Microfluidic and CRISPRi toolkits for elucidating archaeal cytoskeleton

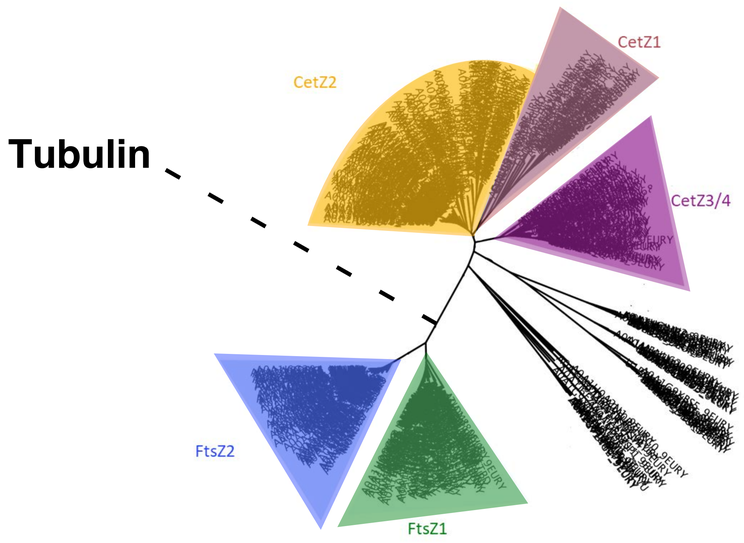
John Mallon[1], Alex Bisson[1]

1. Department of Biology, Brandeis University, Waltham, MA

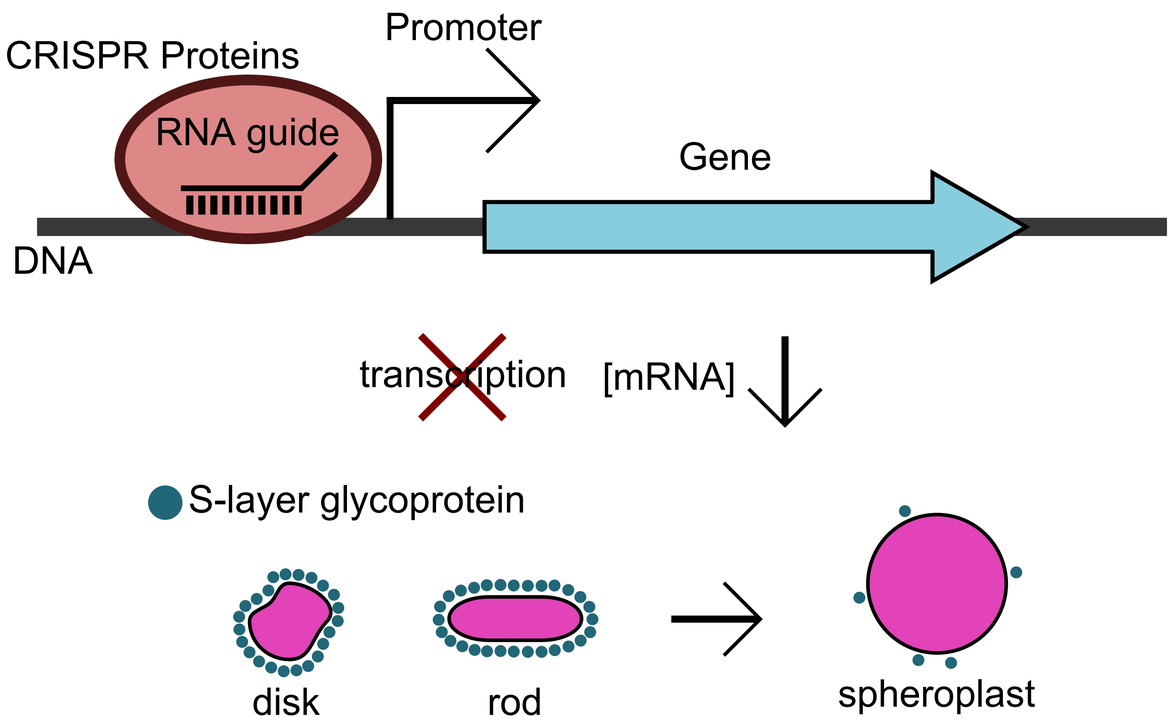
Brandeis bioinspired MRSEC

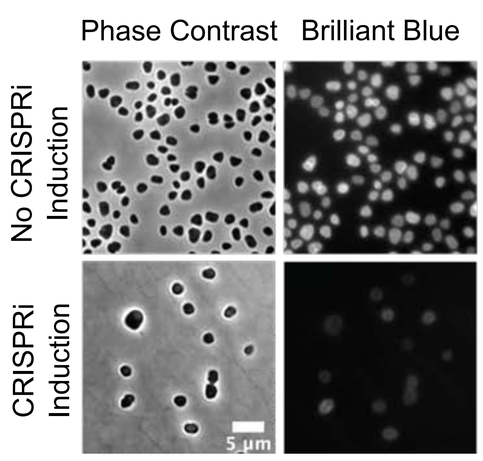
Rationale

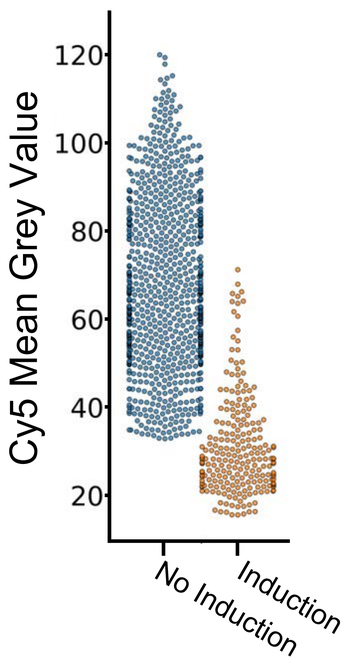
Archaea have many tubulin homologs, some of which are only found in the archaeal domain. Furthermore species will have many of these tubulins within their chromosome. To inspire new materials for active fluids research we look to develop tools for studying the properties of these archaeal tubulins



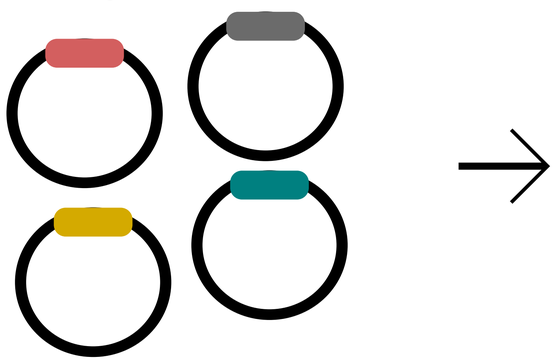
CRISPRi knockdown of S-layer



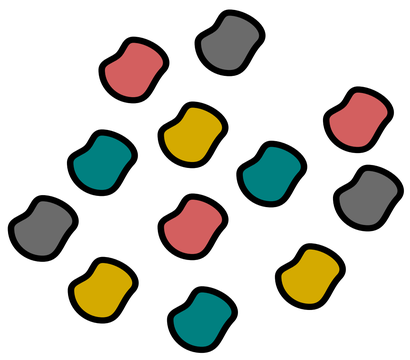




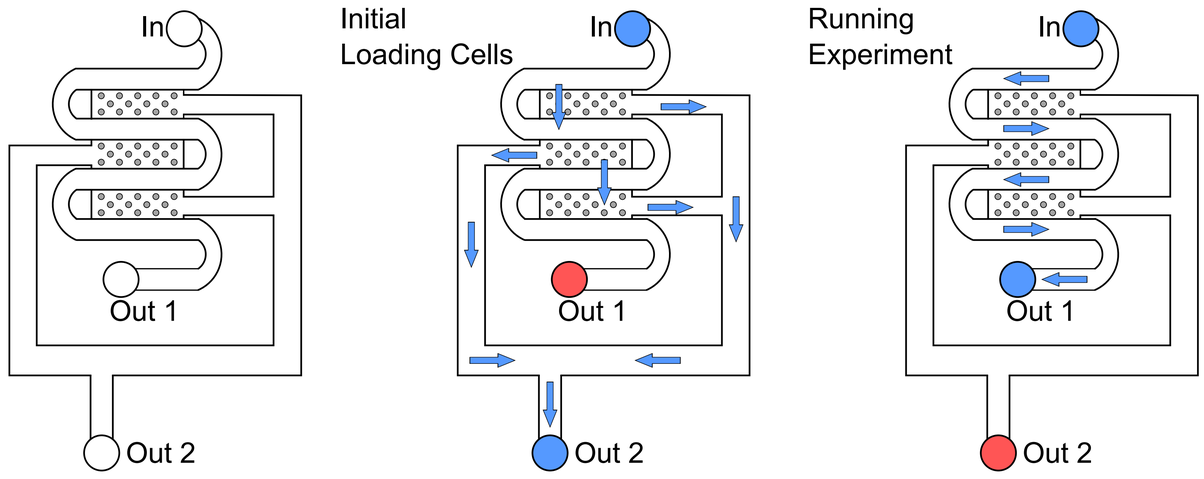
Library of ~8k RNA guides in plasmid form targeting *Haloferax volcanii* genome

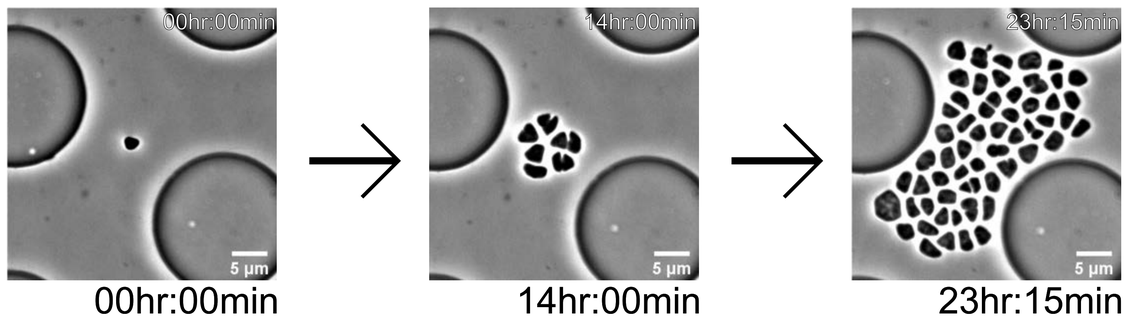


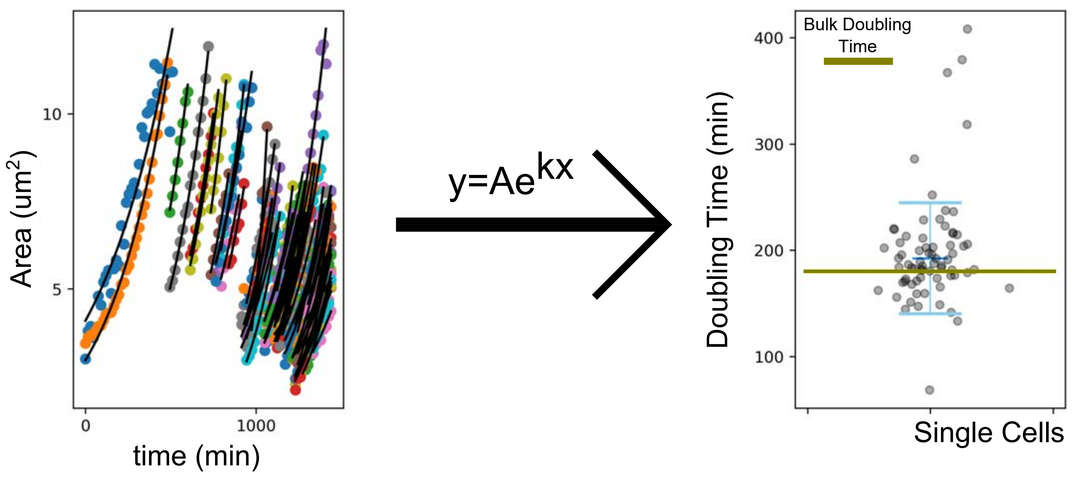
Currently scaling up our transformation protocol for oversampling of library



Microfluidics is growing cells successfully







Conclusions

- Current fluidics system with two outlet design gives single cell growth rates comparable to bulk measurments
- CRISPRi strain can successfully knockdown the highest expressed gene (S-layer) in *Haloferax volcanii*

References

1. Bisson-Filho A.W., Hsu Y.-P., Squyres G.R., *et al.*Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division. *Science*. 2017 Feb 17;355(6326):739-743.

2. Duggin I.G., Aylett C.H., Walsh J.C., *et al.* CetZ tubulin-like proteins control archaeal cell shape. *Nature*. 2015 Mar 19; 519(7543): 362-365

3. Stachler A.E., Marchfelder A. Gene Repression in Haloarchaea Using CRISPR (Clustered Regularly Interspaced Short Palandromic Repeats)-Cas-I-B System. *J Biol Chem*. 2016 Jul 15;291 (29):15226-42.