

## It's all in the movement: exploring the flexibility and binding interactions of *Vibrio fischeri* biofilm regulatory proteins

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Biofilms are necessary for *Vibrio fischeri* to successfully participate in a symbiotic relationship with the Hawaiian bobtail squid *Euprymna scolopes*. As a critical component of these biofilms, the production of the exopolysaccharide SYP is controlled by a complex regulatory mechanism. SypA and SypE comprise one aspect of the SYP regulatory circuit, with SypA playing a central and critical positive role in SYP-dependent biofilm formation, and SypE modulating SypA activity. Compared to homologous proteins from *Bacillus subtilis* that regulate transcription, SypA and SypE appear to control biofilm formation through a similar partner-switching mechanism but completely separate from transcriptional regulation. Using biochemical, biophysical, and computational approaches, we are building on years of cellular and genetic work to determine detailed molecular mechanisms of the SYP regulatory pathway. Here, we use a combination of AlphaFold structure predictions and computational modeling to investigate the SypA-SypE binding interface and molecular mechanisms for SypA and SypE activity. These efforts are being coupled with biochemical and biophysical analysis to determine how SypA and SypE regulate SYP-dependent biofilm formation. Our findings suggest that SypA is more flexible than *B. subtilis* homologs. This flexibility may play a significant role in the SypA regulatory mechanism and protein-protein interactions.

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