SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural and Molecular Microbiology

has the honour to invite you to the public defence of the PhD thesis of

Sander E. VAN dER VERREN

to obtain the degree of Doctor of Bioengineering Sciences

ORDER AT THE SURFACE
Electron cryo-microscopy of the curli assembly system and *Bacillus anthracis* S-layer

Promotor:

Prof. Dr. Han Remaut

The defense will take place on

Thursday, July 9, 2020 at 17:00
and will be held in front of limited audience. Live stream will be available as specified here[1].

[1] https://forms.gle/NnunXjwZzuNPn9Ws8

Members of the jury

Prof. Dr. Stefan Weckx (VUB, chairman)
Prof. Dr. Ir. Wim Versées (VUB, secretary)
Prof. Dr. Han Remaut (VUB, promotor)
Prof. Dr. Tom Lenaerts (VUB)
Prof. Dr. Ariane Briegel (IBL, ULeiden, NL)
Prof. Dr. Sheena Radford (Astbury centre, ULeeds, UK)

Curriculum vitae

Sander E. Van der Verren (1992) obtained his B.Sc. and M.Sc. in Biochemistry and Biotechnology at Ghent University, completing his master thesis in Sweden at Uppsala University.

In 2015, he joined the lab of Han Remaut as a FWO aspirant fellow. His research focussed on the structural biology of bacterial cell surfaces, which led to 3 publications in international peer-reviewed journals and 1 patent. Furthermore he used his gained expertise in cryo-EM through several collaborations with other Belgian laboratories. He supervised 1 thesis student and presented his work at several international conferences, being awarded 1 poster and 1 speaker price.

Abstract of the PhD research

The bacterial cell surface forms the first interface between a bacterium and its environment and is directly related to virulence, adhesion and recognition. Two very different surfaces were studied during this PhD; the ubiquitously produced curli by Gram-negative bacteria and the S-layer surface of the Gram-positive pathogen *Bacillus anthracis*, causative agent of anthrax.

Curli assemble into functional amyloid fibres on the outside of the bacterial cell. These sticky fibres are used in adhesion and meshing of the biofilm matrix which enables bacteria to colonise diverse surfaces. Curli are secreted by a specialised pathway called the curli assembly system. In this thesis, the role and function of the extracellular curli secretion factor CsgF was investigated. Multiple cryo-EM structures of the CsgG:CsgF complex show CsgF to form an intricate secretion complex with CsgG, the curli secretion channel. The N-terminus of CsgF (FCP) ads a second constriction to the CsgG pore, which proves to be useful in nanopore sequencing. Interestingly, the created CsgG:FCP nanopore prototype improves homopolymer sequencing compared to the CsgG nanopore. Next to the constriction peptide CsgF also features a flexible neck and head substructure, which we show are essential to form cell surface-associated curli fibrils. Our results indicate that the CsgF head is intrinsically flexible and possibly only orders as an actively secreting complex, likely through interacting with the minor curli subunit CsgB. Finally, electron tomography (cryo-ET) shows that curli fibres form a dense curli halo around the cell, but without any apparent connections to the cell surface. Considering both the dynamics of the CsgF head and the transient CsgF:CsgB interaction, a model is proposed where the stochastic fall-off of CsgB fibres is an intrinsic feature of the curli assembly machinery.

In a second part of this PhD the Sap S-layer is structurally characterised. Sap is one of two S-layer proteins forming the S-layer in *B. anthracis*. In this thesis, the Sap S-layer is shown to be a viable target for anti-S-layer biologicals in the form of nanobodies. Nanobodies destabilising and depolymerising the S-layer are obtained, leading to cell morphology defects and impaired growth of the bacilli as well as offering a protective effect in a mouse infection model. In order to better understand the action of the nanobodies, further structural insights were sought. Two dimensional projection maps show that the solved Sap X-ray structure needs to rearrange in order to fit in the obtained *p2*-lattice. Through cryo-ET and crosslinking analysis we next proceeded to present a tentative lattice model. Our results contribute to the understanding of S-layer topology in *B. anthracis* and form the prerequisite for the future design of biologicals combatting anthrax.