# A bacterial surface layer protein exploits multistep crystallization for rapid self-assembly

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Surface layers (S-layers) are crystalline protein coats that encapsulate a variety of bacteria and nearly all archaea. Recent insights into the surface layer protein (SLP) from Caulobacter crescentus, RsaA, have revealed both the high-resolution structure of the S-layer lattice (von Kugelgen et al., Cell 2019) as well as its highly efficient self-assembly via calcium-triggered 2D crystallization (Comerci et al., NComms 2019 and Herrmann et al., Biophys J 2017). However, molecular mechanisms governing rapid protein crystallization in vivo or in vitro are largely unknown. Utilizing a combination of x-ray crystallography, static and time-resolved small angle x-ray scattering, and time-resolved electron cryo-microscopy (Cryo-EM), we demonstrate that RsaA achieves rapid self-assembly in vitro via multistep crystallization due to sequential changes within the structure and arrangement of protein domains. This assembly pathway involves two domains serving distinct functions. The C-terminal crystallization domain forms the physiological 2D crystal lattice, but the full-length protein crystallizes multiple orders of magnitude faster due to the N-terminal nucleation domain, which also serves to anchor the S-layer to extracellular lipopolysaccharide. Directly observing the RsaA crystallization pathway using a time-course of Cryo-EM imaging revealed a crystalline intermediate wherein N-terminal nucleation domains exhibit motional dynamics with respect to rigid lattice-forming crystallization domains. Dynamic flexibility between the two domains rationalizes efficient S-layer crystal nucleation on the curved cellular surface. Additionally, enhancing the rate of protein crystallization by a discrete nucleation domain unveils possible evolutionary mechanisms to enhance the kinetics of 2D protein crystallization and may enable engineering of kinetically controllable self-assembled macromolecular nanomaterials.