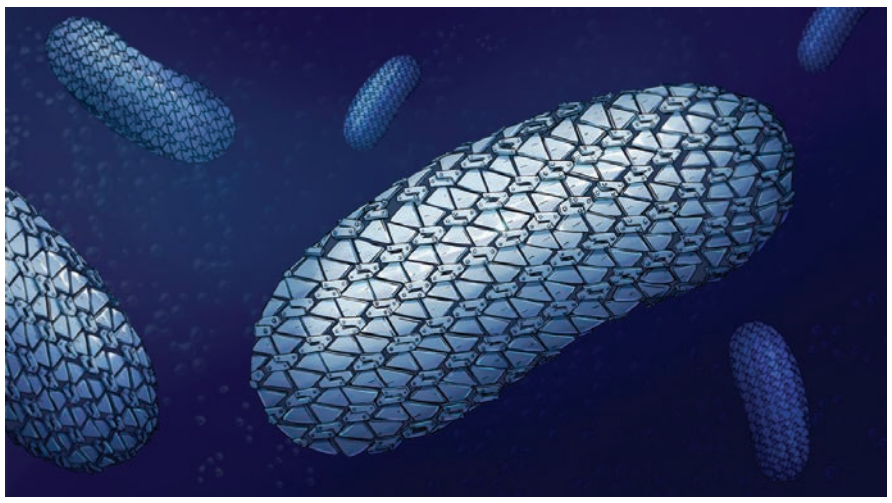
A *diff*icult Crystal to Crack

Dr Paula Salgado, Twitter: @pssalgado, Email: paula.salgado@ncl.ac.uk

Dr Paula Salgado, Senior Lecturer at Newcastle University. (Credit: Paula Salgado)

The S-layer is a two-dimensional protein array that covers the cell surface of many bacteria and archaea. One such bacteria is *Clostridioides difficile*, an antibiotic resistant superbug that is the main cause of antibiotic-associated diarrhoea. Determining the structure and assembly of the S-layer in *C. difficile* (*C. diff* for short) is crucial to not only understand its biology but will open new therapeutic avenues, specifically targeting this pathogen.

Using the full range of MX beamlines at Diamond Light Source, the team lead by Dr Paula Salgado at Newcastle University determined the crystal structure of a lattice-forming protein, SlpA. Like other S-layer (surface layer) proteins, this protein has the ability to form 2D paracrystalline arrays that cover the whole surface of the cell of the important human pathogen, *Clostridioides difficile*.



S-layer assembly around C. difficile cell. (Credit: Lizah van der Aart)

Making a natural 2D array crystallise as a 3D crystal was not easy and the crystals had many problems. The cooperation with MX beamline staff was essential to finally solve the structure, after more than 10 years since the first crystals were obtained, as explained by Dr Paula Salgado

"Even when crystals were obtained, not all diffracted well so Diamond synchrotron was essential for the success of the project. The work relied particularly on I24, the microfocus beamline, to test many hundreds of crystals and screen for the best spot in the best crystal to collect the best data set.

"Getting native data was not the end of the story as there were no models to allow structure determination. Staff across the MX beamlines at Diamond were always eager to help solve this problem and willing to try new approaches and accommodate requests for specialised beamtime.

"Together, we explored and developed new approaches. One possibility we tried was carrying out high-throughput heavy atom soaking to identify suitable anomalous sources for solving the phase problem. We adapted the XChem pipeline and used an acoustic liquid handler (ECHO, Labcyte) that dispenses nanolitre droplets using ultrasonic pulses into plates with crystal-containing drops.

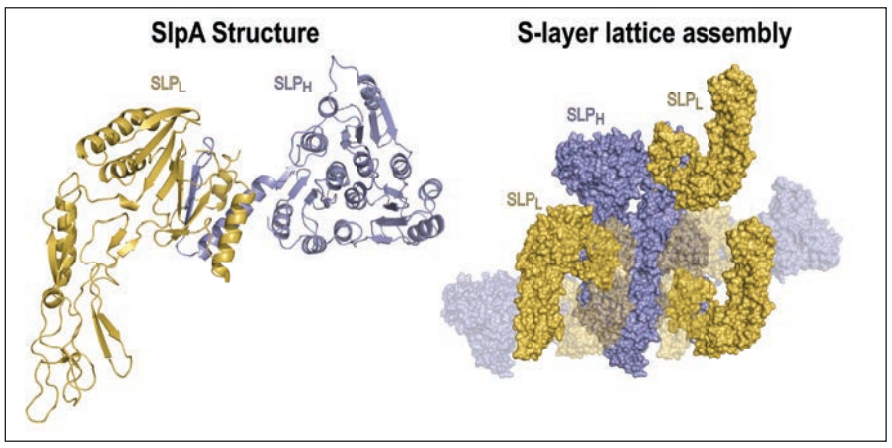
"The crucial experiment, however, was using the unique long wavelength I23 beamline, which allowed using the native sulphur atoms in SlpA as sources of information required to start building a model."

Once phases had been obtained, there were still many intrinsic problems to build a complete model, as explained by PhD student Paola Lanzoni-Mangutchi: "This has been a challenging project and we spent many hours together, culturing the difficult bug and collecting X-ray data at the Diamond Light Source synchrotron."

Dr Barwinska-Sendra who unravelled the structural and functional details of the building blocks of SlpA added: "Working together was key to our success, as I determined the structures of sub-complexes, which allowed Paola to build the full SlpA model."

Together with data obtained by collaborator Dr Fagan from the University of Sheffield using electron diffraction of intact surface layer from *C. difficile* cells, the SlpA allowed the team to describe the structure and assembly of the S-layer in *C. difficile*.

C. difficile or *C. diff* is one of the so-called superbugs, which have multiple ways to resist antibiotics and can combine multiple resistance mechanisms. *C. diff* is a superbug that infects the human gut and is resistant to all but three current drugs. Not only that, but it becomes a problem when antibiotics are taken, as the good bacteria in the gut are killed alongside those causing an infection. As *C. diff* is resistant, it can grow and cause diseases ranging from diarrhoea to death due to massive lesions in the gut. Another problem is the fact that the only way to treat C.diff is to take more antibiotics, so the cycle is restarted and many people get repeat infections.



SlpA structure showing how SLPL (gold) and SLPH (slate) form a tight complex that creates an intricate lattice. (Credit: Paola Lanzoni-Mangutchi, Anna Barwinska-Sendra and Paula Salgado)

The Wellcome Trust-funded BugS-layer Consortium, which also includes Dr Douce from the University of Glasgow, is now focusing on exploring the newly determined SlpA structure and assembly and how it allows the possibility of designing *C. diff*-specific drugs to break the S-layer and fight infections.

Paola Lanzoni-Mangutchi, Oishik Banerji, Jason Wilson, Anna Barwinska-Sendra, Joseph A. Kirk, Filipa Vaz, Shauna O'Beirne, Arnaud Baslé, Kamel El Omari, Armin Wagner, Neil F. Fairweather, Gillian R. Douce, Per A. Bullough, Robert P. Fagan & Paula S. Salgado. "*Structure and assembly of the S-layer in C. difficile*". *Nat Commun 13*, 970 (2022). https://doi.org/10.1038/s41467-022-28196-w

PDB IDs: 7ACV, 7ACW, 7ACX, 7ACY, 7ACZ

Nature Comms. Doi: 10.1038/s41467-022-28196-w

Further Information

Dr Paula Salgado: https://salgadolab.org/
https://www.ncl.ac.uk/medical-sciences/people/profile/paulasalgado.html
Imagery from: https://lizahvanderaart.com/