Establishing a robust FtsZ-based divisome for synthetic cell constriction

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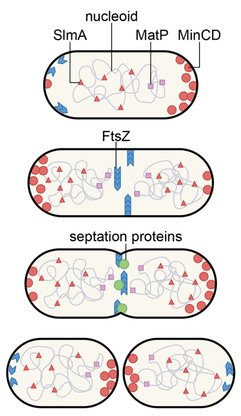
The FtsZ divisome

Membrane constriction forms an essential stage of cell division. The goal of this project is to develop a minimal divisome capable of autonomous liposome constriction. For this we draw inspiration from the conserved FtsZ-based divisome found in bacteria, as well as in archaea. We will start with the best studied example of the FtsZ system in *Escherichia coli. In vivo, E. coli* division proceeds as follows:



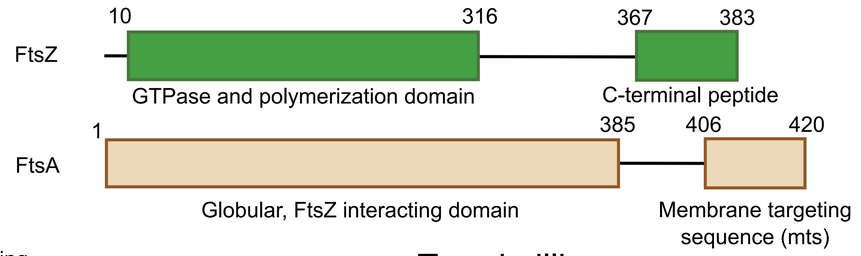
Positioning of FtsZ ring, including MinCDE gradient and nucleoid occlusion

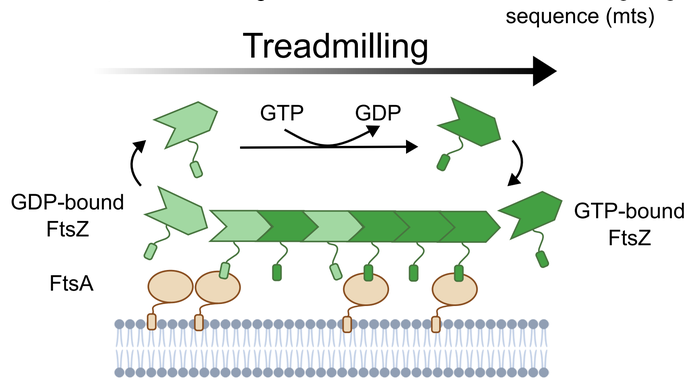
FtsZ filaments form, bundles and condenses into FtsZ-ring at division site

Downstream division proteins recruited, including peptidoglycan synthesis complex, followed by septation

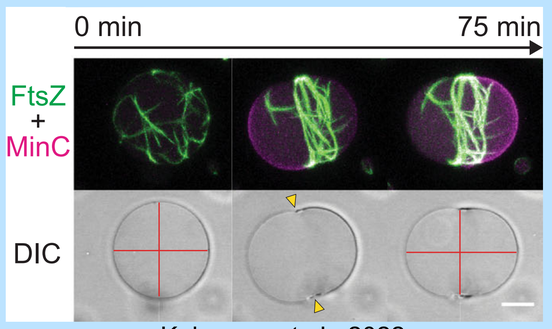
Abscission is completed

Cameron and Margolin, 2023

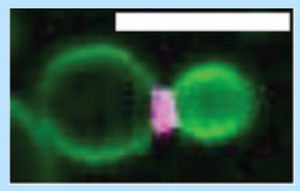




In vitro reconstitution



Kohyama et al., 2022



Godino and Danelon, 2023

Reconstituted FtsZ and FtsA have been shown by Kohyama et al. to progressively constrict liposomes (upper left) and locate at the constriction point of dumbbell liposomes as demonstrated by Godino and Danelon (upper right), but not yet both in the same experiment. Scale bars indicate 10 µm and 5 µm respectivelly.

Research questions

Which FtsZ/FtsA variants most effectively constrict liposomes?

What are the optimal conditions for constriction?

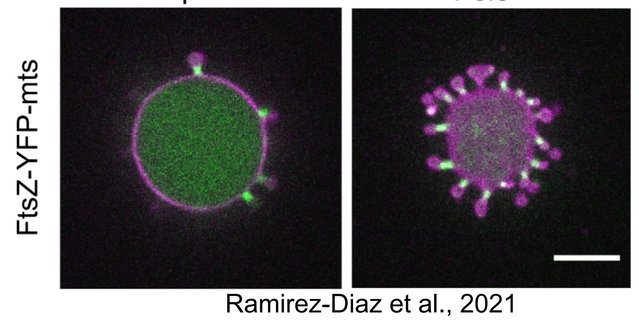
What is the mechanism of force generation?

How can FtsZ-based constriction be combined with chromosome segregation and liposome abscission?

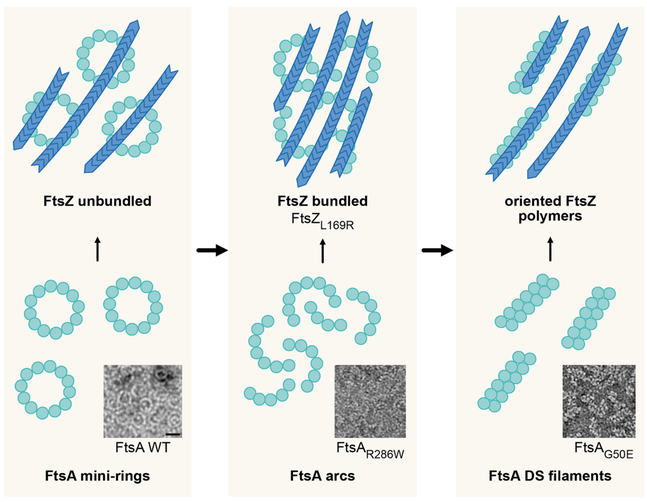
FtsZ/FtsA variants

Membrane targeted FtsZ

FtsZ modified with membrane binding domain functions without FtsA. It was shown to form protrusions with constricted necks in deflated GUVs.
If a single protein is enough for constriction, it would be an attractive simplification of the system.



Ramirez-Diaz et al., 2021

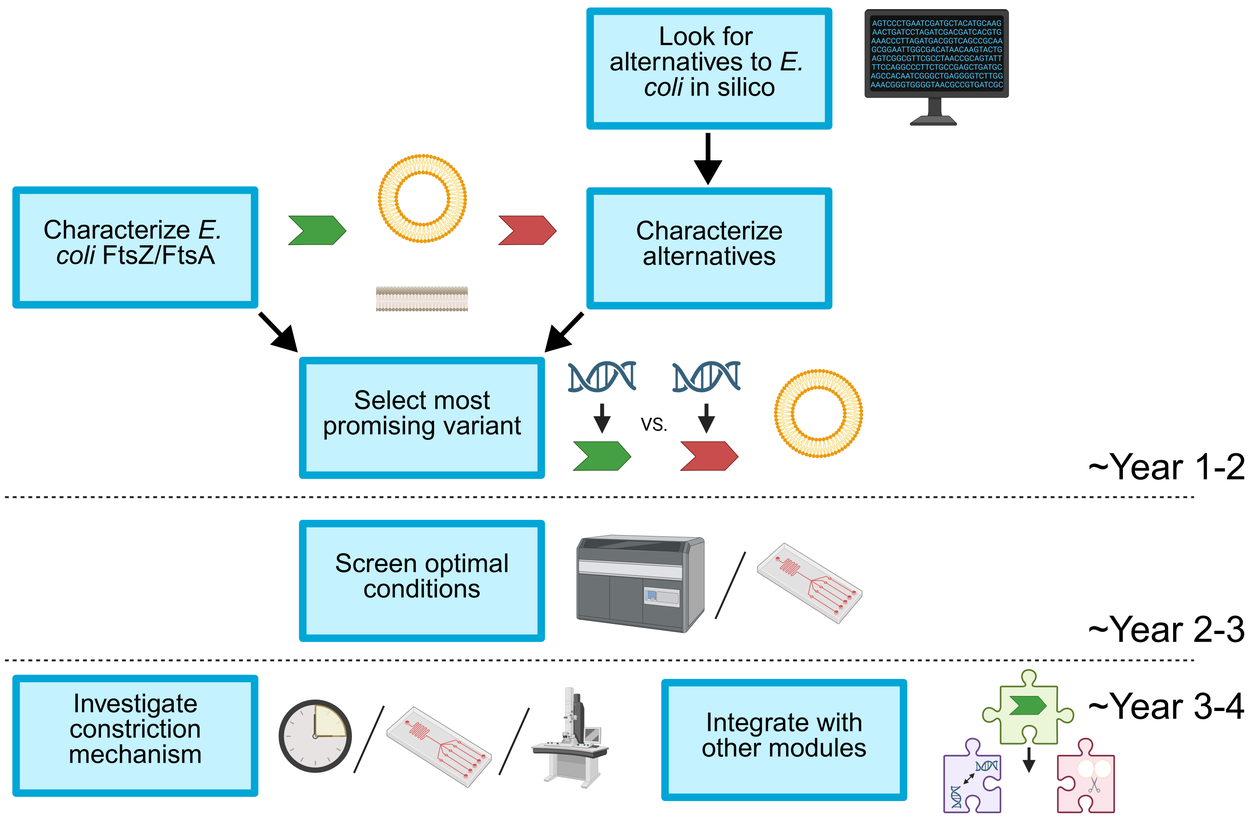


Cameron and Margolin, 2023

FtsA polymerization and FtsZ bundling

FtsA polymerization state influences its ability to promote FtsZ bundling. Several mutants of FtsA have altered polymerization and improve FtsZ bundling.
Using these mutants instead of wildtype FtsA might improve FtsZ ring formation, allowing more robust constriction.

Workflow

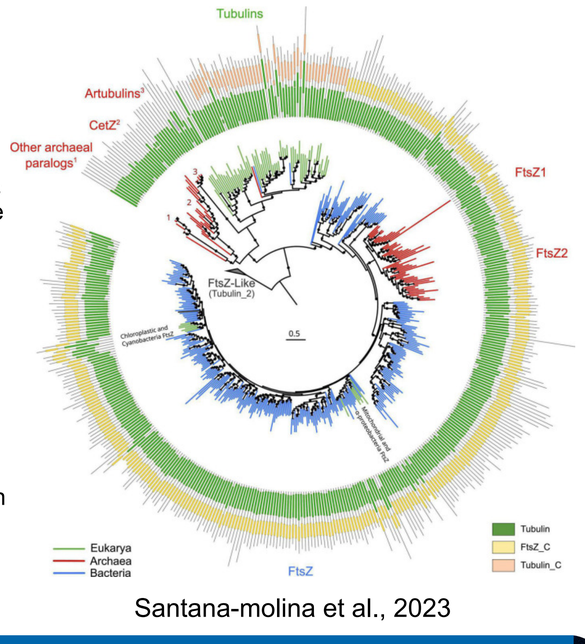


Beyond *E. coli*

FtsZ is also present in most other bacteria as well as in archaea; potential for more suitable FtsZ systems.

Preselect based on:
- Genetic tractability
- FtsZ only division mechanism
- Symmetric division
- Organism shape/size
- Organism cell cycle
- Mesophilic
- No other paralogs of FtsZ

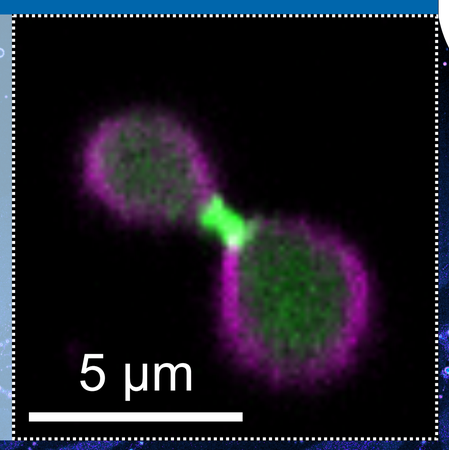
We will compare FtsZ from other species with *E. coli* FtsZ using bioinformatics and simulations to select the most promising candidate for in vitro testing.



Santana-molina et al., 2023

Ongoing work

- Purifying wildtype FtsZ/FtsA
- Cloning FtsZ with membrane targeting sequence
- Optimizing cell-free expression of FtsZ and FtsA and GUV yield for GUVs produced using inverted emulsion method



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