MS11 Opportunities from combining structural biology and fold prediction

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A novel self-assembly mechanism for the S-layer in Lactobacillus acidophilus

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Abstract

The surface layer or S-layer represents the outermost cell envelope in many bacteria and archaea and is formed by individual (glyco)protein subunits. These surface layer proteins organize and self-assemble into highly regular two-dimensional crystalline arrays. In general, S-layer proteins have two functional regions, whereas one region is responsible for the attachment to the cell wall and another region responsible for the self-assembly of the S-layer array. Since the S-layer is in close contact with the environment these arrays fulfil various functions like bacterial adherence to other cells or receptors, protection against life-threatening conditions and maintenance of the cell shape. In *L. acidophilus*, a naturally occurring host of the human gut microbiome, the S-layer is relevant for the adherence to the gut epithelia cells and is associated with probiotic properties.

In this study we present the crystal structure of both major S-layer proteins, SlpA and SlpX, of *L. acidophilus* and SlpA of the close relative *L. amylovorus*. For crystallization, each of the three proteins were split into three functional regions, whereas the two N-terminal parts are responsible for the self-assembly. The crystal structures gave us insight and understanding of the mechanism of how the self-assembly occurs. In combination with AlphaFold Multimer predictions we propose a model for the fully assembled S-layer of SlpA from *L. acidophilus*. Our model suggests several possible interactions that are all important for the formation of the S-layer. The N-terminal region shows a very interesting mechanism of a flexible linker that interacts with the next molecule and the middle region of the protein is crucial for dimer formation, that is responsible for the P2 symmetry of the S-layer. Predictions of the S-layer of close relatives in the Lactobacillus genus support our proposed model. For future research we aim, that our unique model helps in the understanding of the interaction of *Lactobacillus* with the host.