Challenging the archetype: Coupling computational modeling and cellular exploration of probe the protein-protein interaction controlling *Vibrio fischeri* biofilm formation.

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Anti-sigma factor antagonists SpoIIAA and RsbV from *Bacillus subtilis* are the archetypes for single-domain STAS proteins in bacteria. The structures and mechanisms of these proteins along with their cognate anti-sigma factors have been well studied. SpoIIAA and RsbV utilize a partner-switching mechanism to regulate gene expression through protein-protein interactions to control the activity of their downstream anti-sigma factor partners. The *Vibrio fischeri* STAS domain protein SypA is proposed to employ a partner-switching mechanism with its partner, SypE, to regulate biofilm formation. However, the *Vibrio* regulation appears opposite from the canonical pathway, with SypA being downstream to SypE. Additionally, genetic evidence suggests that SypA and SypE are not involved in transcriptional regulation like classic anti-sigma factor antagonists/anti-sigma factor pairs. Rather, SypA and SypE are hypothesized to regulate biofilm formation downstream of transcription. Here we explore the commonalities and differences between SypA and the canonical single-domain STAS proteins SpoIIAA and RsbV. We use a combination of AlphaFold2 structure predictions and computational modeling to investigate the SypA-SypE binding interface. We then test a subset of our predictions in *V. fischeri* by generating and expressing SypA variants for their ability to form biofilms. Our findings suggest that, while SypA shares many sequence and structural traits with anti-sigma factor antagonist STAS domain proteins, there are significant differences that may account for SypA's distinct regulatory output.