Discovering and targeting *Bacillus anthracis'* Achilles heel: the new way to fight anthrax

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Anthrax is a highly resilient and deadly disease caused by the spore-forming bacterial pathogen *Bacillus anthracis*. Today, anthrax mostly affects wildlife and livestock, but remains a concern for human public health primarily in persons handling contaminated animal products and as a bioterror threat due to the high resilience of spores, the high case-fatality rate even with the aggressive use of antibiotics and the lack of a civilian vaccine program. As part of its immune evasion strategy, the bacterium presents a dynamic cellular surface with a complex composition. In its vegetative form, the cell surface of *B. anthracis* is covered by one of two protective paracrystalline protein arrays known as the Sap or EA1 S-layer (surface layer), present during exponential and stationary growth phase, respectively.

The self-assembling characteristic of these S-layer proteins has thus far hampered their detailed structural and biophysical characterization. Here, we applied Nanobodies (Nbs) as a bio-tool to control Sap polymerization and to accomplish its crystallization and structure determination, unveiling a new class of S-layer proteins. The Sap assembly domain consists of six β-sandwich domains that organize into a flat, tile-shaped unit that self-assembles independent of calcium. Amongst the isolated Nbs we identified inhibitory nanobodies that prevented Sap assembly and depolymerized existing Sap S-layers *in vitro*. When applied *in vivo,* nanobody-mediated destruction of the Sap S-layer resulted in severe morphological defects and proved bacteriostatic unlike the genetic knockout of *sap*. In a mouse model of ongoing *B. anthracis* infection, subcutaneous administration of Sap inhibitory Nanobodies resulted in clearance of infection and a cure of lethal anthrax disease. These findings expose, for the first time, the disruption of S-layer integrity as a mechanism with therapeutic potential in S-layer carrying pathogens.

Reference:

Fioravanti A.*, Van Hauwermeiren F., Van der Verren S., Jonckheere W., Goncalves A., Pardon E., Steyaert J., De Greve H., Lamkanfi M. and Remaut H.*. **Structure of the S-layer protein Sap reveals a mechanism for therapeutic intervention in anthrax**. Manuscript accepted in Nature microbiology - NMICROBIOL-18081671B *Corresponding author.