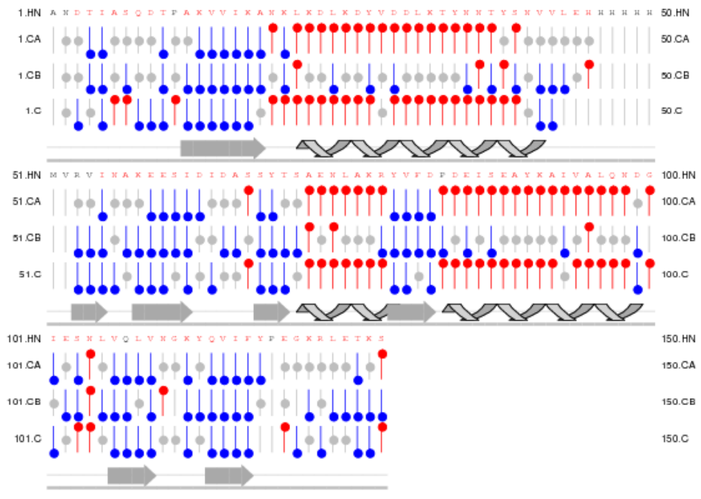




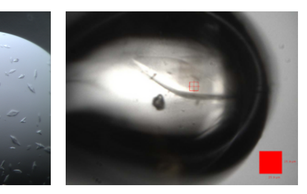


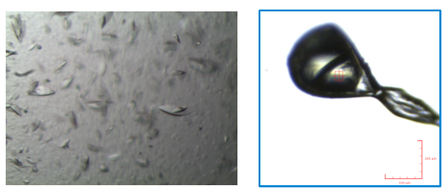
- P2, 8 molecules asymmetric unit
- Phases too weak to build model

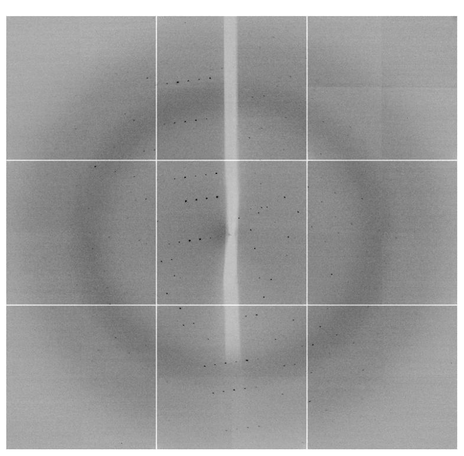
- NMR studies
  - Backbone assignment: 91% complete
  - Side chains assignment: 75% aliphatic 100% aromatic
- Structure calculations ongoing



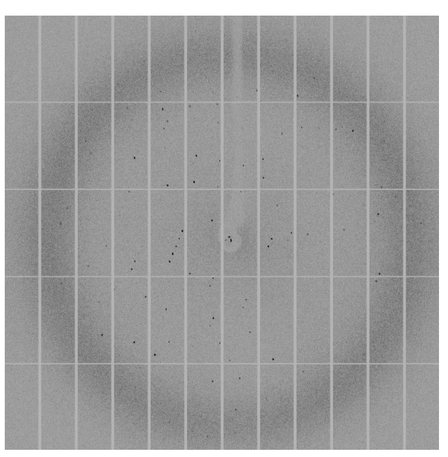
LMW-HMW complex:





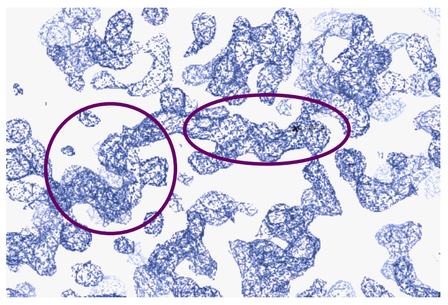


Native



SeMet derivative

- P1, 2 molecules asymmetric unit
- Low resolution (~3.0Å)
- Weak anomalous signal for SeMet
- No suitable heavy atom derivative



Compound	Co-xtal	Soak	Processable	Resolution	Anomalous signal
K_2PtCl_4	no	yes	3 no, 2 yes	< 4	weak
K_2PtBr_4	no	yes	1 Pt no, 2 Br yes	< 6	weak
$NaAuCl_4$	no	yes	no	-	-
$(Ch_3)_3Pb(CH_3COO)$ (PbTMA)	yes	no	2 yes, 2 no	7 - 8	weak

Future work:

- Finalise LID-HID NMR model
- Determine and refine LID-HID crystal structure
- Study S-layer organisation and assembly by EM
- Optimise crystallisation with PbTMA
- Investigate incorporation of other heavy atoms
- Improve crystal quality using micro-seeding and dehydration
- Introduce Met residues to increase SeMet anomalous phasing power

References

- Fagan, R.P., et al., (2011) *J Med Micro*, 60, 1225-8
- Fagan, R.P., et al., (2009) *Mol Microb* 71,1308-22
- Calabi, E., et al. (2001) *Mol Microbiol* 40: 1187–1199.

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