



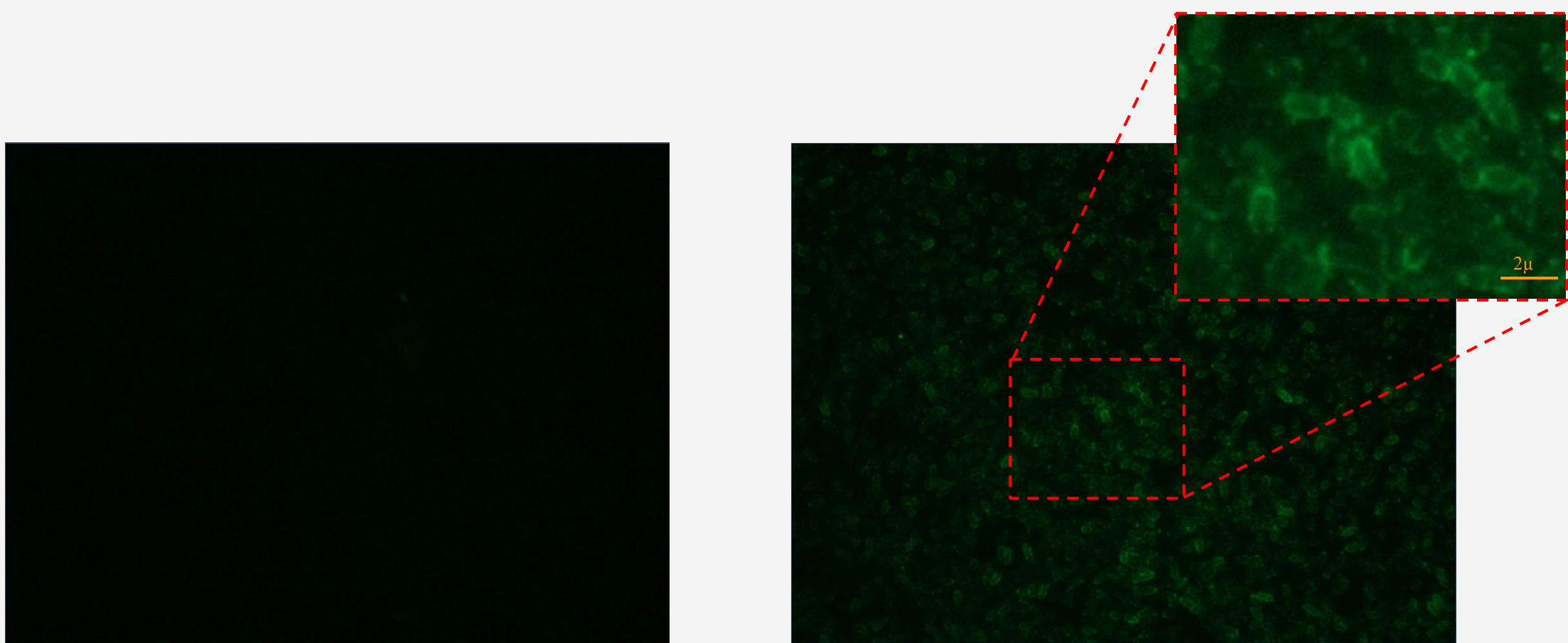
Directed Spatial Structure and Patterning in Synthetic Communities

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Abstract

Microbial communities play a foundational role in system regulation. The spatial structure of these natural communities in the environment is something that cannot be controlled in synthetic cultures yet. In order to achieve this, the adhesin pair of SpyTag and SpyCatcher was used. In *Synechococcus elongatus* (*S. elongatus*), SpyTag-FLAG was used for immunofluorescence, confirming the expression, transport, and display of the protein on the outer membrane (OM) via SomA. With that confirmation, *Escherichia coli* BL21 (DE3) was induced after transformation to produce SpyTag-mNG and SpyCatcher-mNG. Sonification and purification were performed, yielding the proteins to be used in a time series binding assay. The gel showed the covalent bonding between SpyTag and SpyCatcher as the combination band of the bound proteins increased as their individual bands decreased. With these promising preliminary results, further experimentation can be performed with the goal of introducing SpyCatcher-mNG to *S. elongatus* expressing SpyTag via SomA, and introducing SpyTag-mNG to *Escherichia coli* W expressing SpyCatcher via Intimin to test direct binding of the two proteins. Further study includes having both *S. elongatus* and *Escherichia coli* W express their proteins simultaneously and combining them in co-culture to test cell binding. Through this project, specific binding can be achieved, providing a toolbox to control the aggregation of the microbes in synthetic communities.

SpyTag-FLAG displayed on outer membrane of *S. elongatus*



Uninduced; 2xKO ($\Delta wzt \Delta slpA$)
SpyTag-FLAG, anti-FLAG

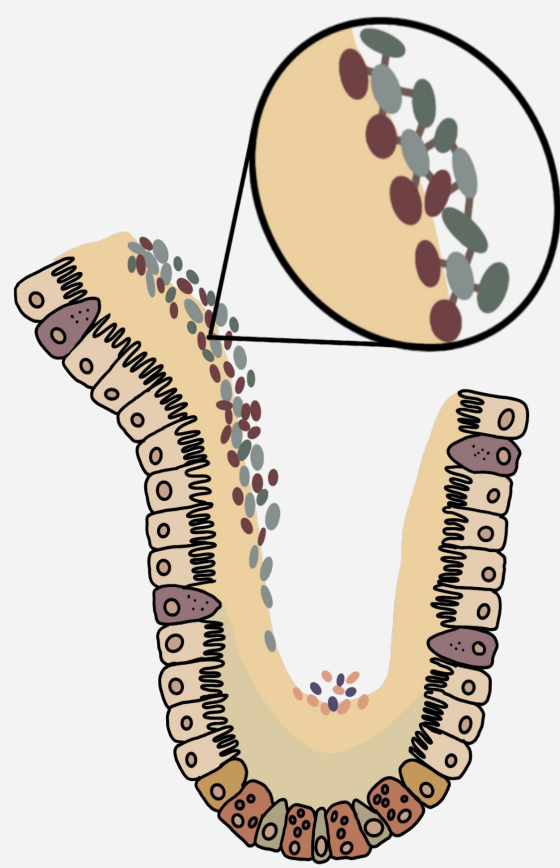
Induced; 2xKO ($\Delta wzt \Delta slpA$)
SpyTag-FLAG, anti-FLAG

Conclusions

- SpyTag-FLAG was confirmed to be displayed on the OM (SomA) of *S. elongatus* via immunofluorescence
- SpyTag-mNG and SpyCatcher-mNG were purified from *E. coli* BL21
- SpyTag-mNG and SpyCatcher-mNG were confirmed to bind together via time series gel assay

Spatial structure plays a defining role in microbial communities

- Natural communities have spatial structure and patterning
- Spatial structure cannot be controlled in synthetic communities
- Goal: Direct aggregation and structuring of chosen cells



Protein purification of SpyTag-mNG and SpyCatcher-mNG



E. coli BL21 with protein

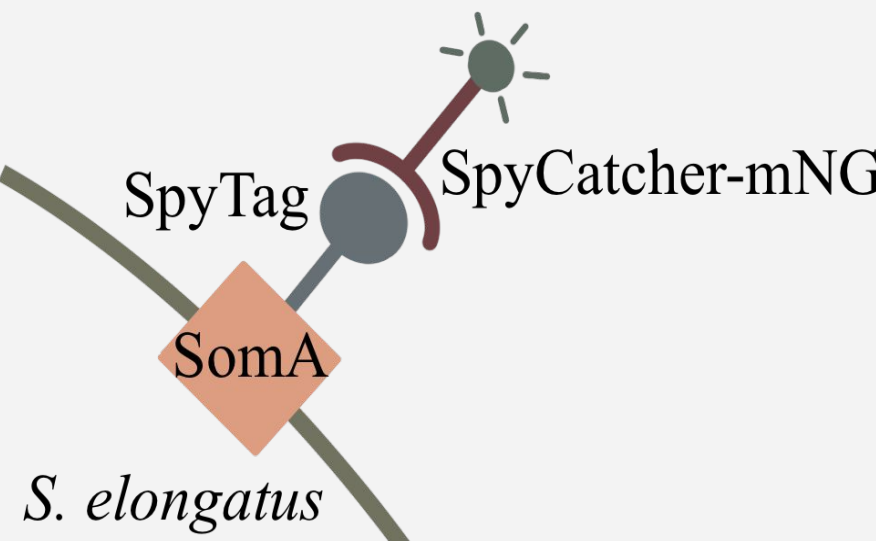
Sonication

Ni-column

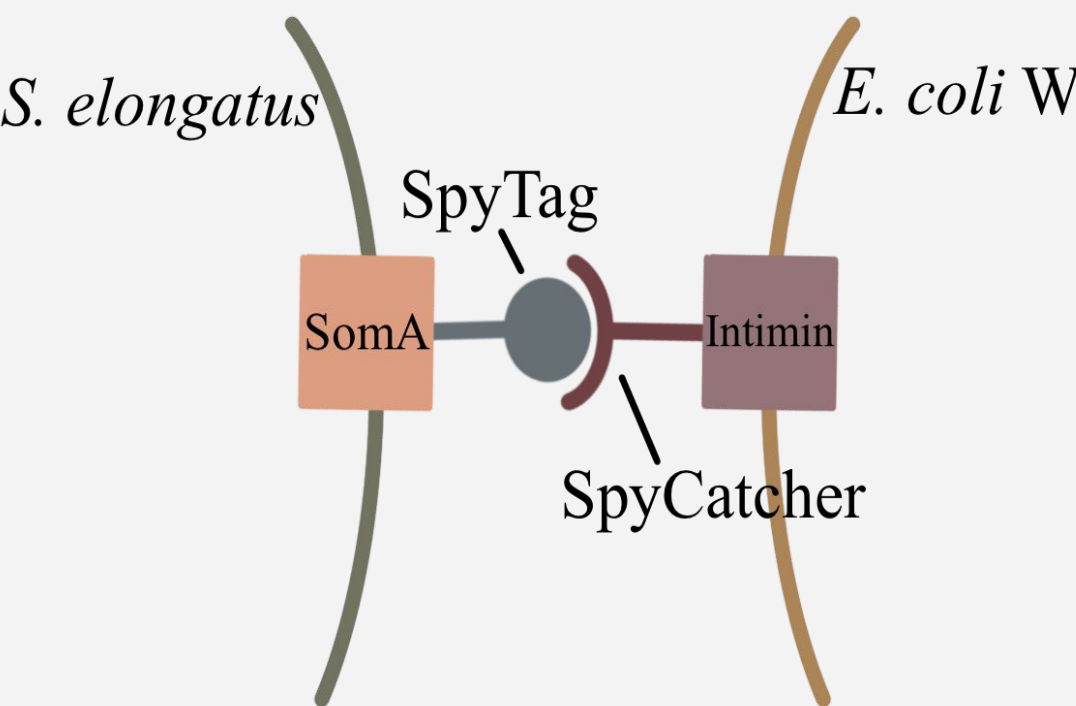
Purified protein

Protein gel

Future steps



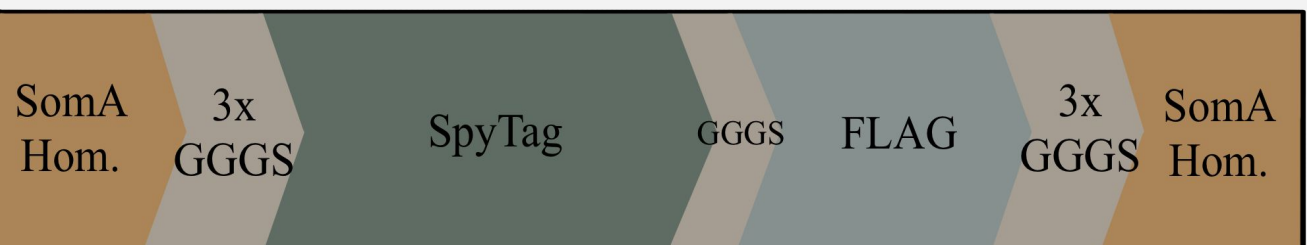
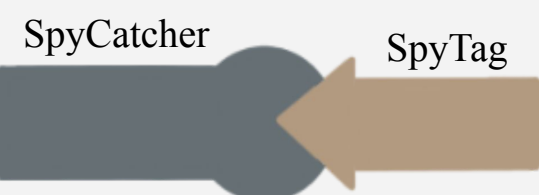
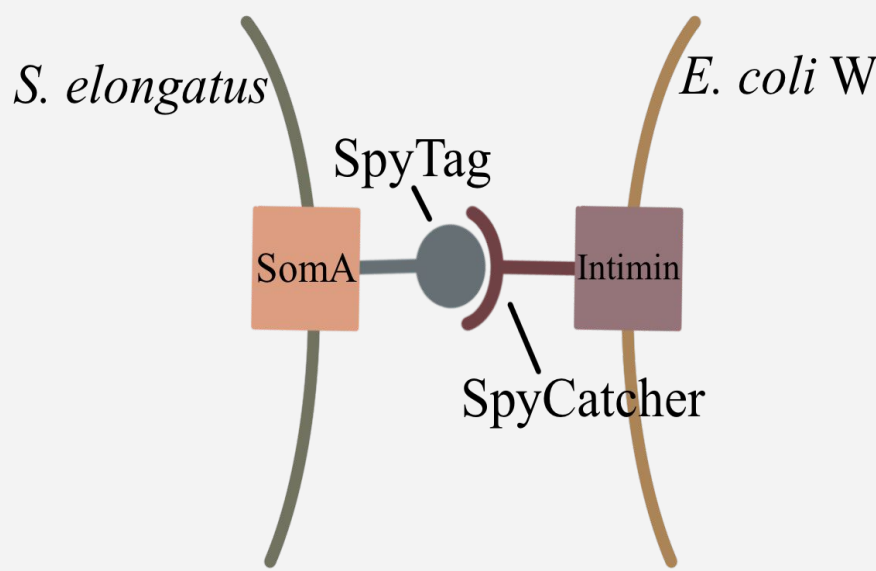
Validate surface display of *S. elongatus*



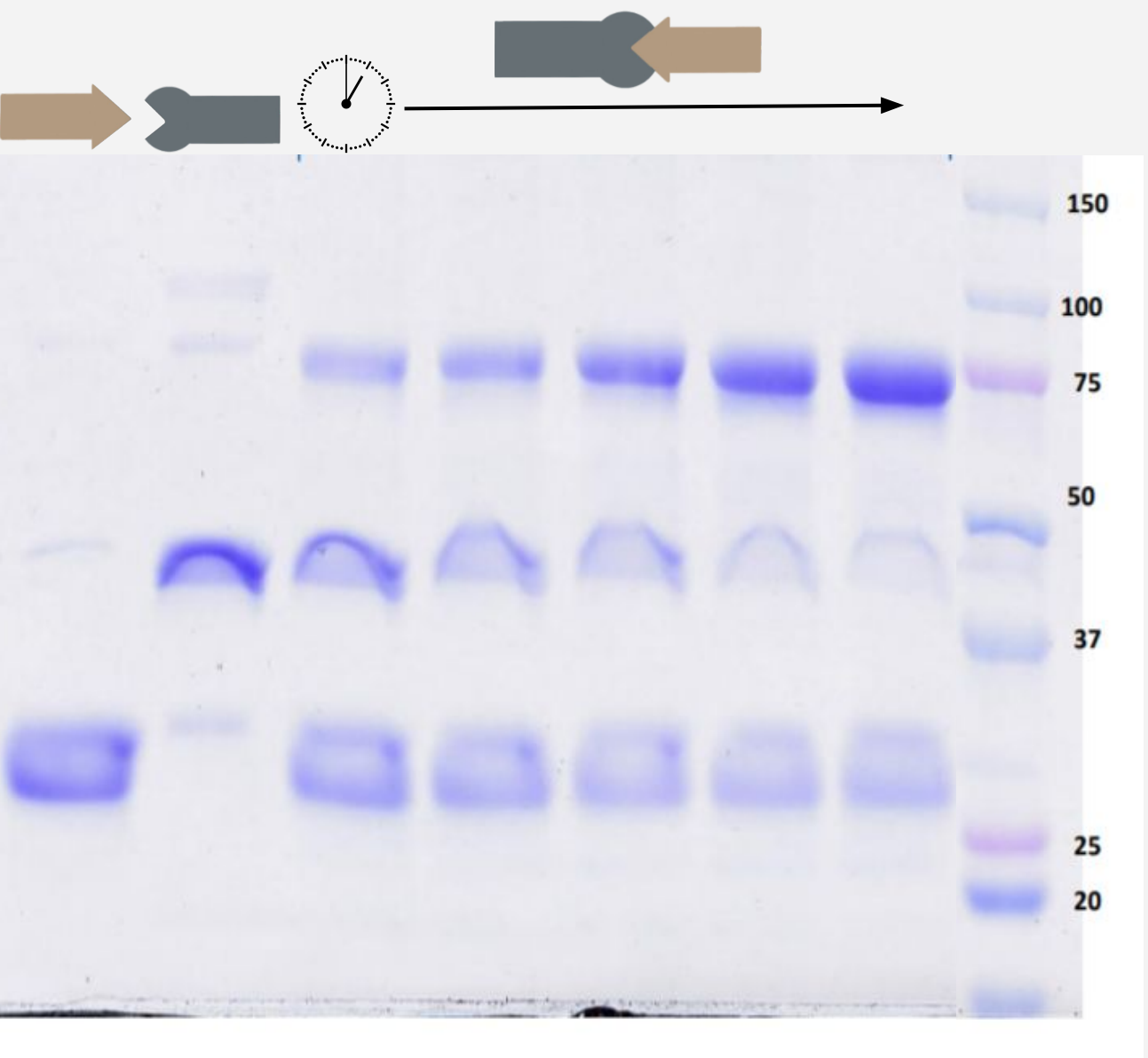
Physically attach cells

Specific approach for cell-cell binding to create aggregates

- Use adhesin pair SpyTag and SpyCatcher on OM
- *Synechococcus elongatus* (*S. elongatus*) and *Escherichia coli* W (*E. coli* W)



Do SpyTag and SpyCatcher bind in vitro?



- Time series SpyTag/Spycatcher reactions
- SpyTag and SpyCatcher individually decreasing
- Combination band increasing

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