Structural Glycobiology and Inhibition of S-Layer Anchoring by Secondary Cell Wall Polymer

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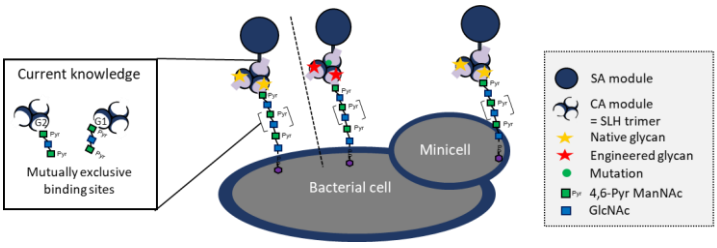
S-layers are 2D crystalline cell envelope structures of many prokaryotes and are potential targets for therapeutic inhibition. Many S-layers are glycosylated and, in Gram-positive bacteria, can be attached through the interaction of an S-layer homology (SLH) domain trimer with peptidoglycan-linked secondary cell wall glycopolymer (SCWP).

Our hypothesis is that the native glycosylation of the *Paenibacillus alvei* SLH domain trimer located in the two binding grooves influences the molecular logic of S-layer anchoring to the cell wall and that the terminal pyruvylated *N*-acetylmannosamine S-layer binding epitope for SCWP is an ideal starting point to design inhibitors of proper cell wall assembly. The influence of the glycosylation of the SLH domain trimer on binding to SCWP by analyzing its interaction with the SCWP is investigated in ligand in a bottom-up approach of increasing complexity, *in vitro* and *in vivo*. Furthermore, we aim to identify small molecule inhibitors of the SLH domain trimer-SCWP interaction. Our project features chemical synthesis of SCWP fragments and tailored analogues, rational S-layer (glyco)protein engineering and *P. alvei* cell design, biophysical protein-carbohydrate interaction analyses, X-ray crystallography, and cryo-electron tomography/microscopy, and is supported by molecular modelling and simulation.

This project will yield a detailed mechanistic model of S-layer anchoring in Gram-positives. Understanding the governing glycoprotein-carbohydrate interactions at a molecular level will open avenues for their disruption, thereby contributing to the advancement of molecular glycobiology in bacteria–a field of increasing importance in the context of bacterial pathogens.



Scenarios for investigating structural glycobiology of S-layer anchoring by SCWP. © Christina Schäffer & Markus Blaukopf, BOKU Vienna.