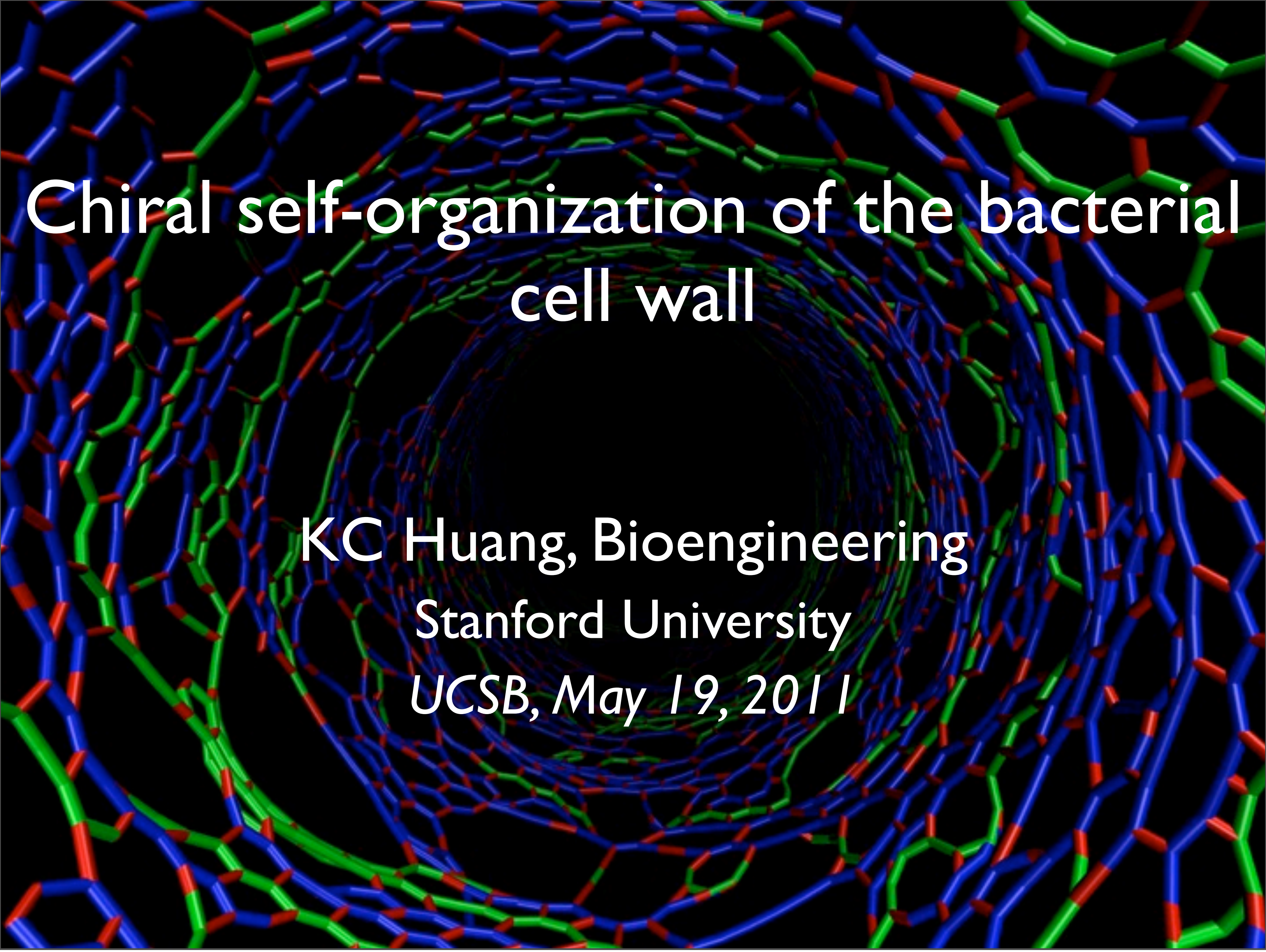


Chiral self-organization of the bacterial cell wall

KC Huang, Bioengineering
Stanford University

UCSB, May 19, 2011

Thanks

Ned Wingreen
Josh Shaevitz
Steven Wang

What rules govern how cells choose their shape?

What rules govern how cells grow?

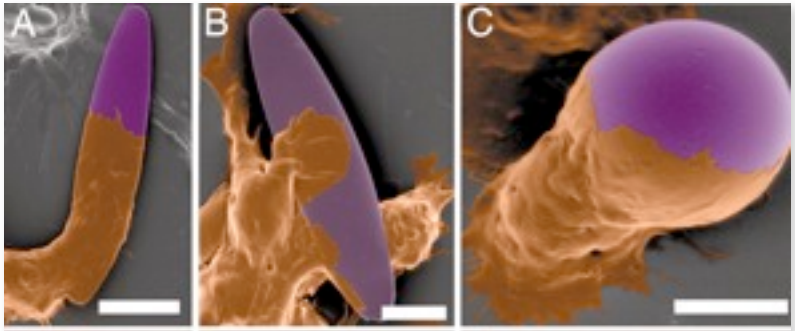
The Selective Value of Bacterial Shape

Kevin D. Young*

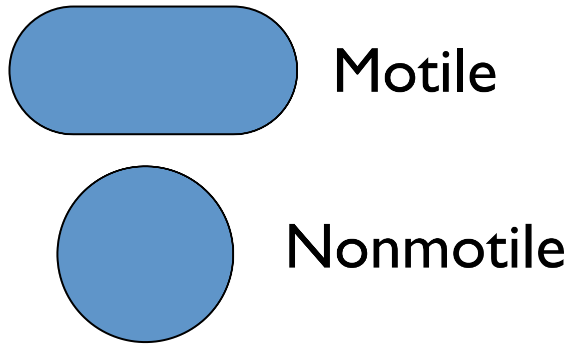
INTRODUCTION

It's not in the open we feel comforted but in the shadows. . . . We can't feel at home with the infinite sky above and around us. Space must be cut off, shaped, defined, for us to inhabit. From cradle to coffin, it's enclosure that defines us.
—Robert Morgan (221)

To be brutally honest, few people care that bacteria have different shapes. Which is a shame, because the bacteria seem to care very much. A simple way to verify this is to take a leisurely stroll through *Bergey's Manual of Determinative Bacteriology* (133) or *The Prokaryotes* (65, 313), pausing to admire the surprising and bewildering riot of shapes, sizes, and aggregates, some of which are illustrated in Fig. 1. There are cells

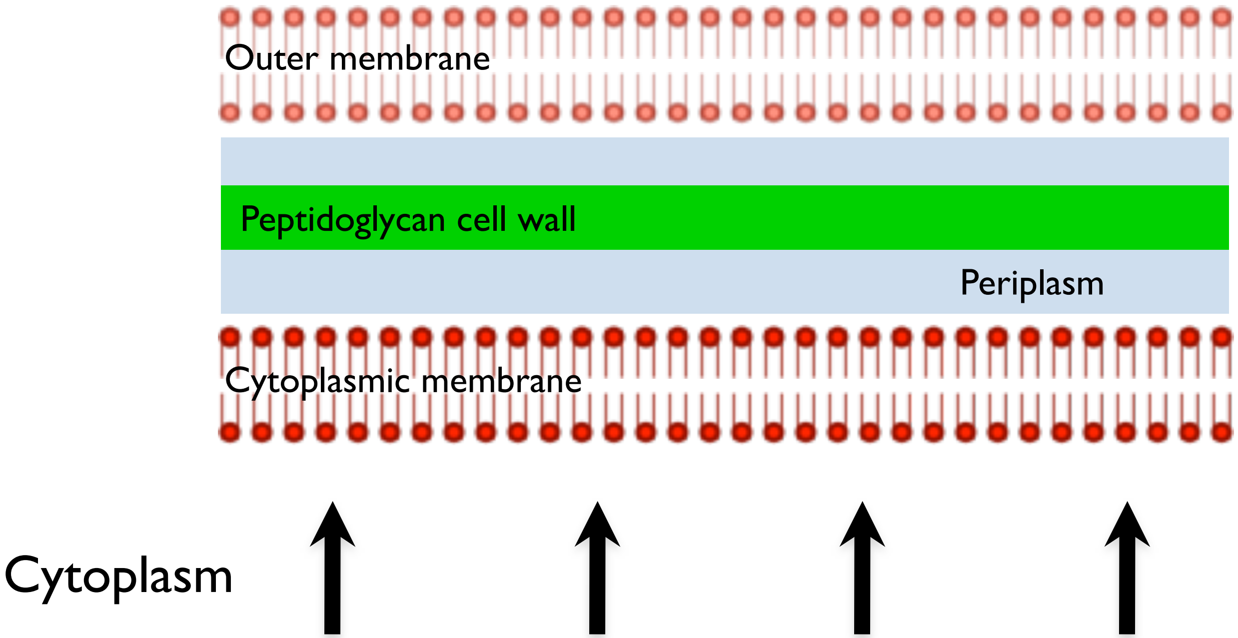


(Champion *PNAS* 2006)


Motile
Nonmotile

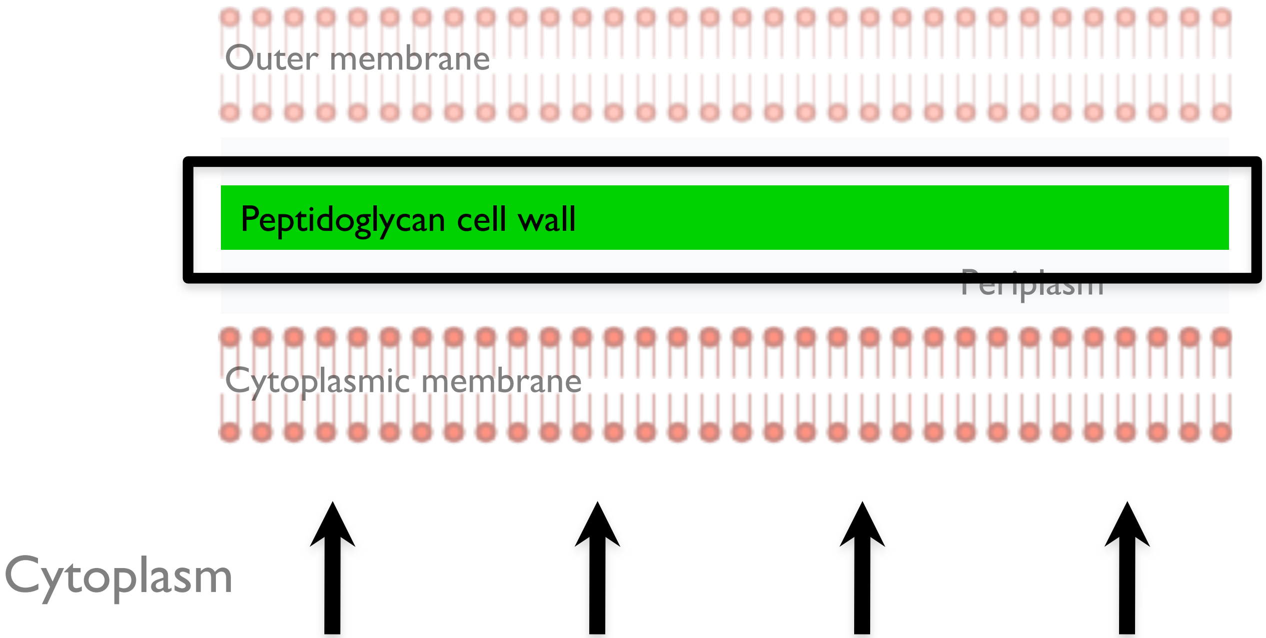
- Importance of shape to cellular processes
  - Predation, immunity, differentiation, …
- Introduction to the bacterial cell wall
- Modeling the *E. coli* cell wall
  - Robustness of cell shape to damage
  - Cell shape determination
  - Cell shape maintenance
- How do cells grow?

Cell wall determines cell shape



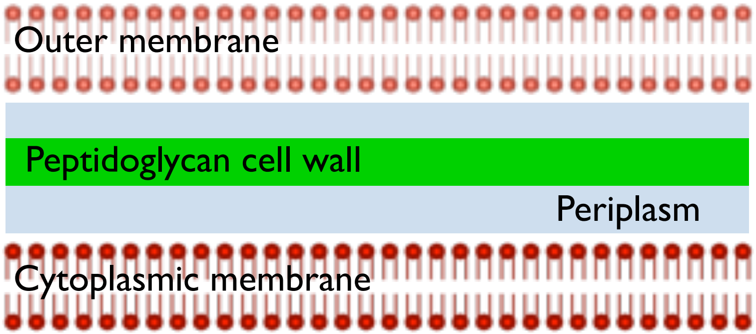
Turgor pressure (>1 atm)

Cell wall determines cell shape

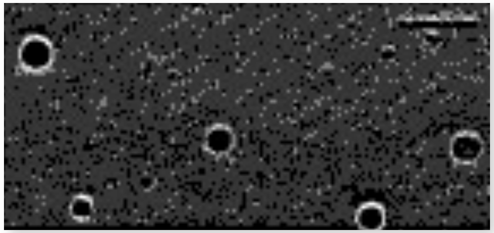


Turgor pressure (>1 atm)

Cell wall determines cell shape

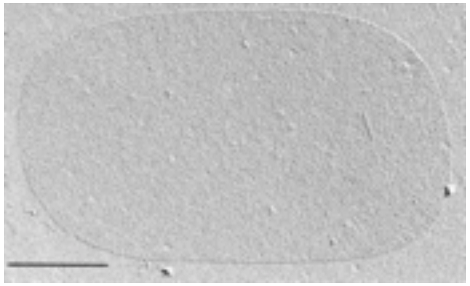


Spheroplast (membrane)



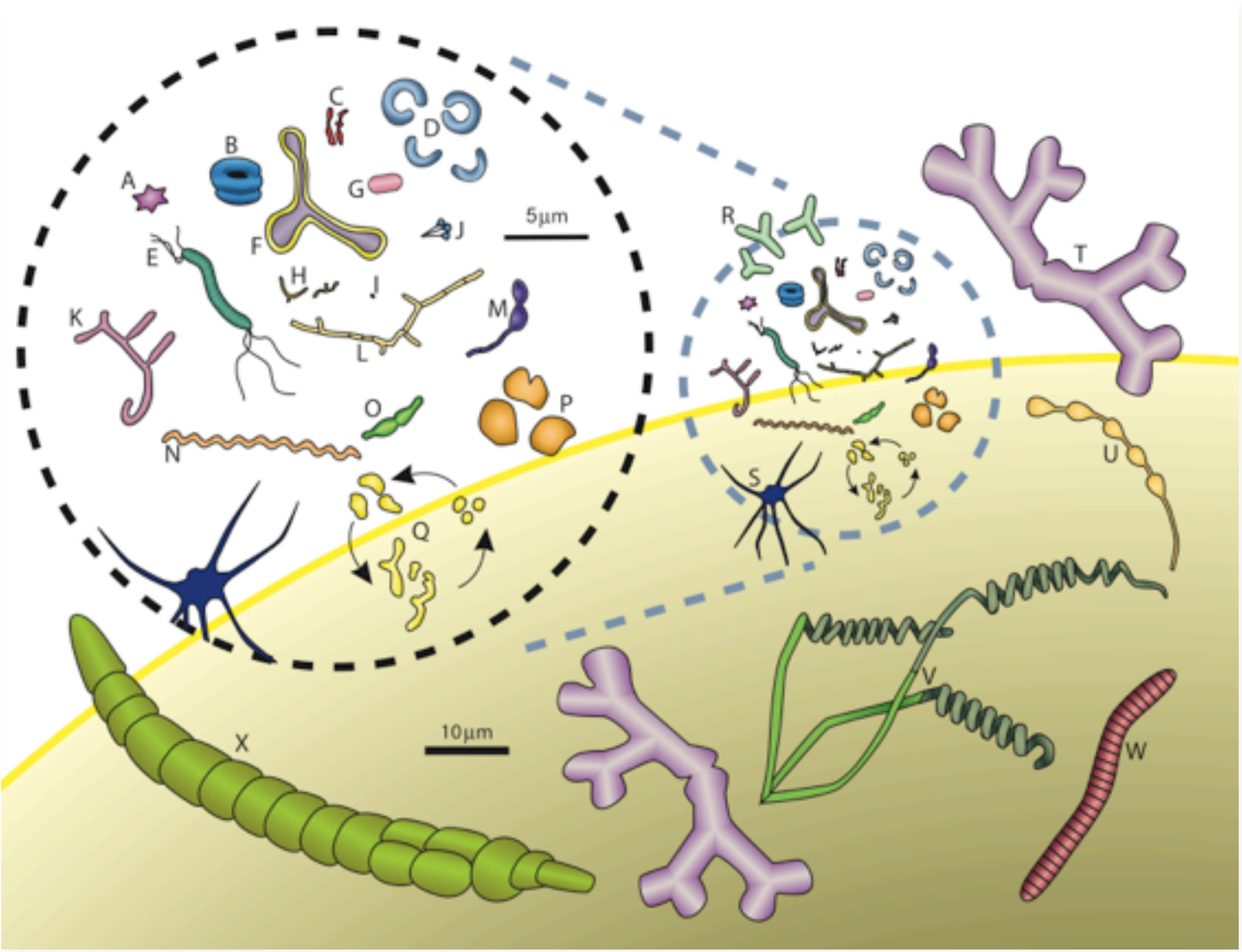
Martinac *PNAS* 1987

Sacculus (cell wall)

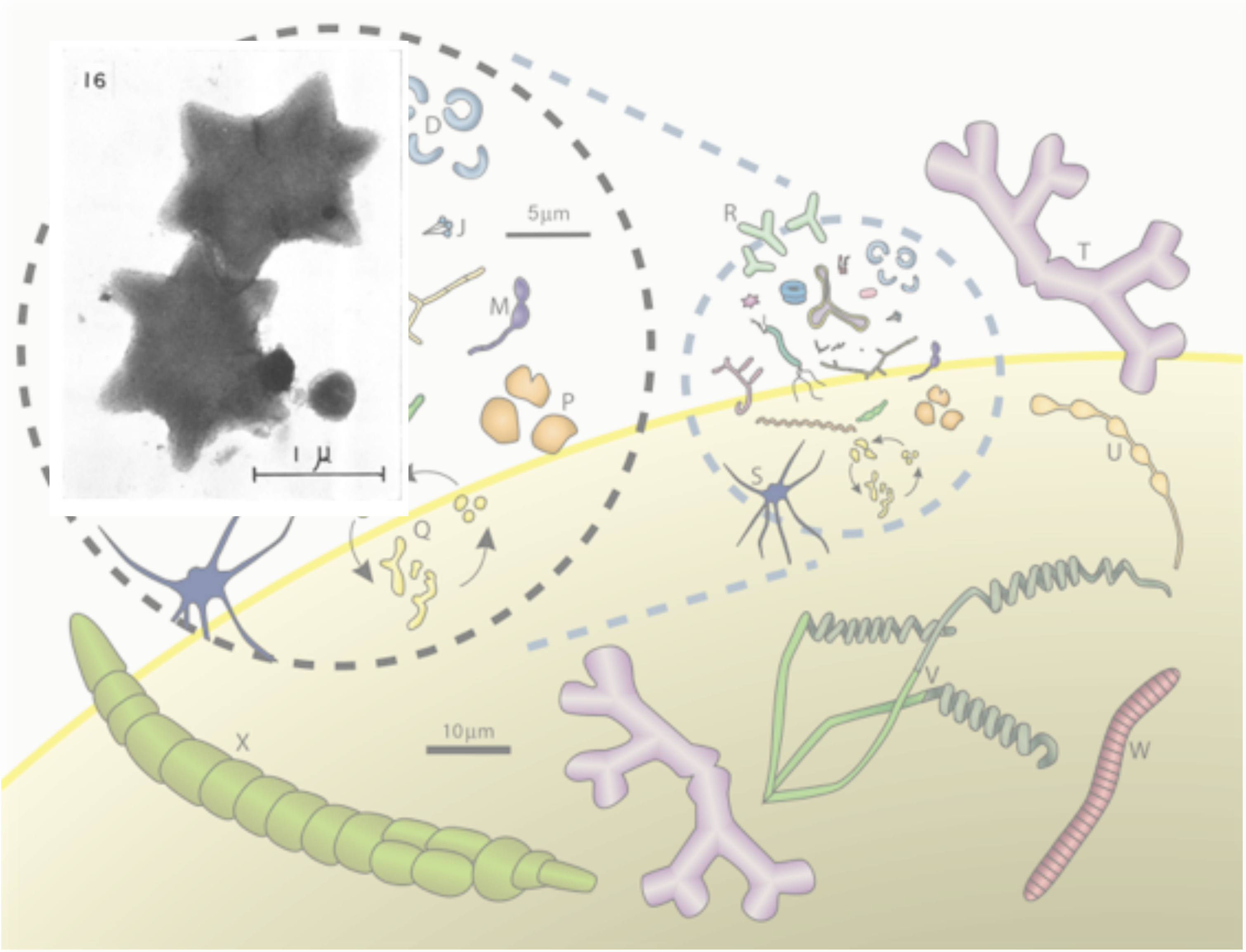


Holtje *MMBR* 1998

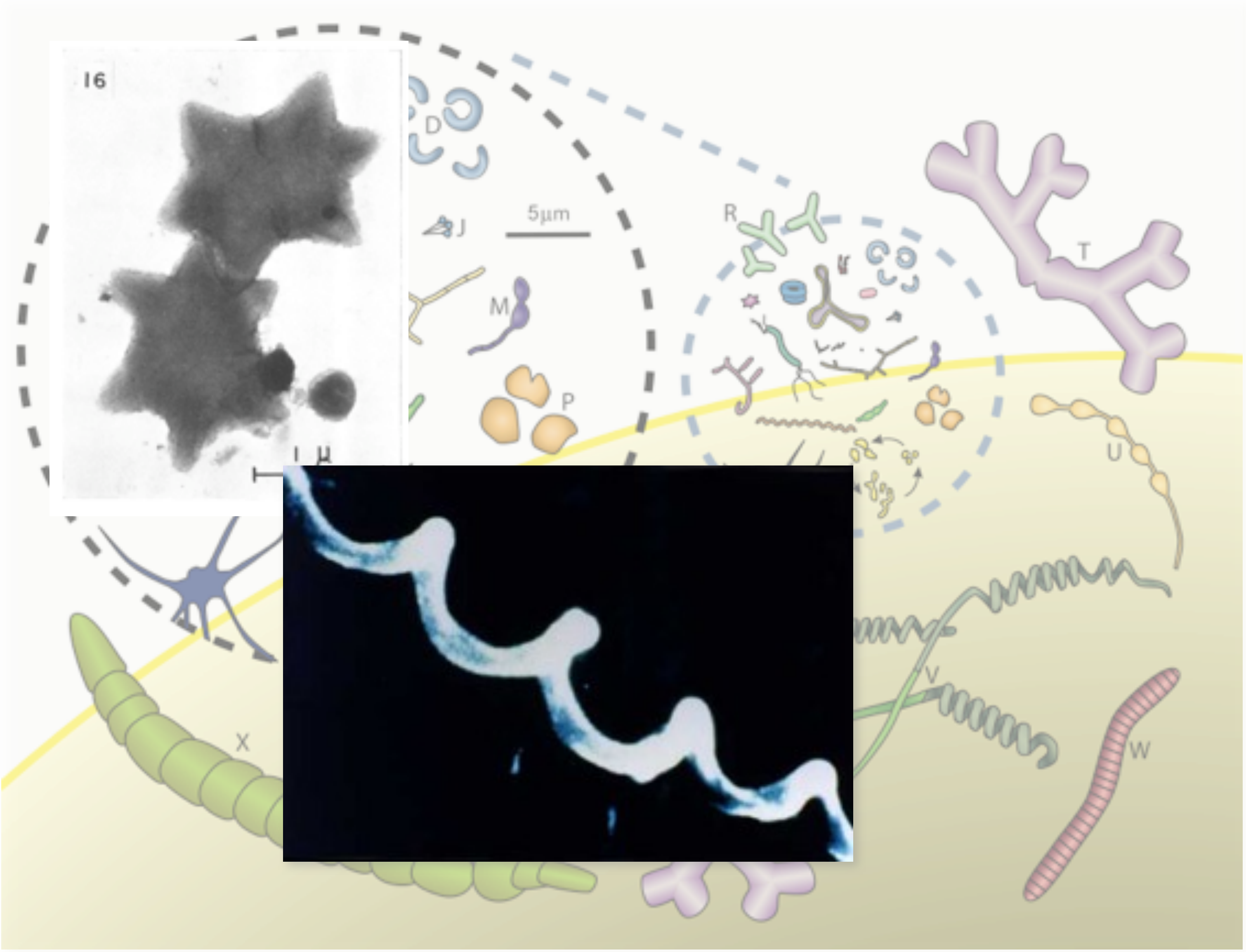
The cell wall is necessary and sufficient for cell shape determination



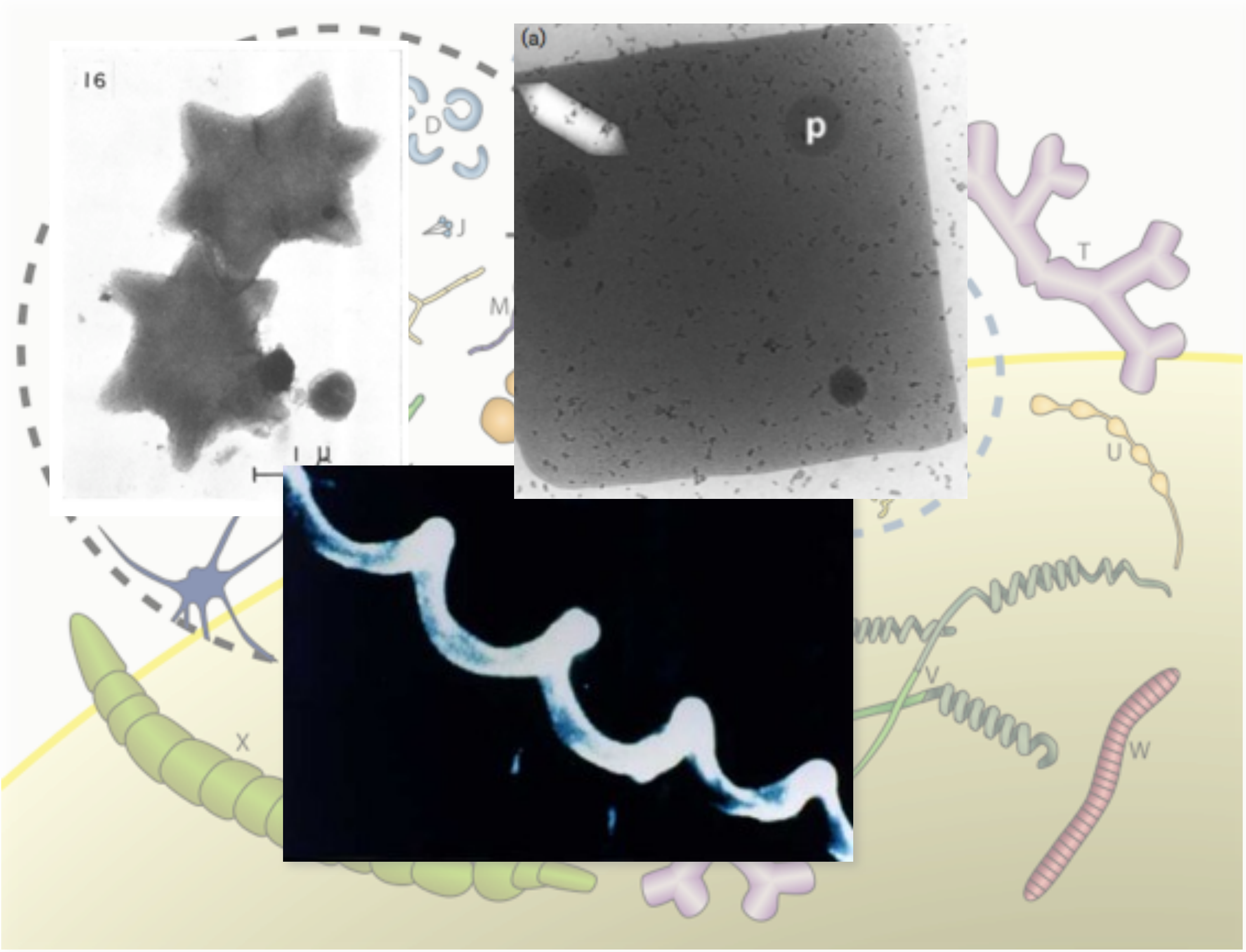
Young *MMBR* 2006, microbiological-garden.net



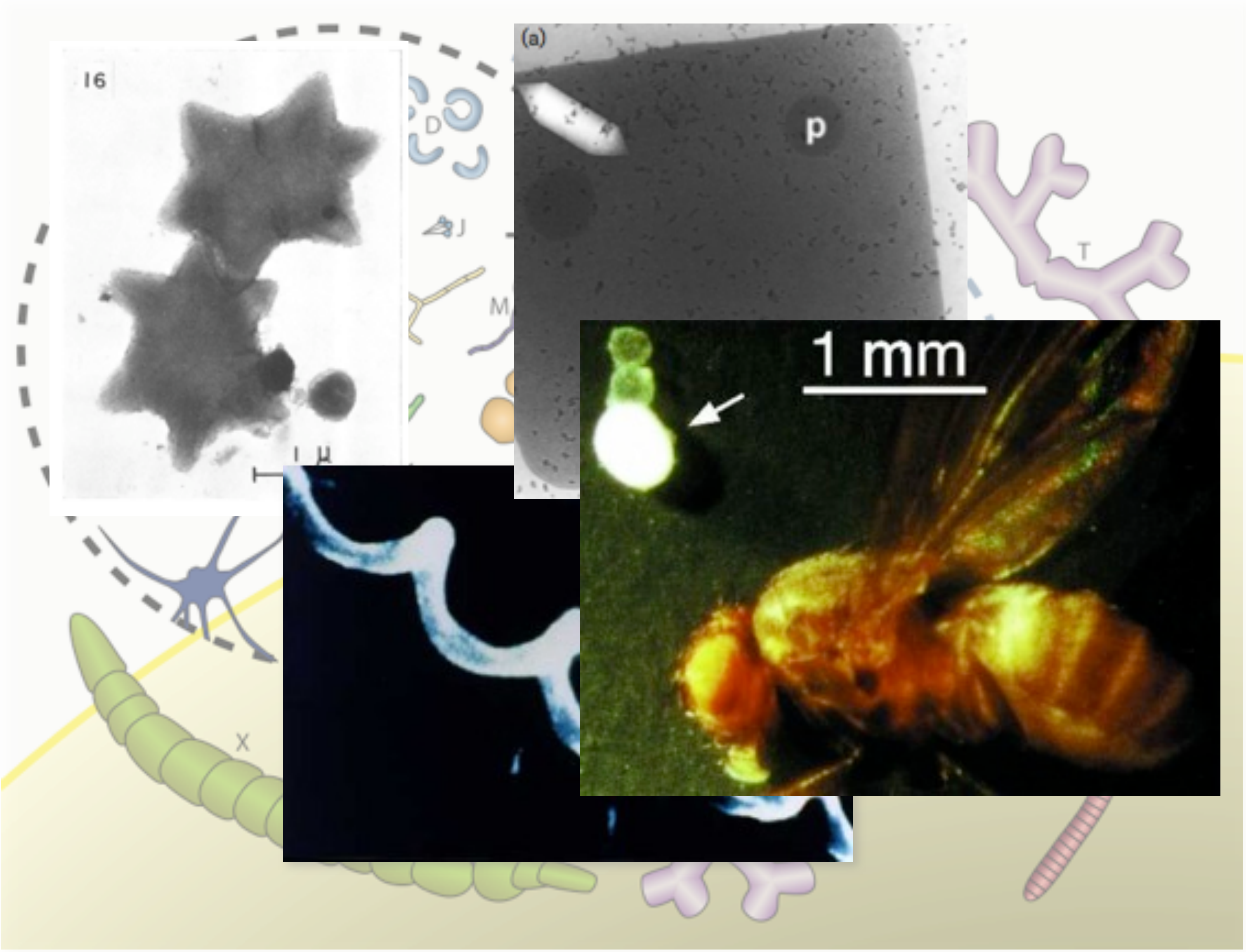
Young *MMBR* 2006, microbiological-garden.net



Young *MMBR* 2006, microbiological-garden.net



Young *MMBR* 2006, microbiological-garden.net

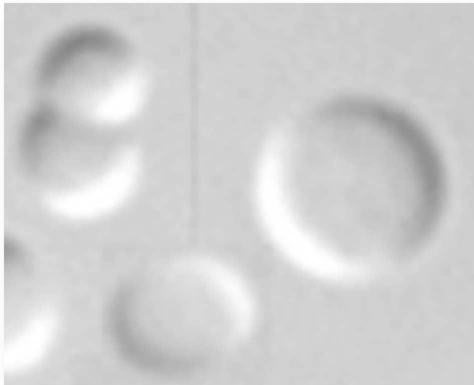


Young *MMBR* 2006, microbiological-garden.net

Exponential phase

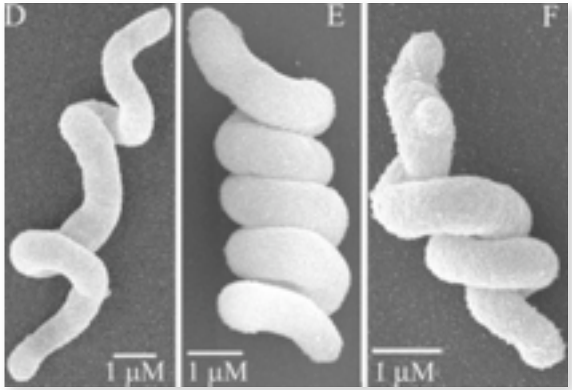
Astragraphics

Stationary phase, MreB knockout



Shih *MolMicro* 2005

Point mutations

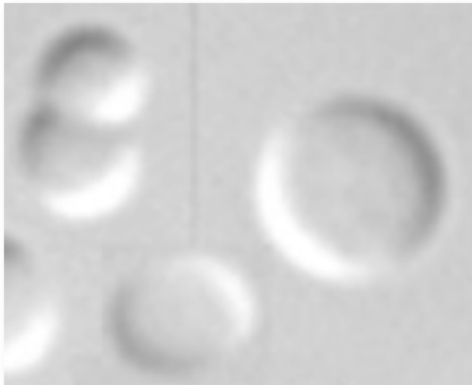


Varma *JBact* 2007

Exponential phase

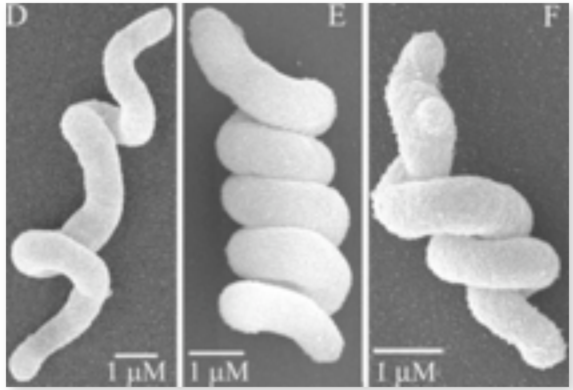
Astragraphics

Stationary phase, MreB knockout



Shih *MolMicro* 2005

Point mutations

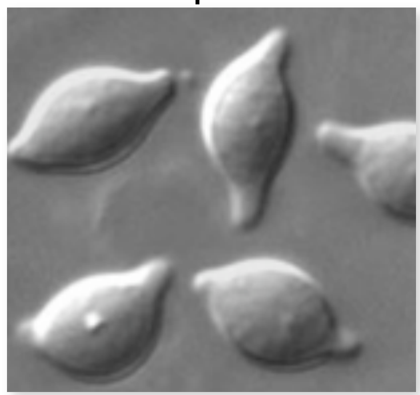


Varma *JBact* 2007

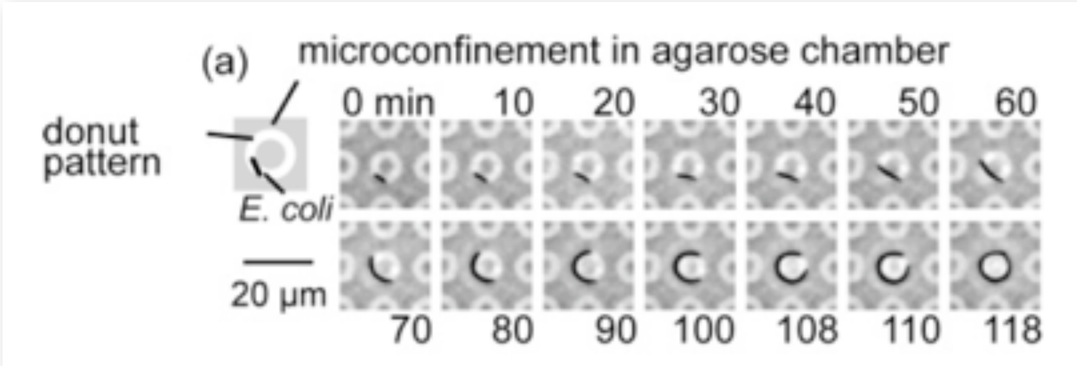
Genetic knockout

Varma *JBact* 2008

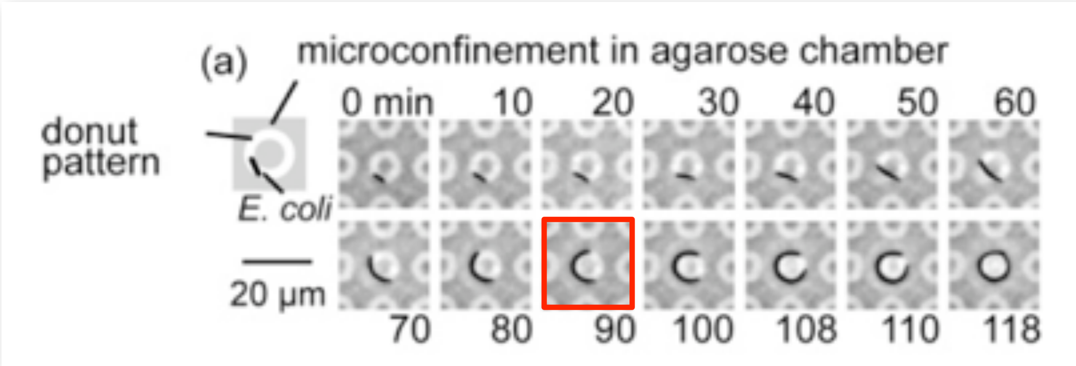
Chemical perturbation



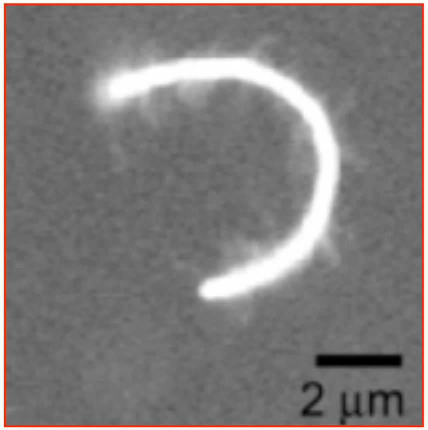
Varma *JBact* 2007

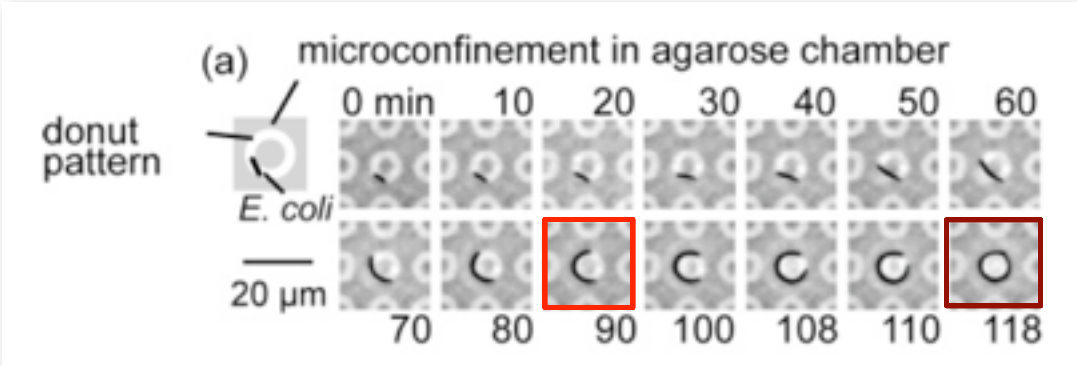


Takeuchi *NanoLett* 2006

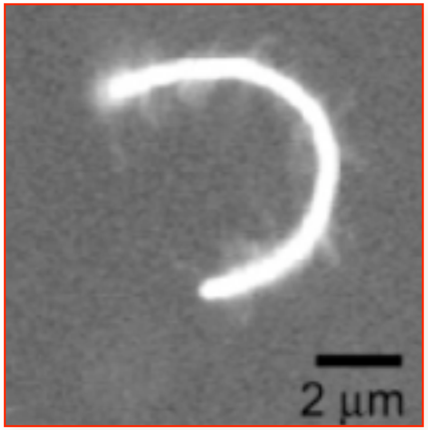


Takeuchi *NanoLett* 2006





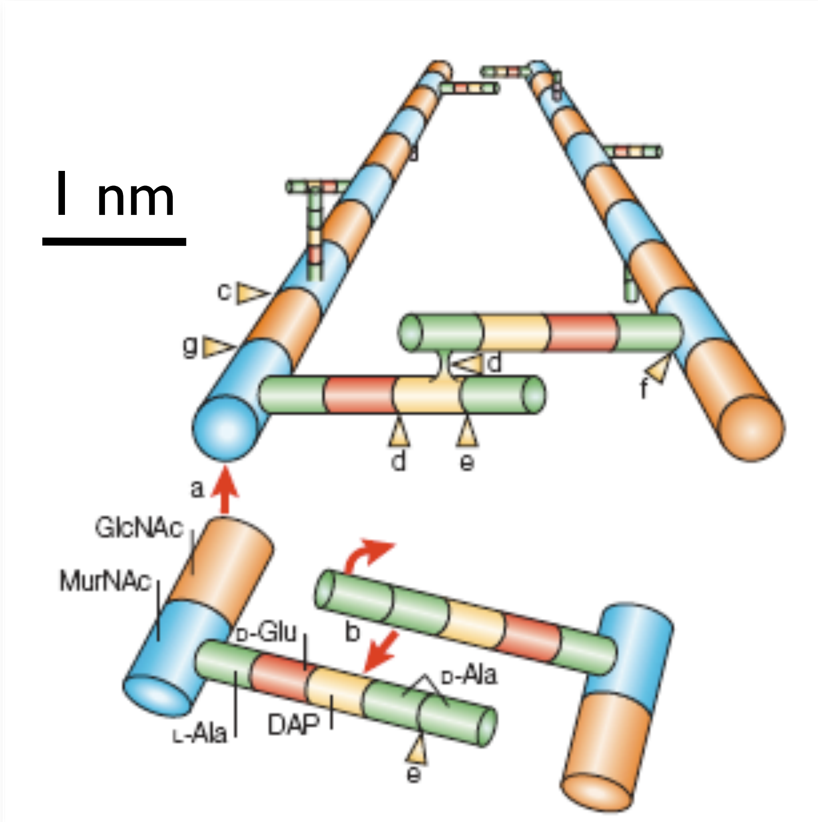
Takeuchi *NanoLett* 2006



Cells grow into (and maintain) shapes defined by the chamber

Cell wall is the stress-bearing structure

Cell wall is a peptide-sugar network (peptidoglycan)



Cabeen and Jacobs-Wagner NatureRevMicro 2005

- *E. coli* cell wall is 75-80% single layered (2-3nm thickness).

structure

- Glycans polymerized as strands of 50-60 subunits.

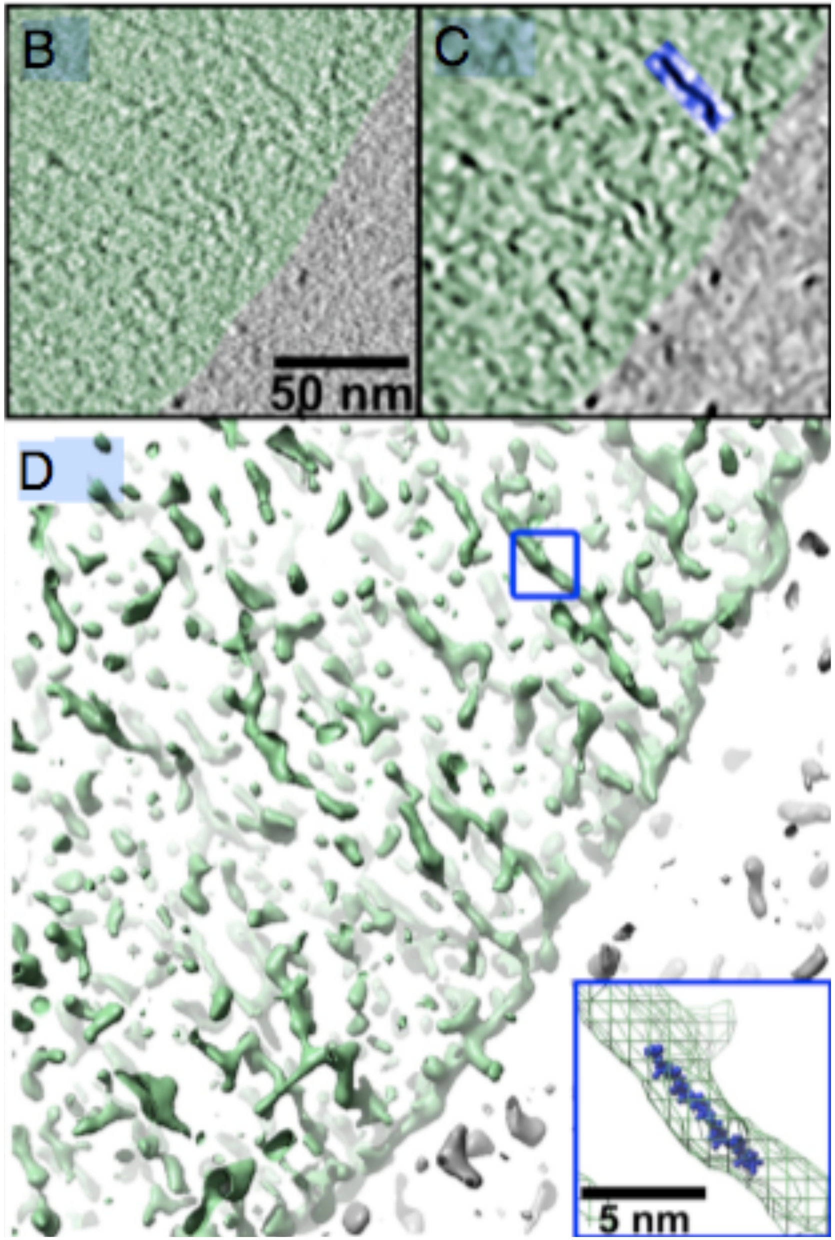
biochemistry

- Approximately 50% of peptides are crosslinked.

biochemistry

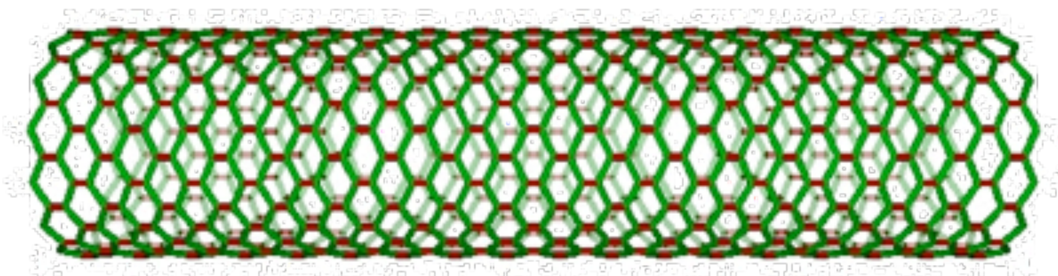
- Isolated cell walls (sacculi) are 2-3 times more deformable along the long axis than in the perpendicular direction.

mechanics

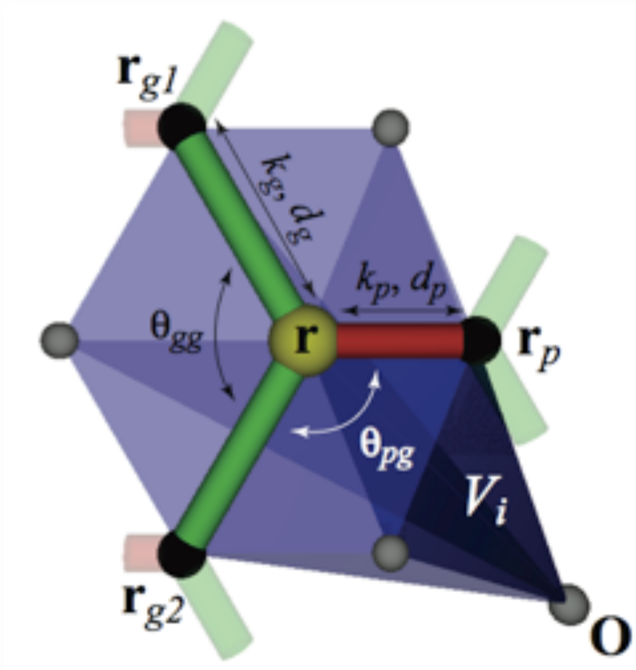


(Gan *PNAS* 2008)

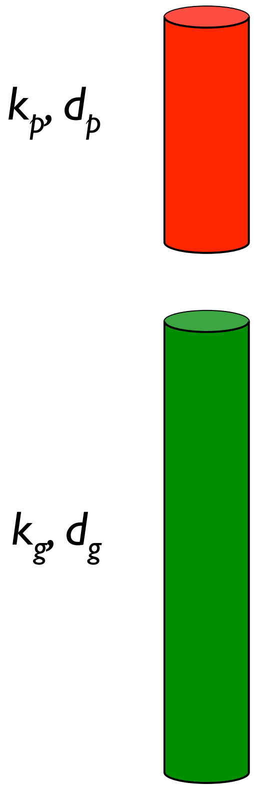
• Spring network of peptides and glycans with hoops of glycans oriented along the circumference
• Osmotic pressure acts on the surface (**F** = Π *d*V/*d***r**)



(Holtje, Koch, Beveridge, Pink, …)

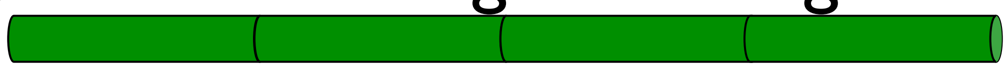


Macroscopic observables lead to microscopic parameters

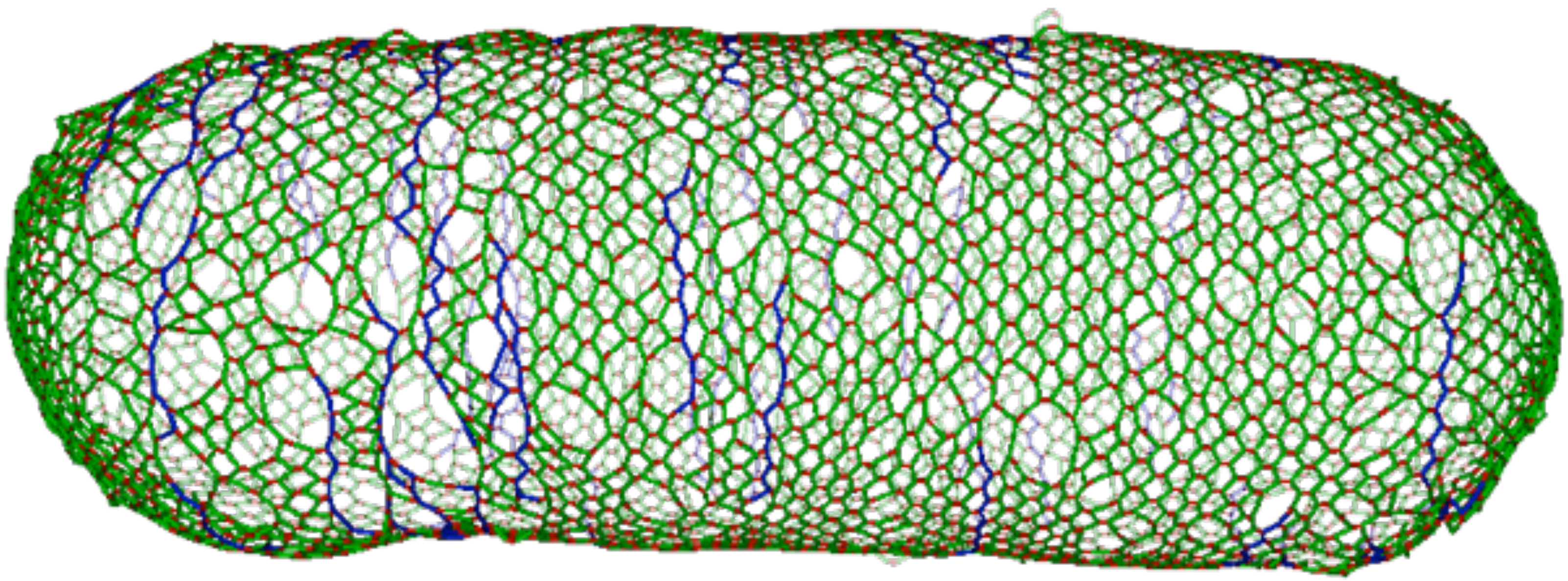


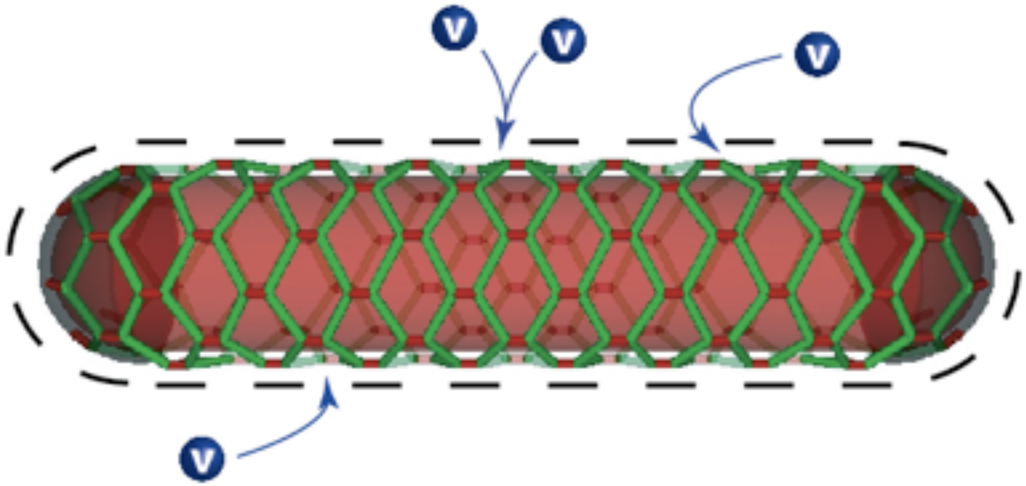
- Lengths (d_p, d_g~1nm, 2nm)
- Young's modulus → stiffness
  (k_p~10pN/nm, $k_g \gg k_p$)
- Persistence length → bending
  (ζ ~ 1-10nm, κ ~ 10pNnm)

- Glycan strand lengths → organization



Bacterial cell wall




v
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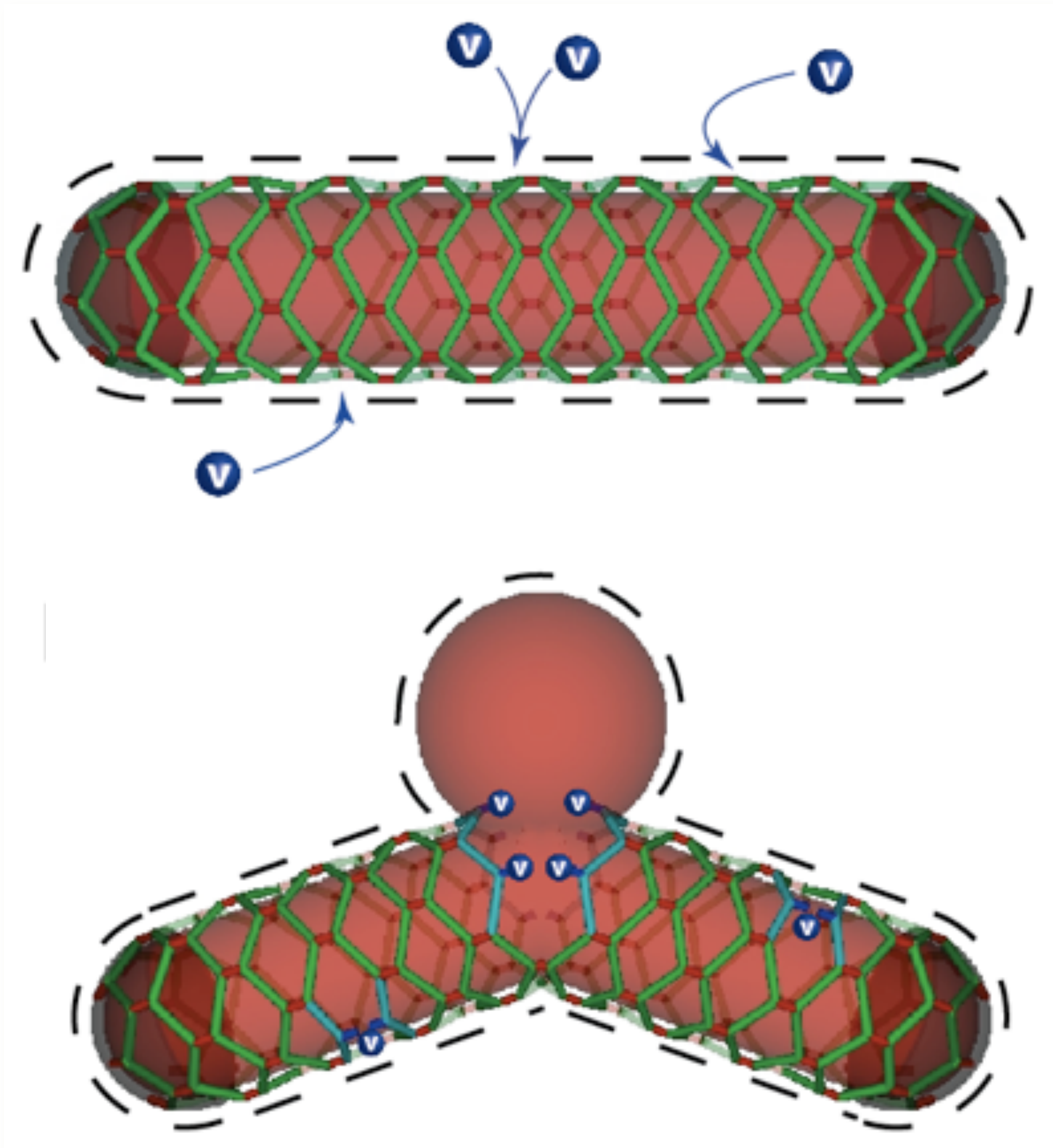
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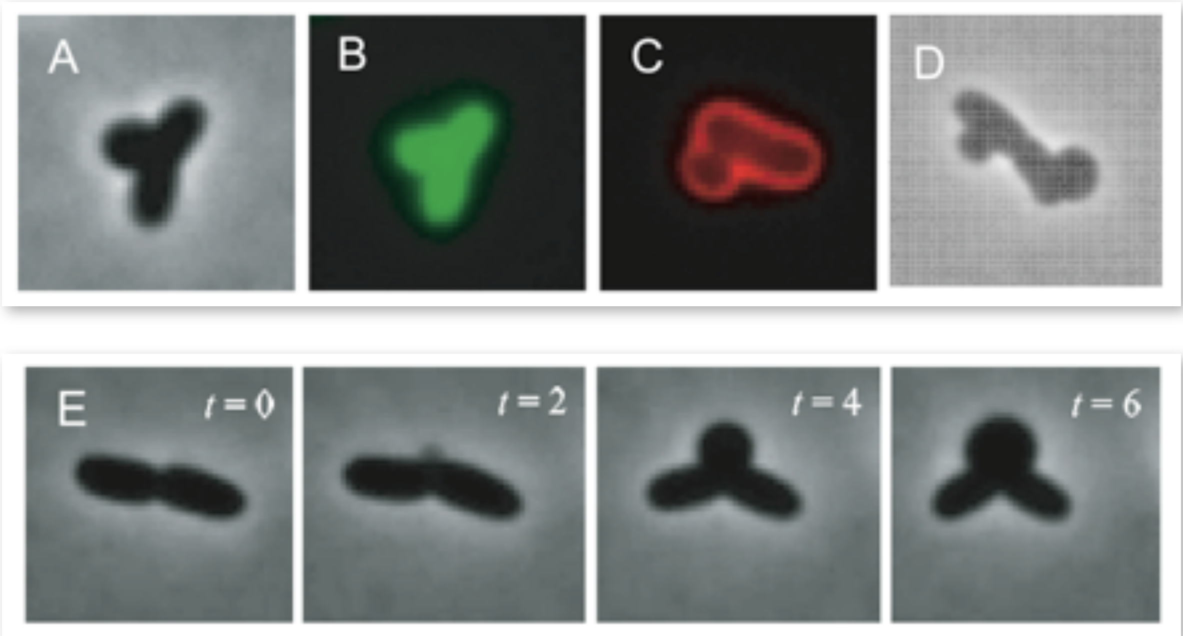
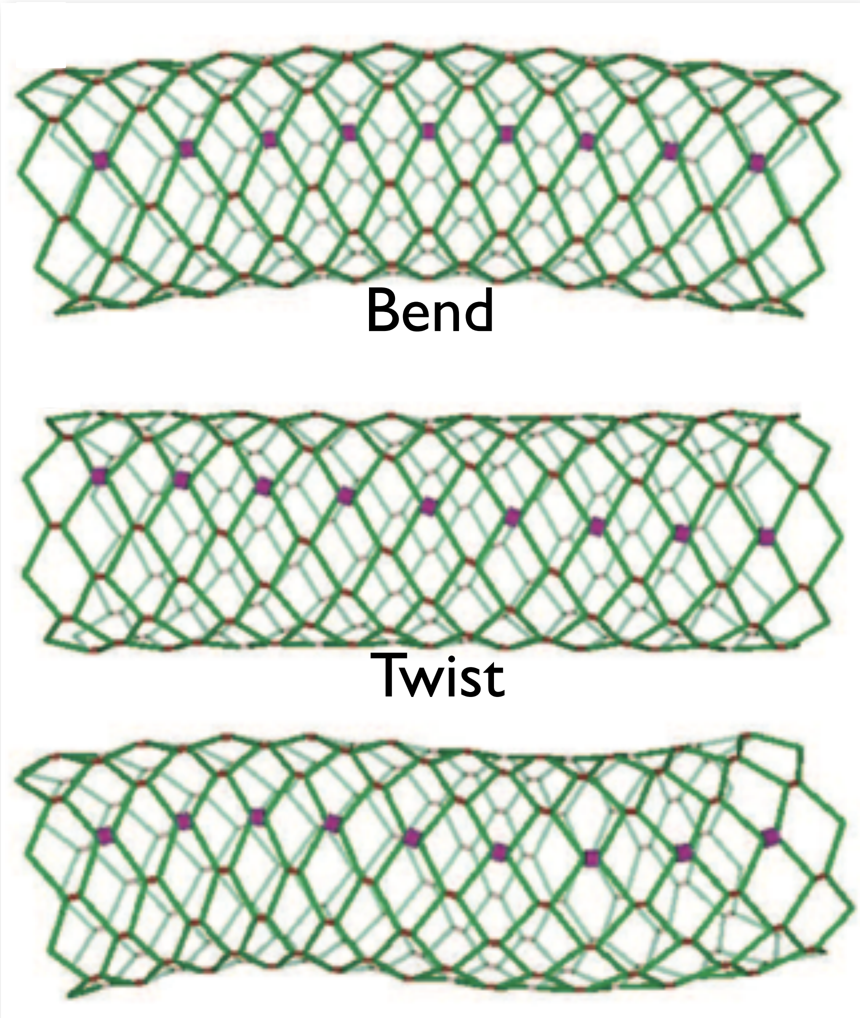


(*imp4213* strain: Sampson *et al*, Genetics 1989, Tom Silhavy)

In response to vancomycin, cells "crack" around the bulge

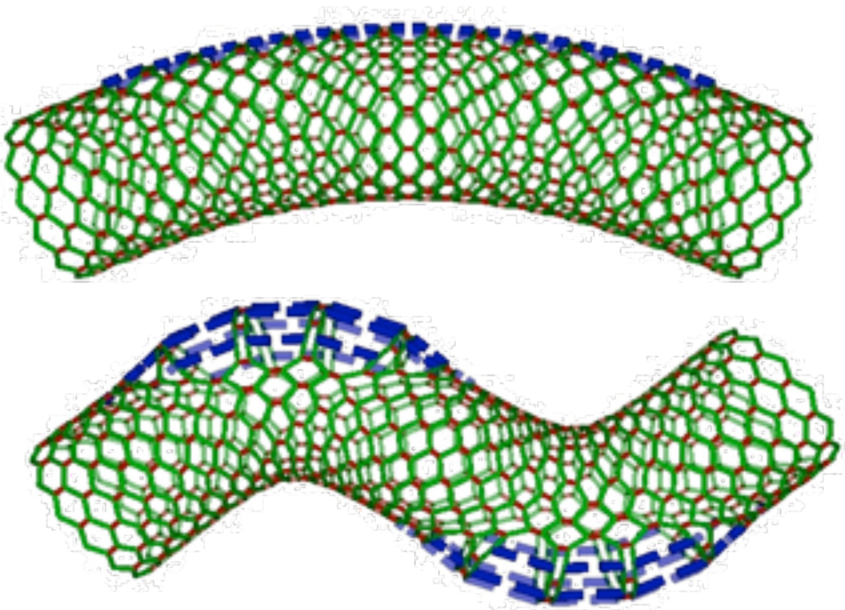
Normal modes of cell shape



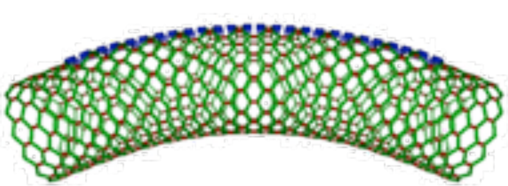
- Single peptide defect couples strongly to the bend mode

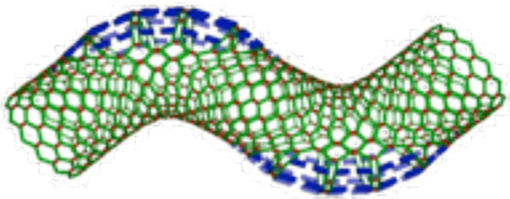
- Single glycan defect couples to both twist and bend modes

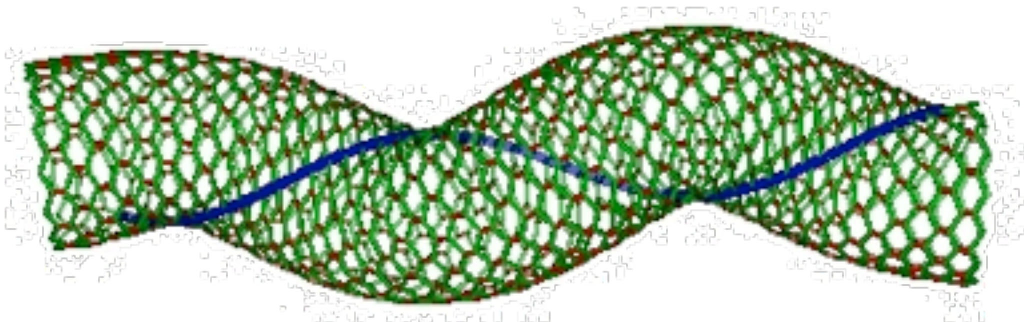
Common cell shapes via spatial patterning of defects

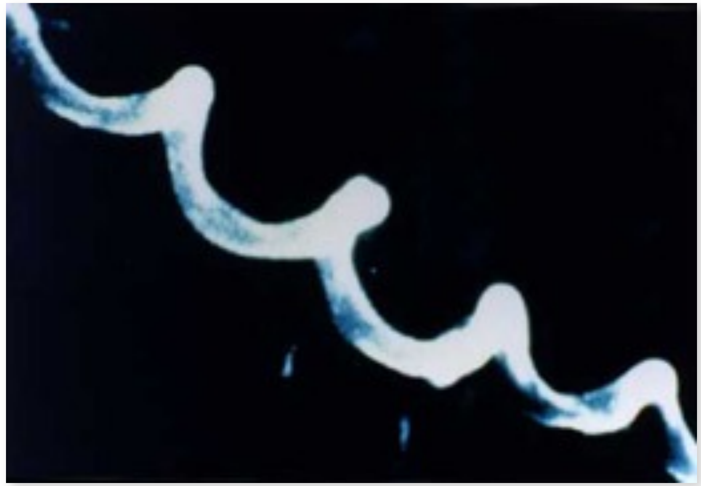


Caulobacter crescentus









Diane Caporale

Spirochaetes

Cell

Peptidoglycan Crosslinking Relaxation Promotes *Helicobacter pylori*'s Helical Shape and Stomach Colonization

Laura K. Sycuro,[1,3] Zachary Pincus,[4] Kimberley D. Gutierrez,[2] Jacob Biboy,[5] Chelsea A. Stern,[2,3] Waldemar Vollmer,[5] and Nina R. Salama[2,3,*]
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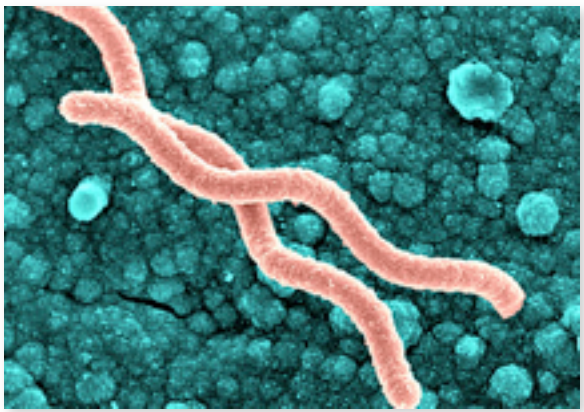
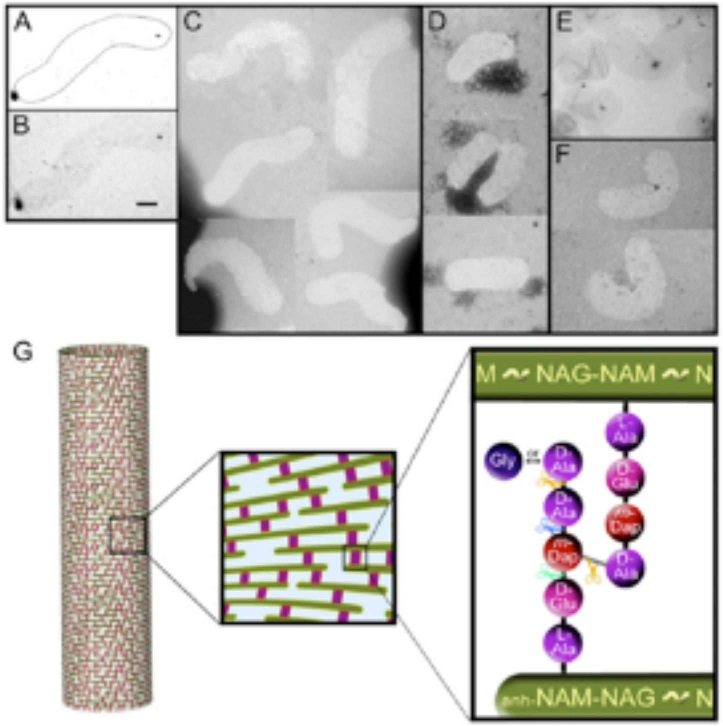
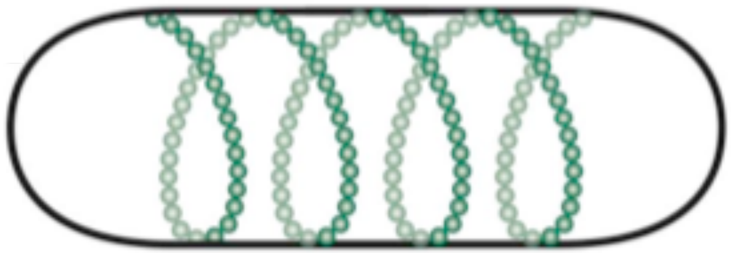
[5]Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, New
*Correspondence: nsalama@fhcrc.org
DOI 10.1016/j.cell.2010.03.046



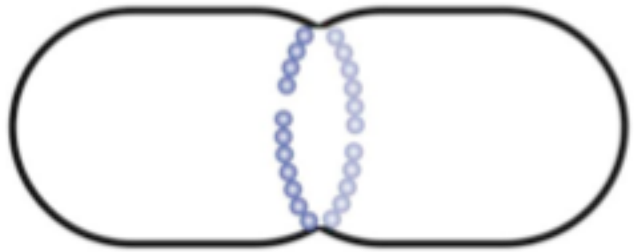


Hypothesis:
Spatial patterning of new insertion affects shapes of growing bacteria

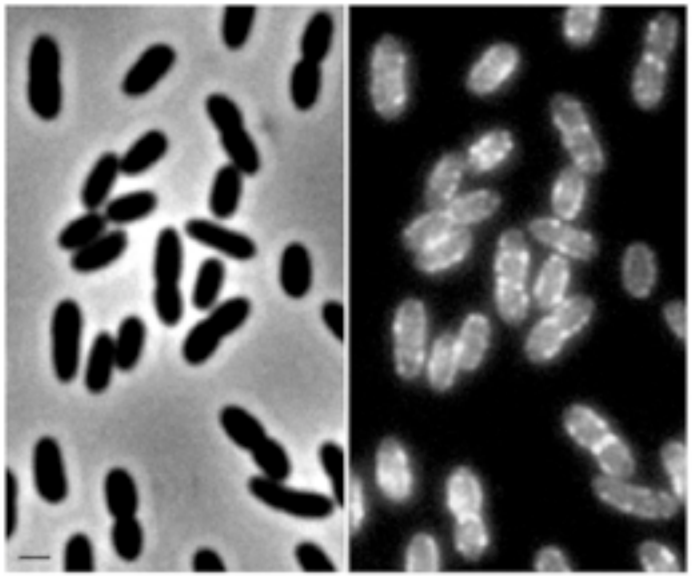
Modes of growth



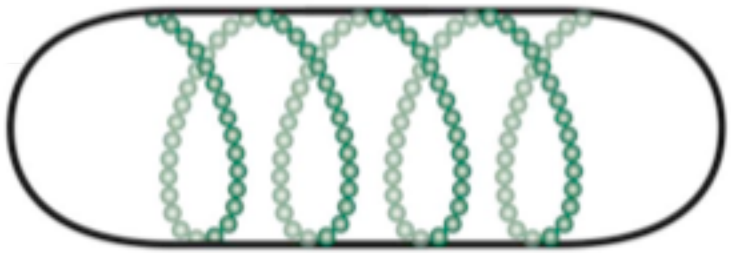
Elongation (PBP2)



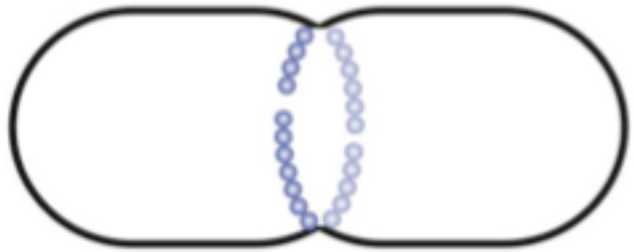
Division (PBP3)



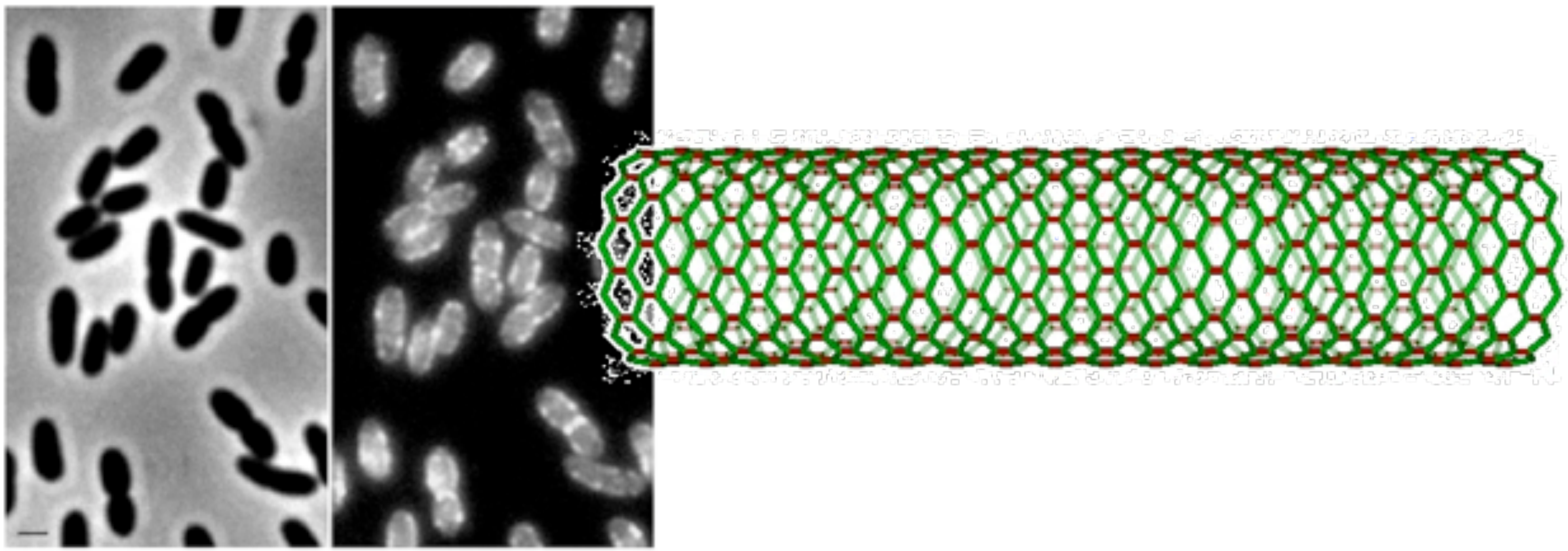
Modes of growth



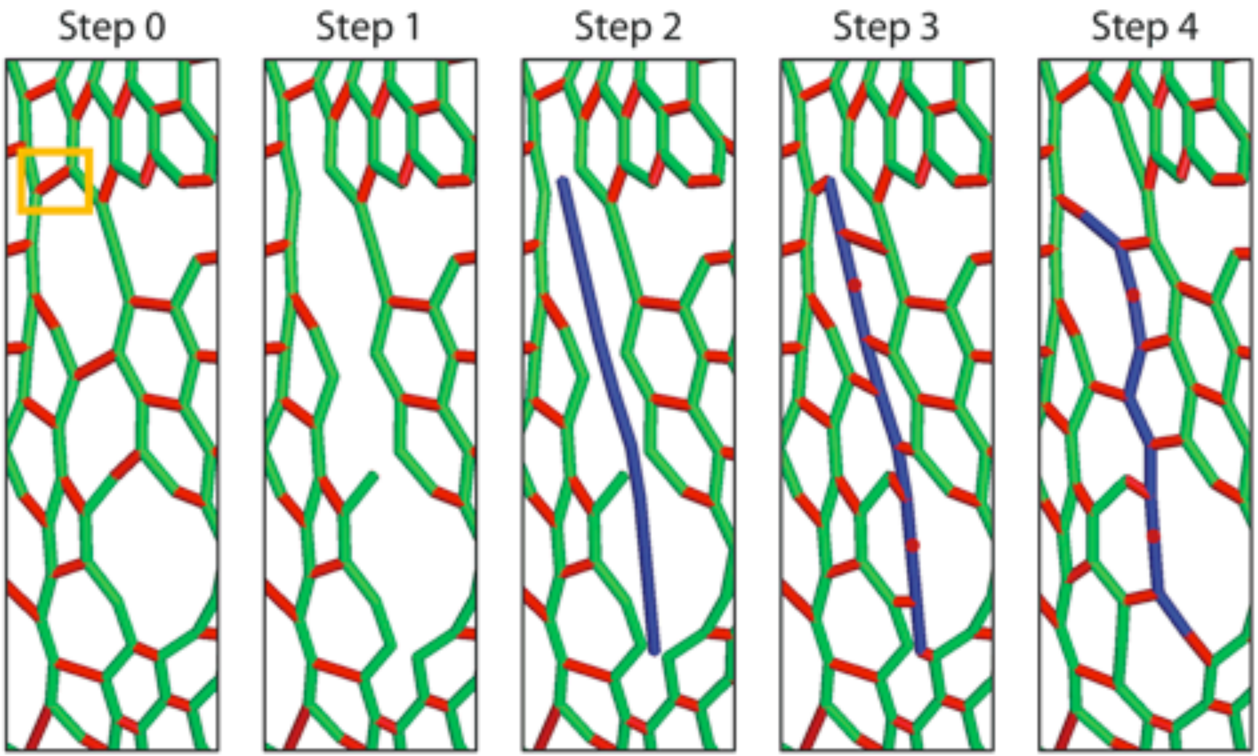
Elongation (PBP2)



Division (PBP3)

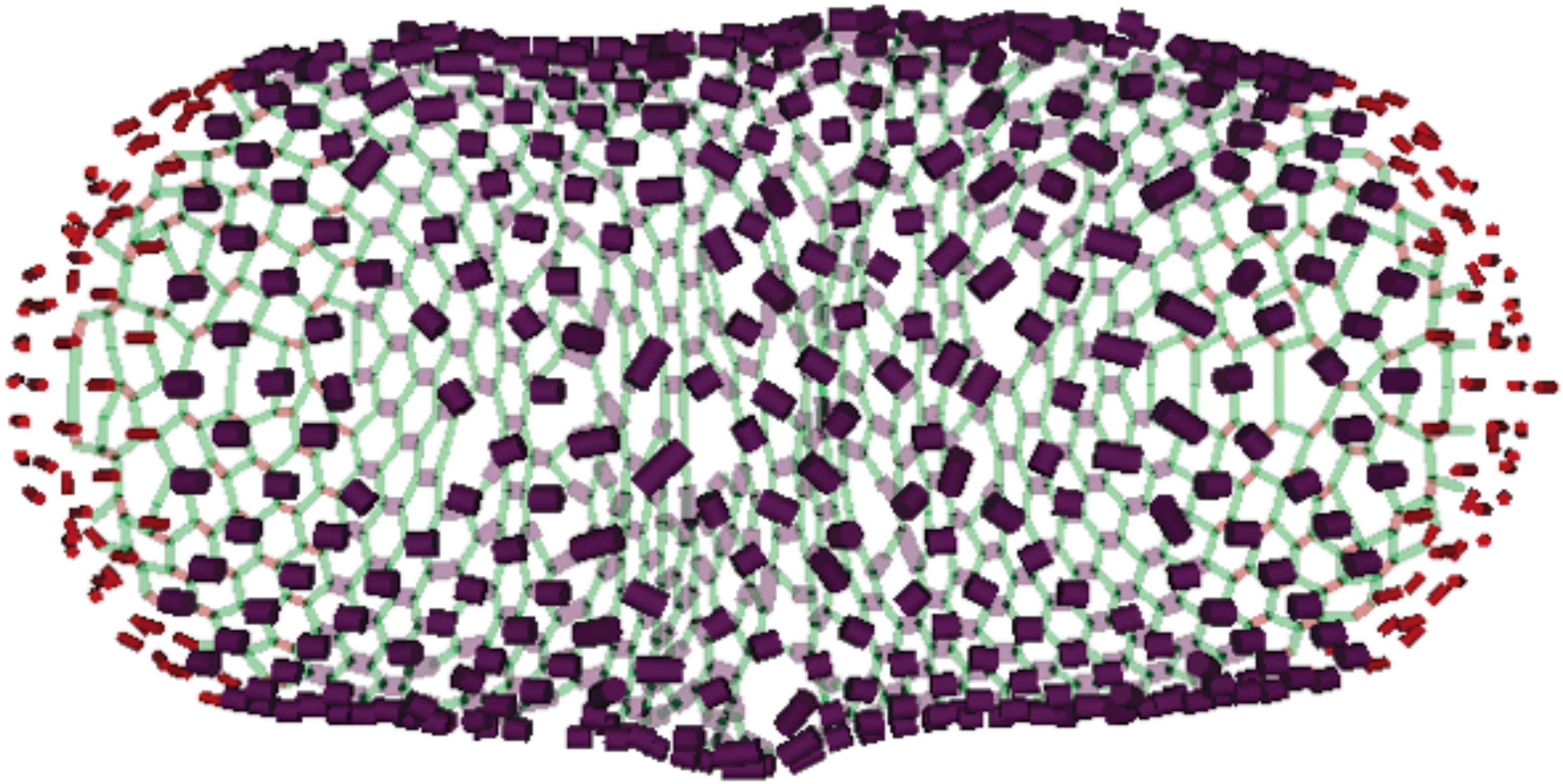


Insertion of new material

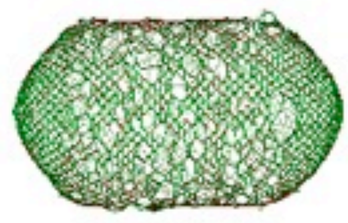


Vary spatial, biochemical, and mechanical characteristics

Random insertion



Random strand insertion

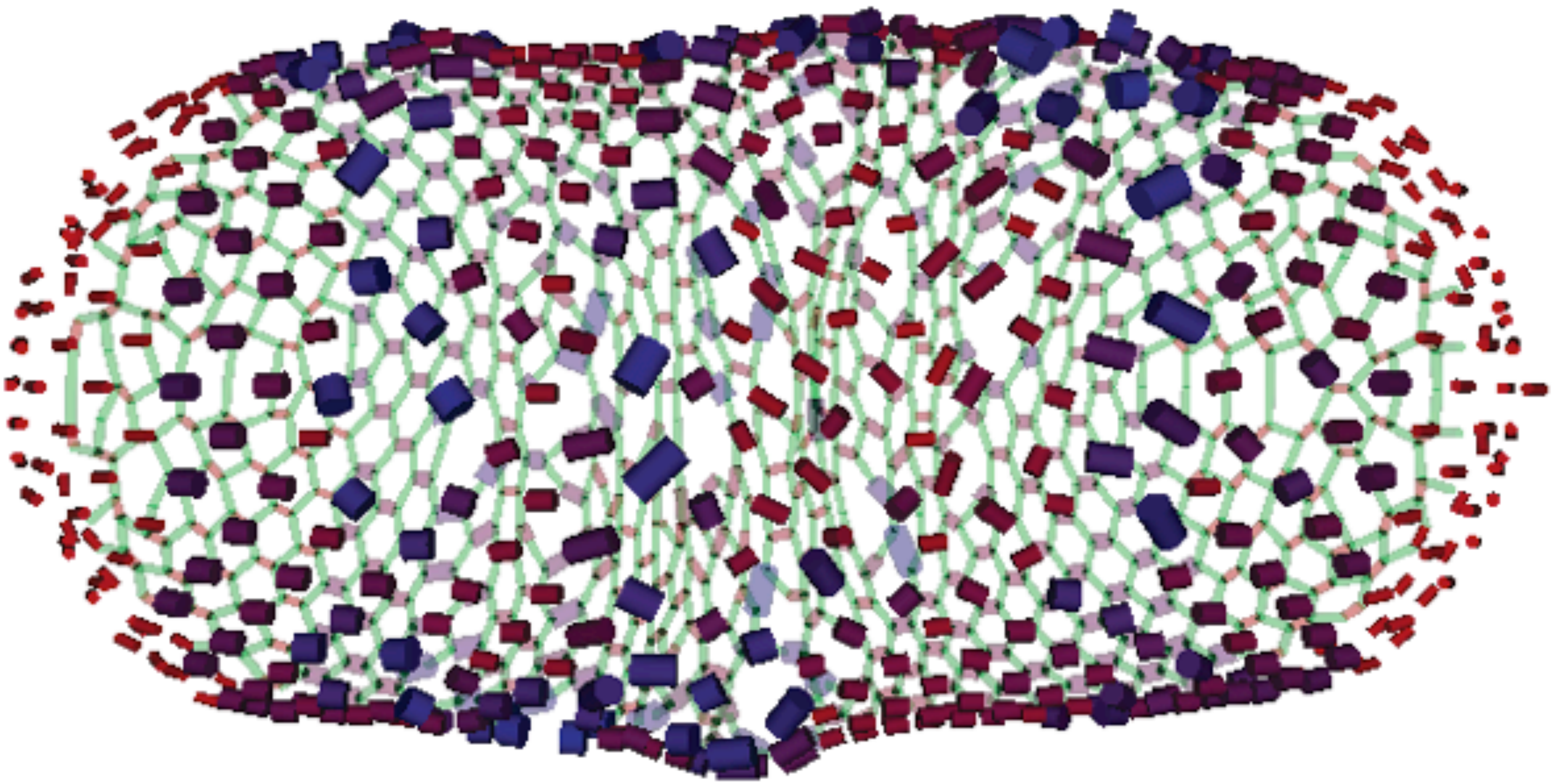


6X growth

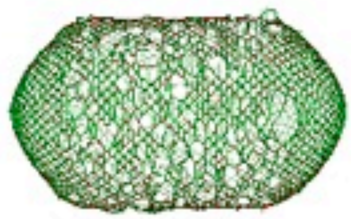
Decouple wall density and insertion

Decouple wall density and insertion

Decouple wall density and insertion



Uniform density insertion preserves cell shape



What biological factor could be responsible?

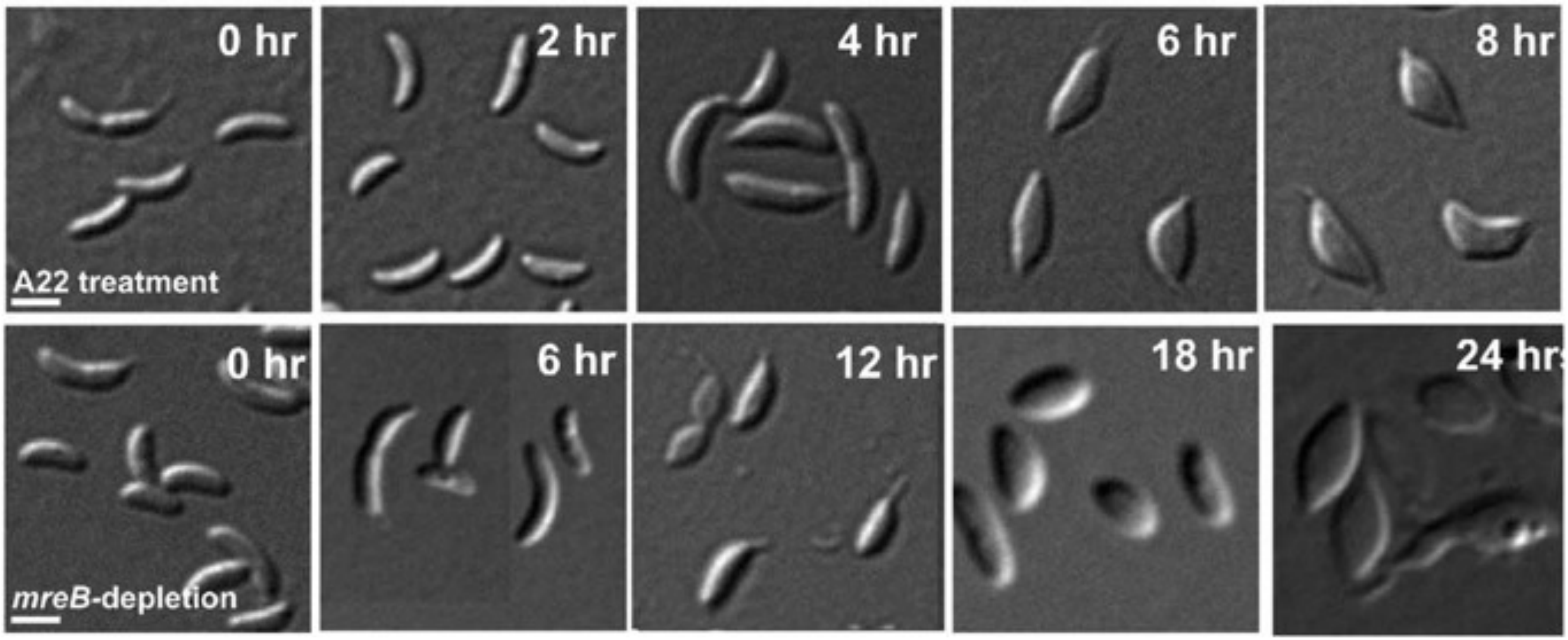
- Patterns cell-wall growth
- Insensitive to fluctuations in cell shape
- Deletion affects cell shape
- Conserved across bacteria

Cell growth without MreB

- Bacterial actin homolog
- Found in rod-shaped bacteria
- ATP binding, inhibited by A22

Cell growth without MreB

- Bacterial actin homolog
- Found in rod-shaped bacteria
- ATP binding, inhibited by A22

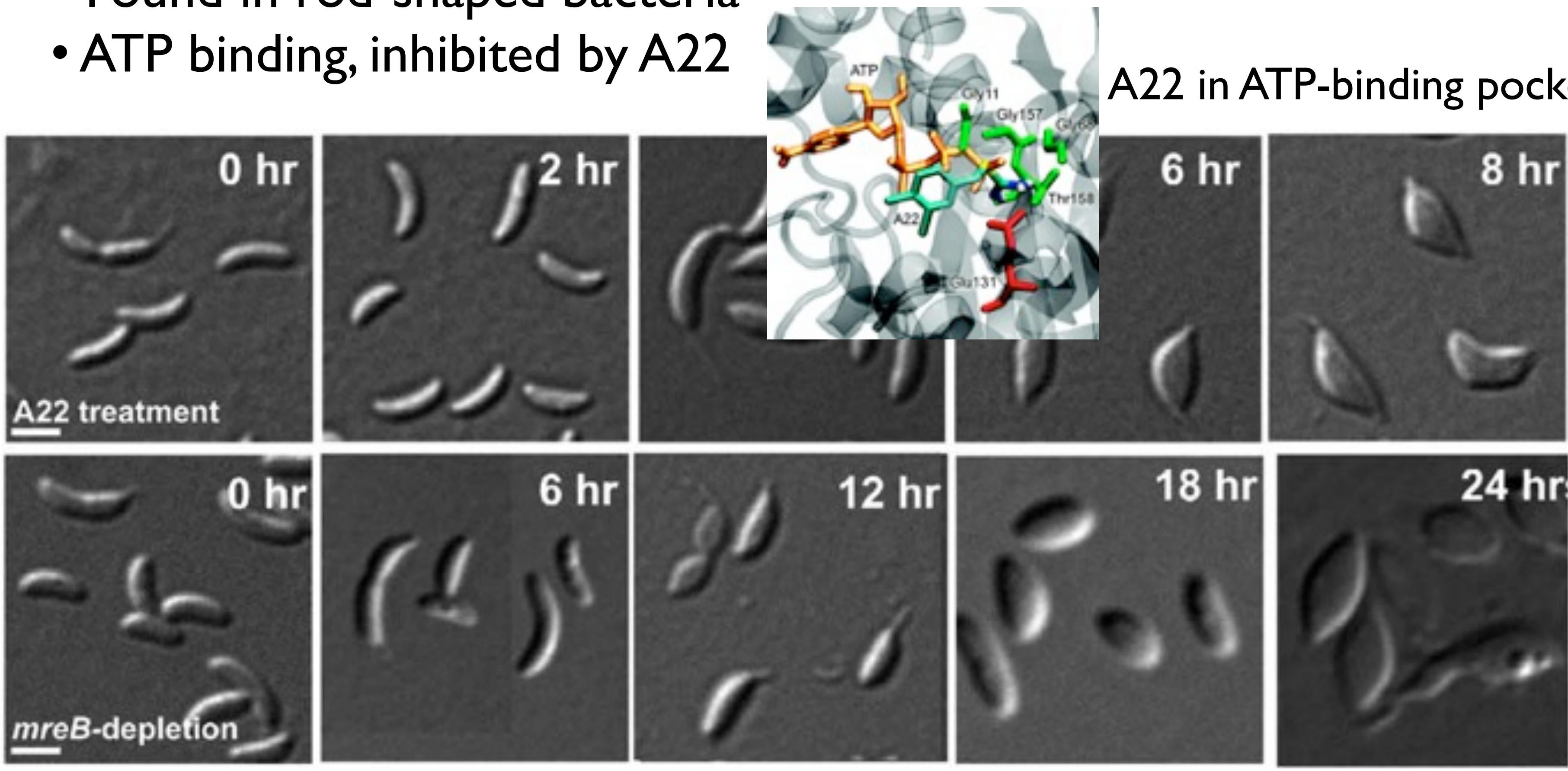


Gitai et al. (2005) Cell 120:329.

Cell growth without MreB

- Bacterial actin homolog
- Found in rod-shaped bacteria
- ATP binding, inhibited by A22

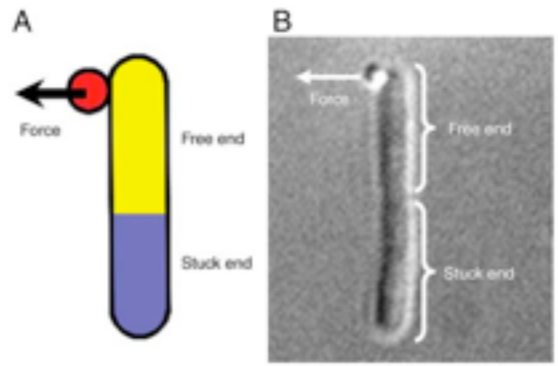
A22 in ATP-binding pocket

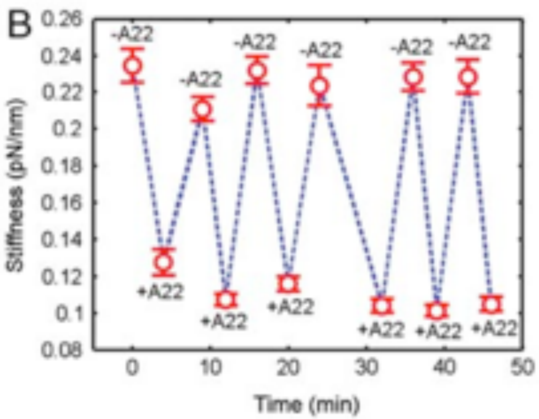


Gitai et al. (2005) Cell 120:329.

Role of MreB in rod-shaped growth

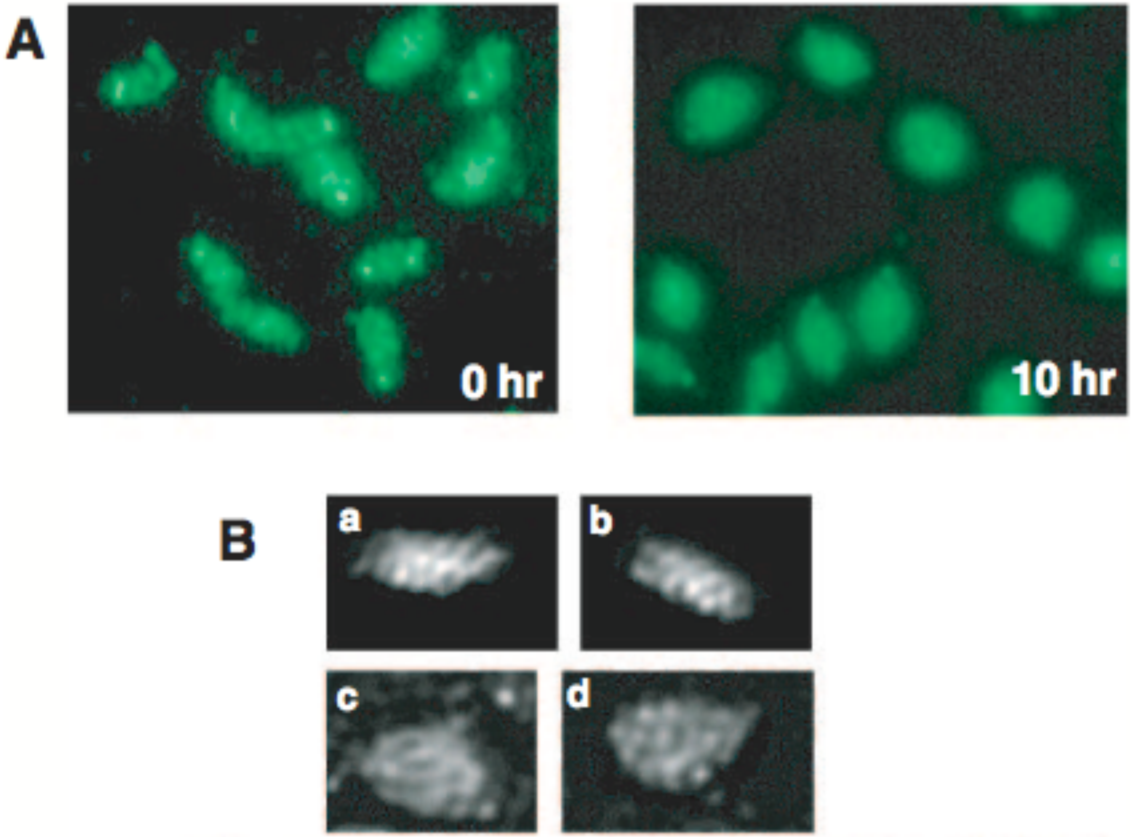
Stiffness is comparable to cell wall





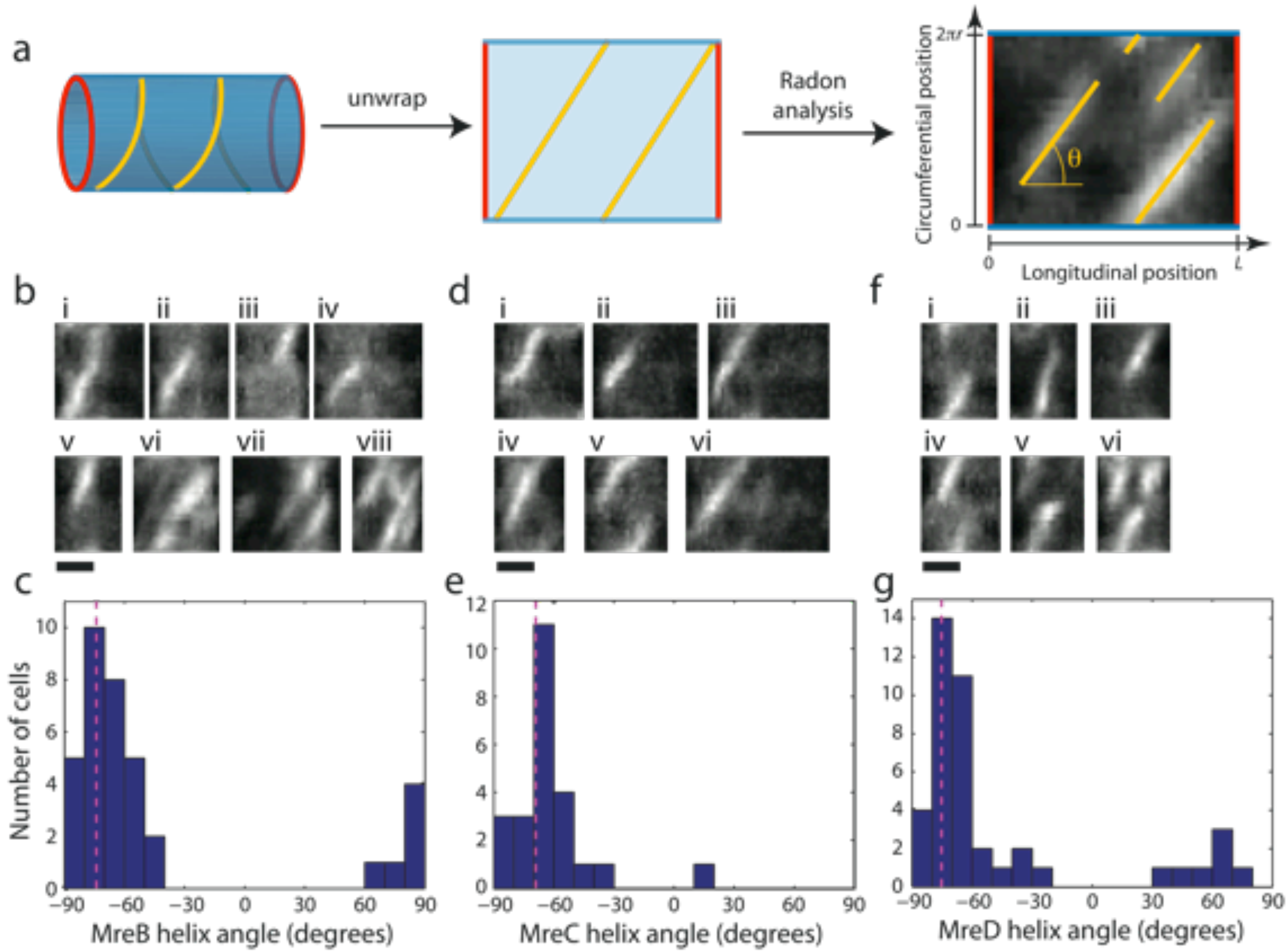
Wang et al. (2010) PNAS 107:9182.

MreB controls PBP2 localization

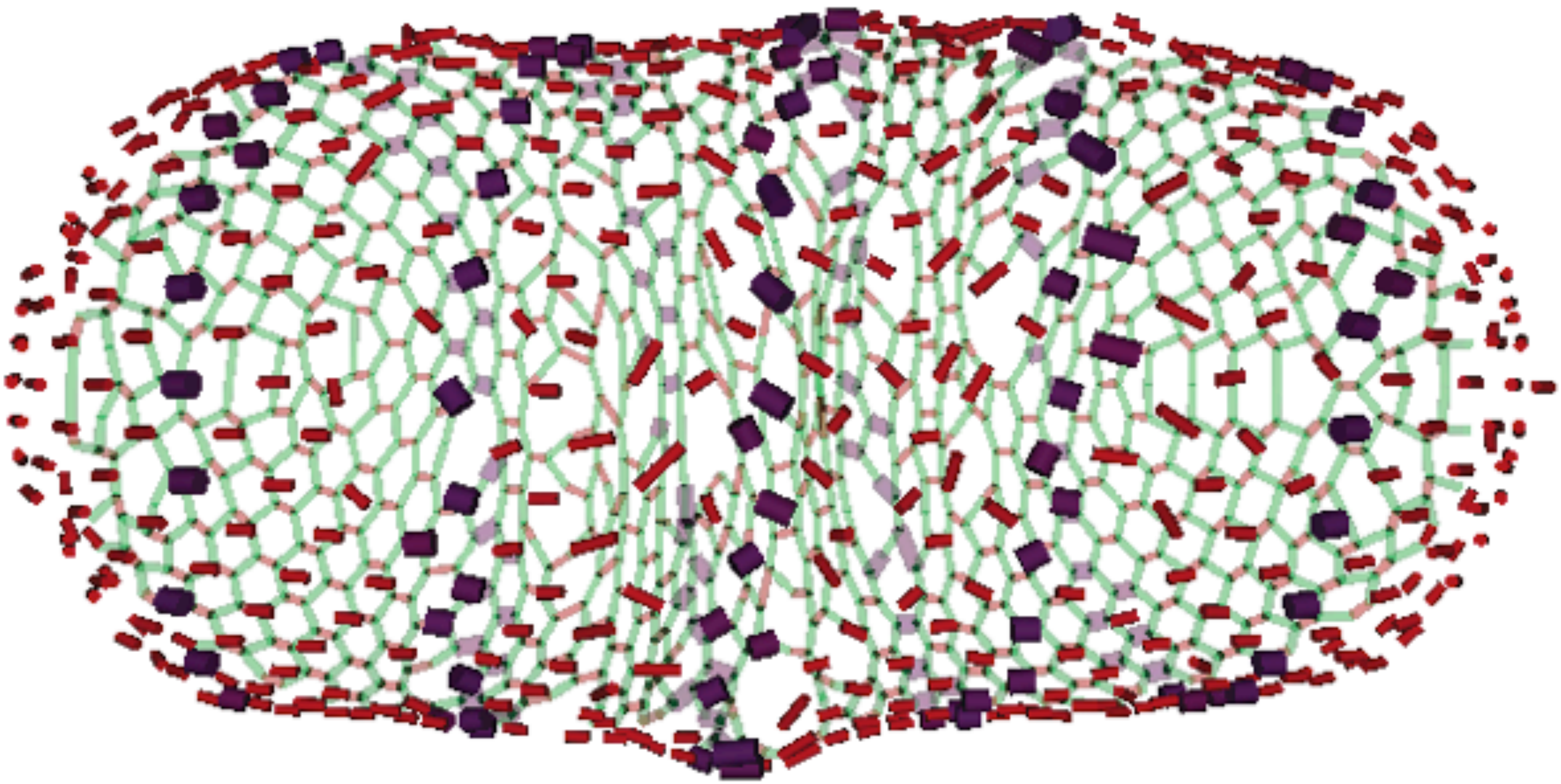


Figge *MolMicro* (2004)

MreB is left-handed!



Helically patterned insertion

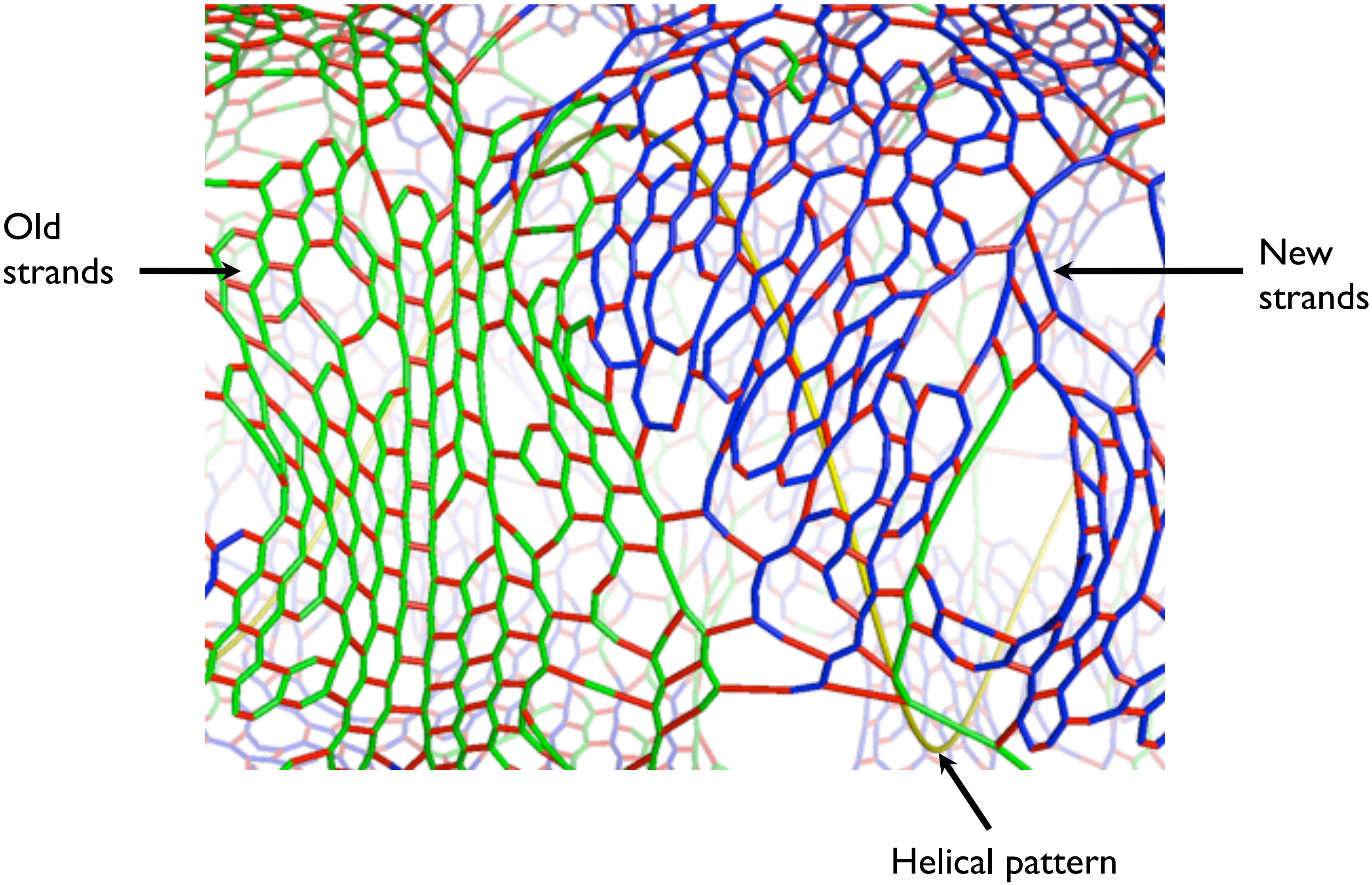


Helical-insertion growth preserves shape

6X growth

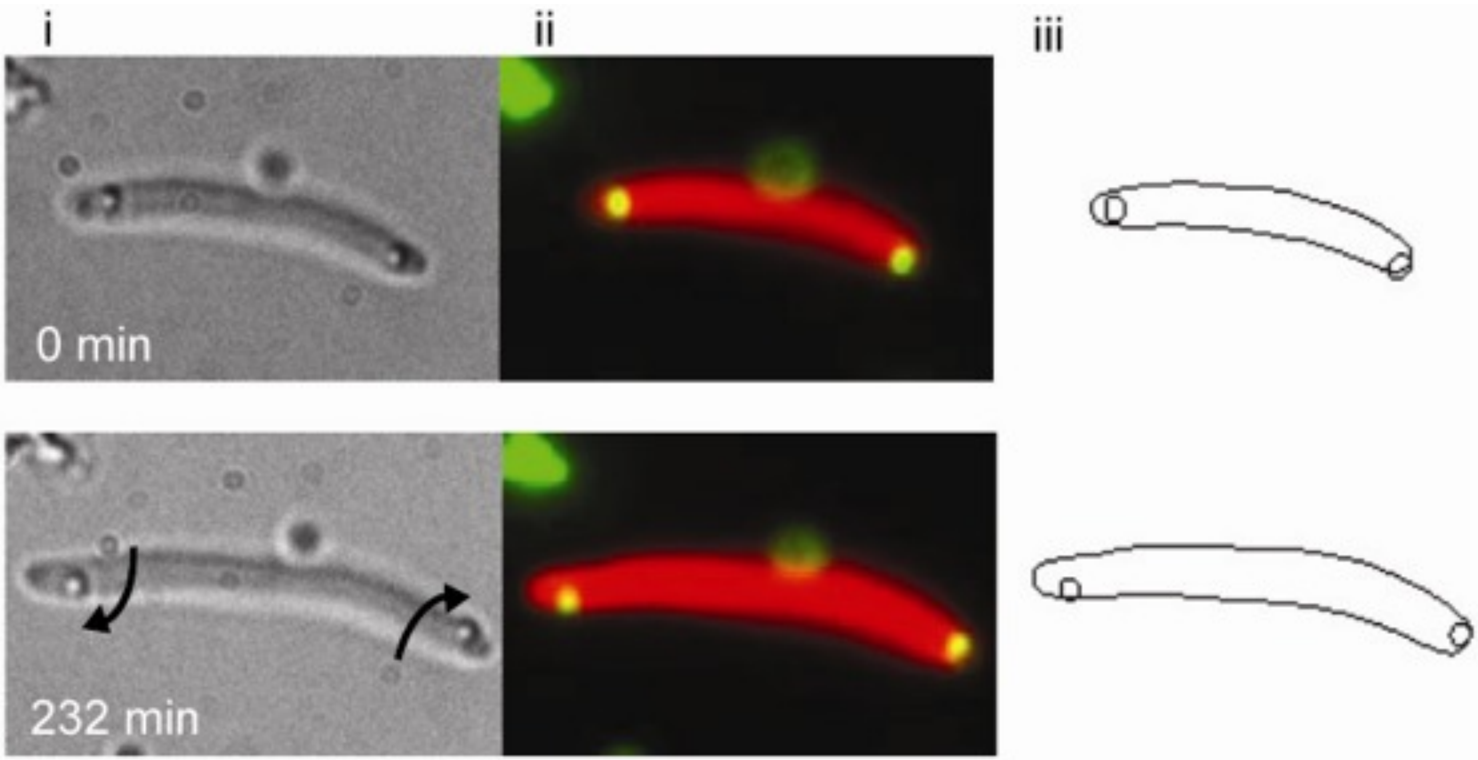
What is the effect of helical insertion?

New strand reorientation



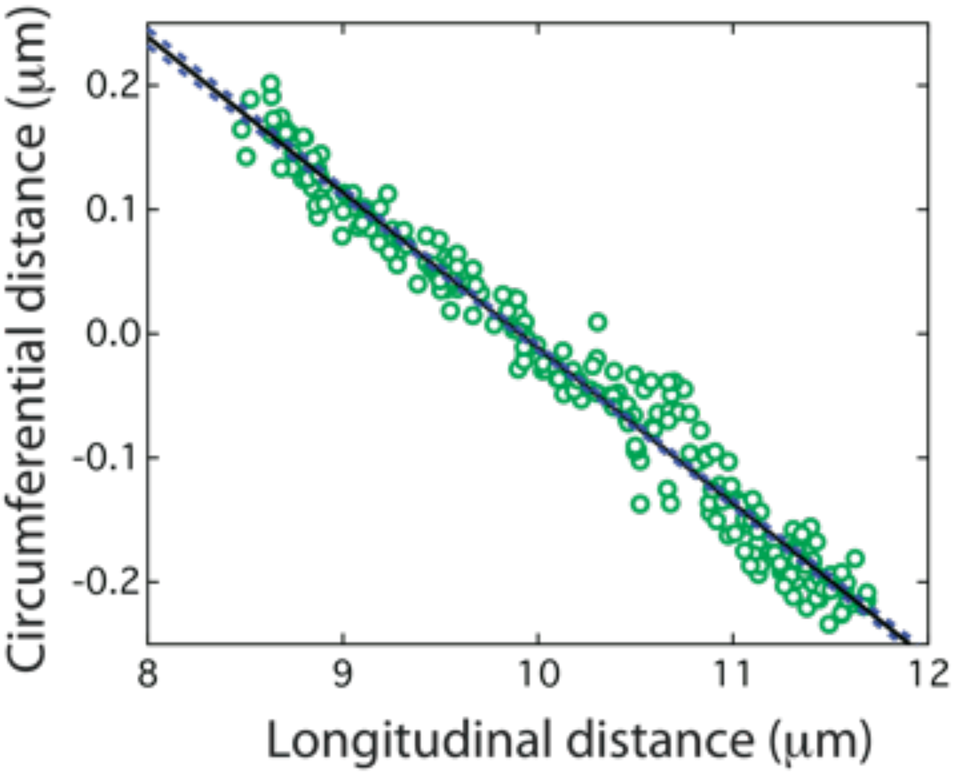
Cell wall rotation

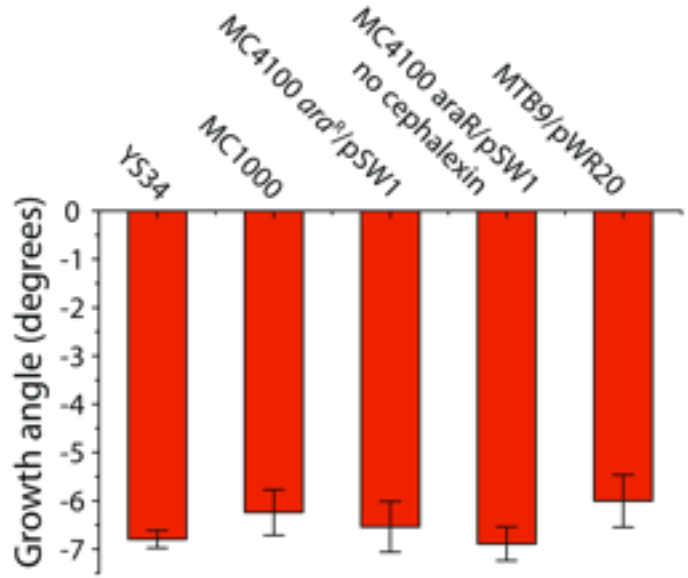
Left-handed rotation during growth



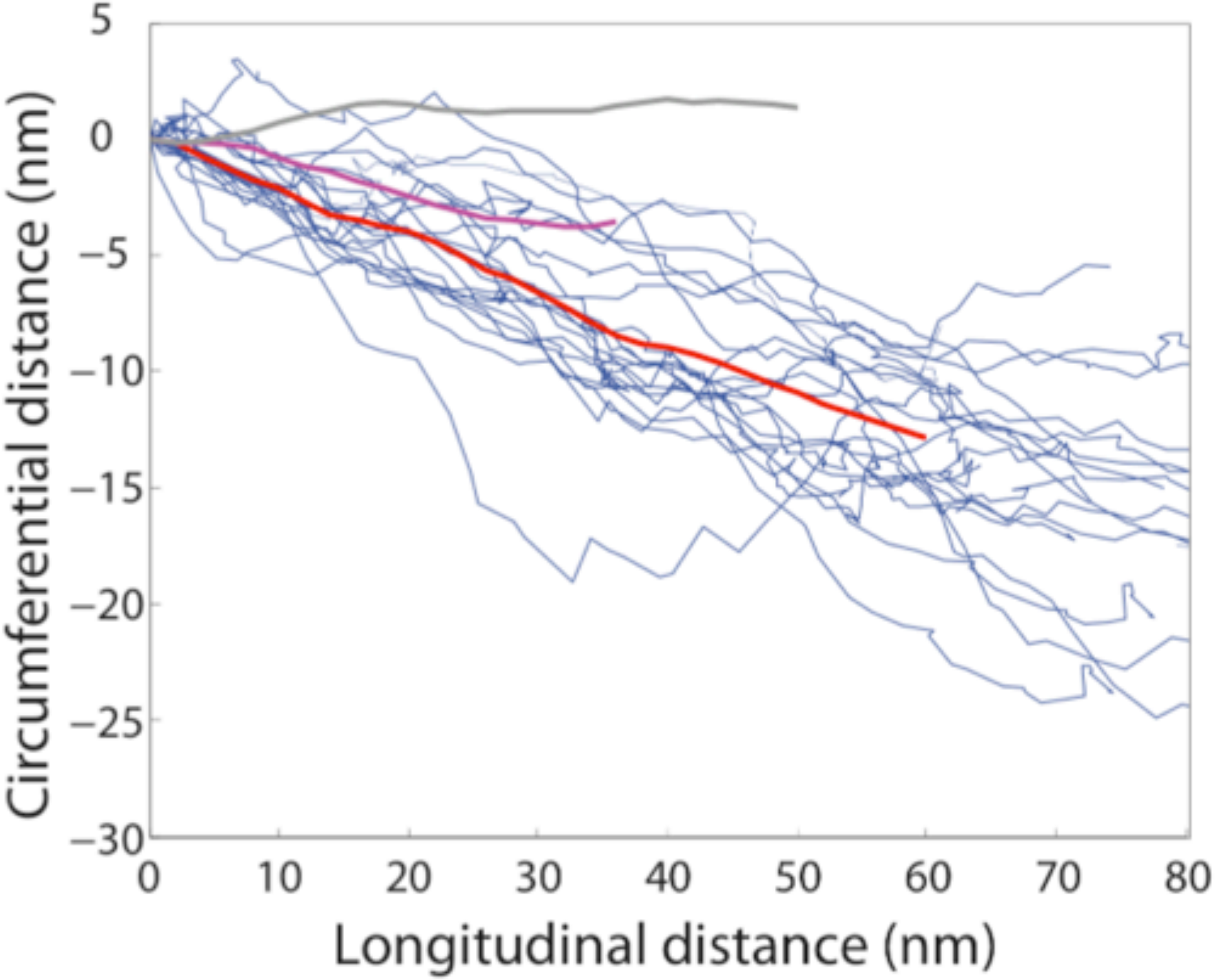
(Steven Wang and Josh Shaevitz, Princeton)

Rotation is always left-handed!

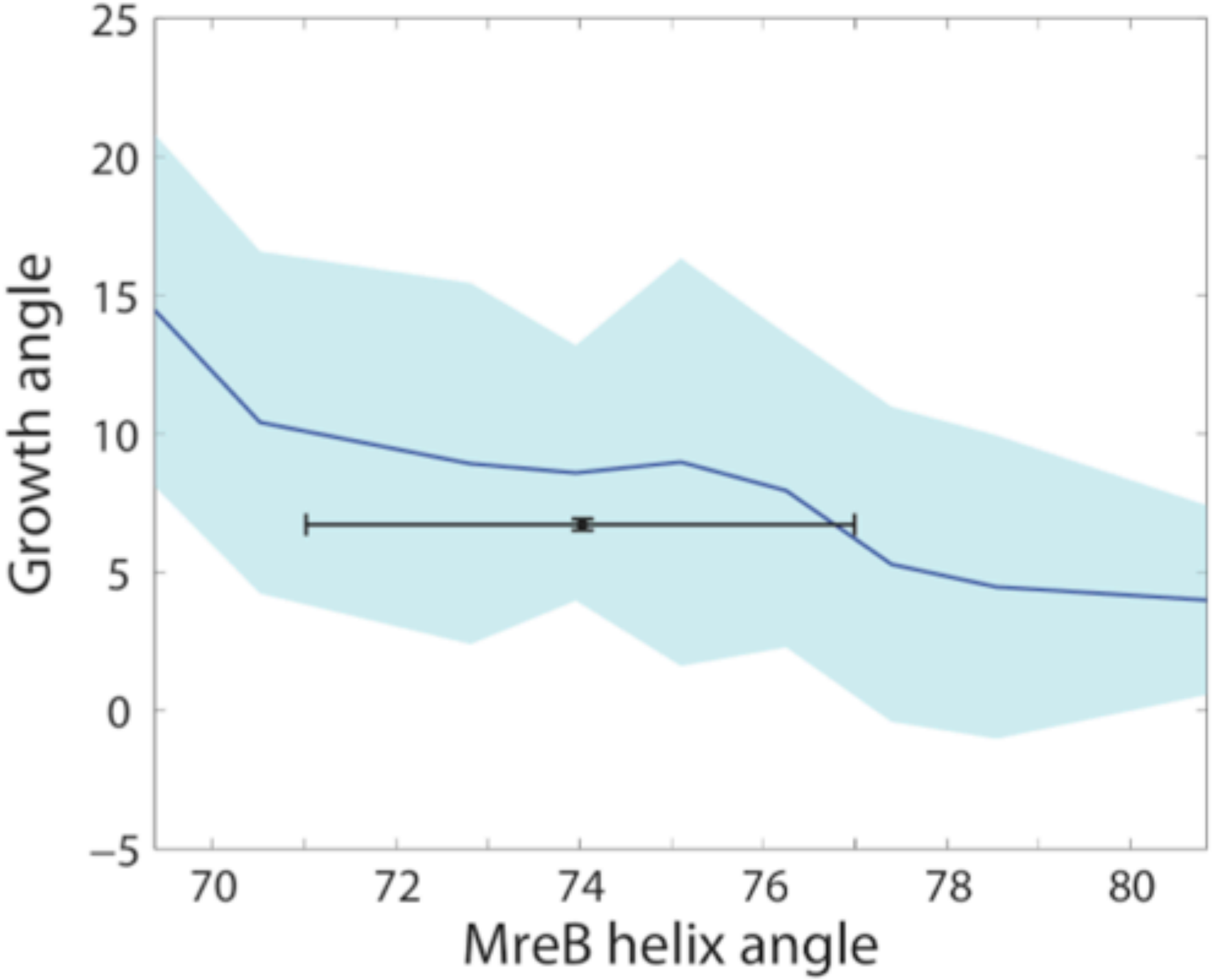




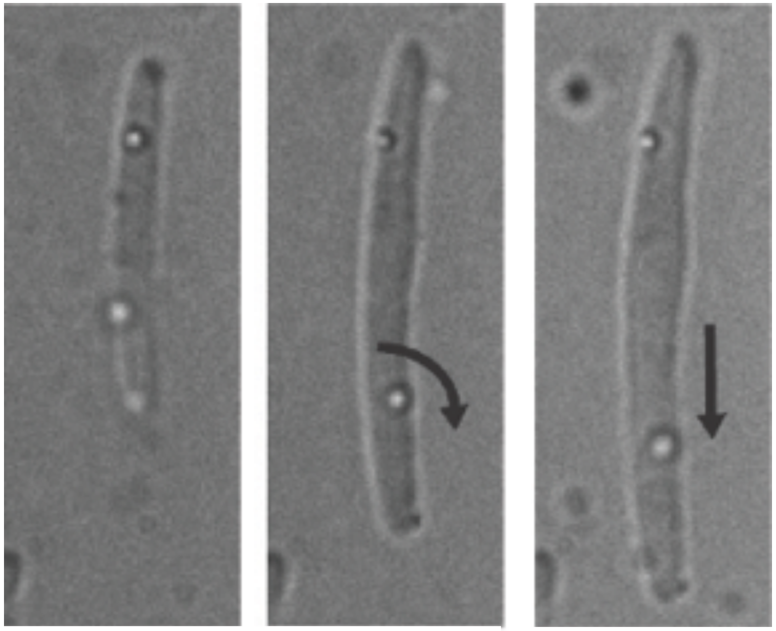
Bead twisting



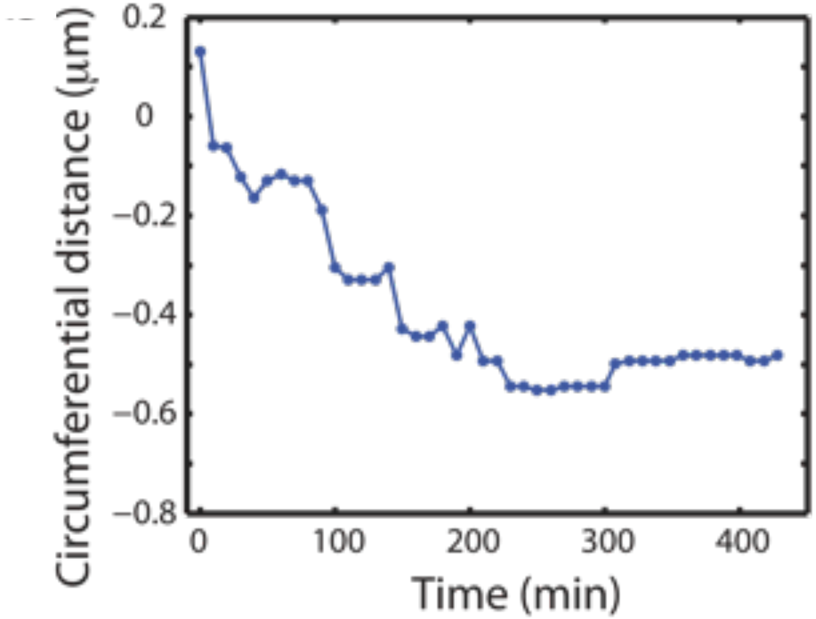
Helix angle vs. twist angle



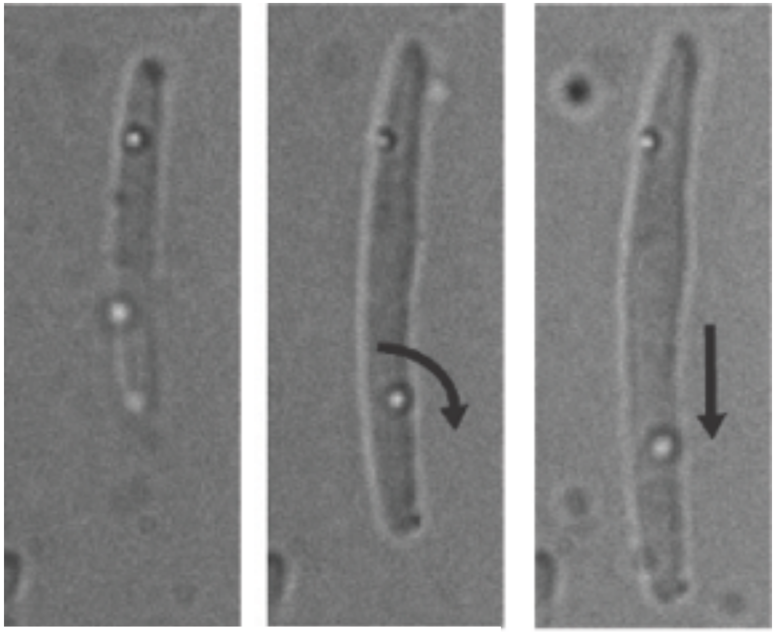
Rotation is MreB-dependent



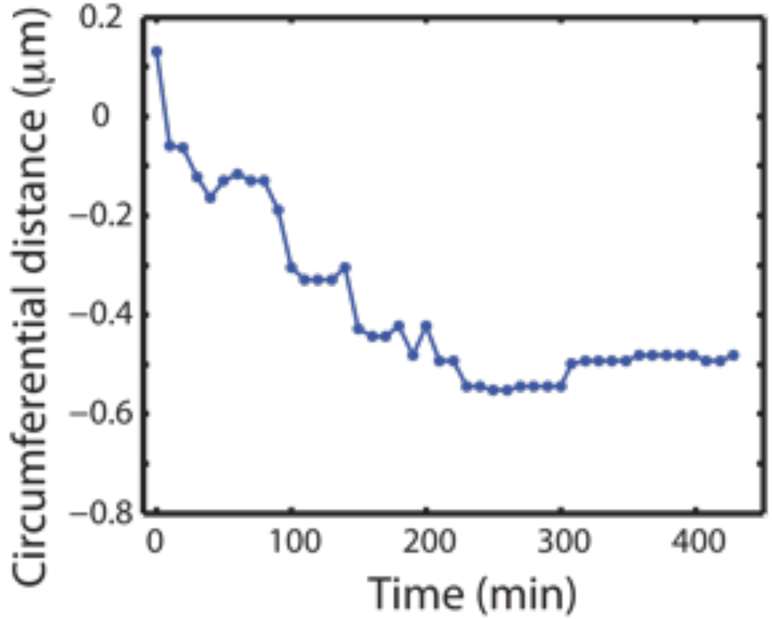
0 hours   5 hours   7 hours



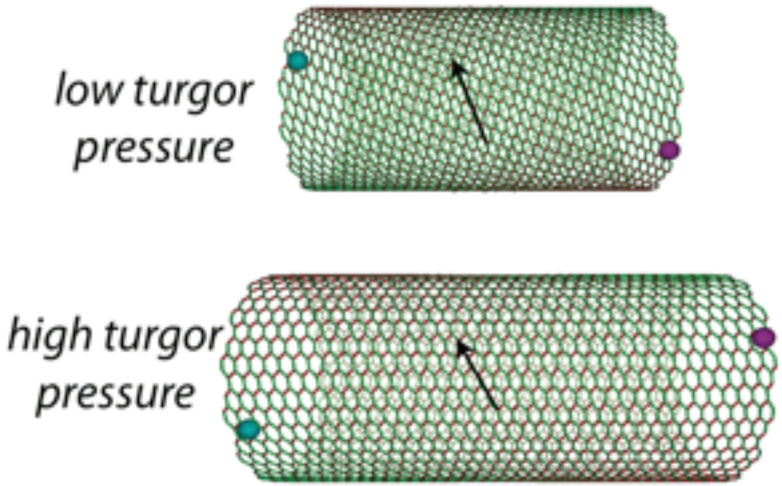
Rotation is MreB-dependent



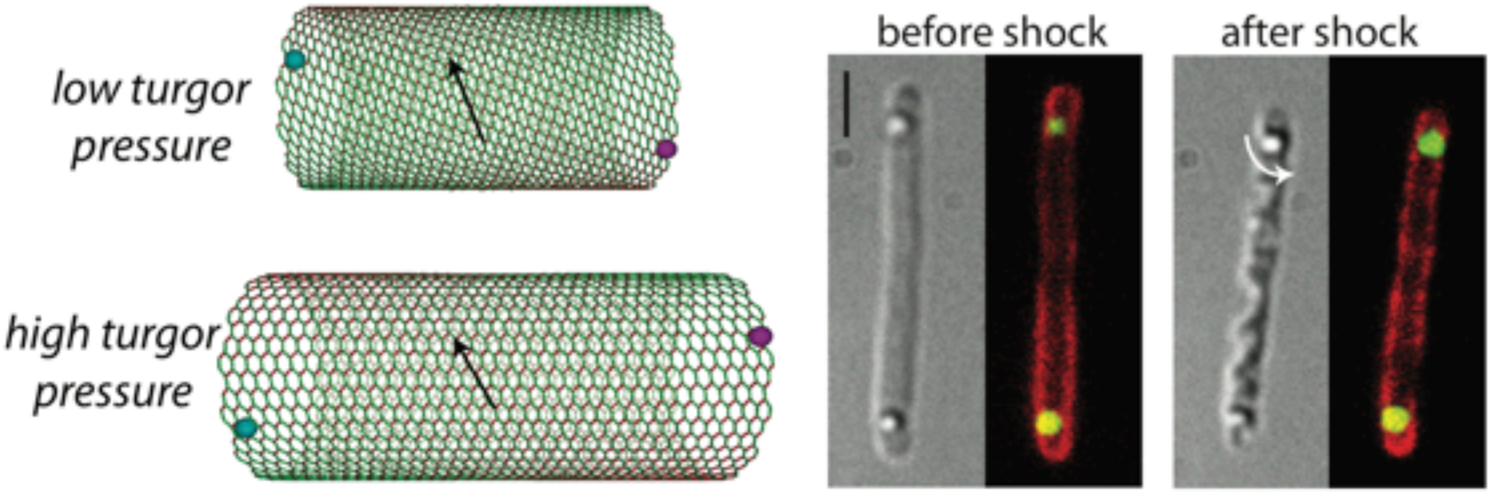
0 hours 5 hours 7 hours



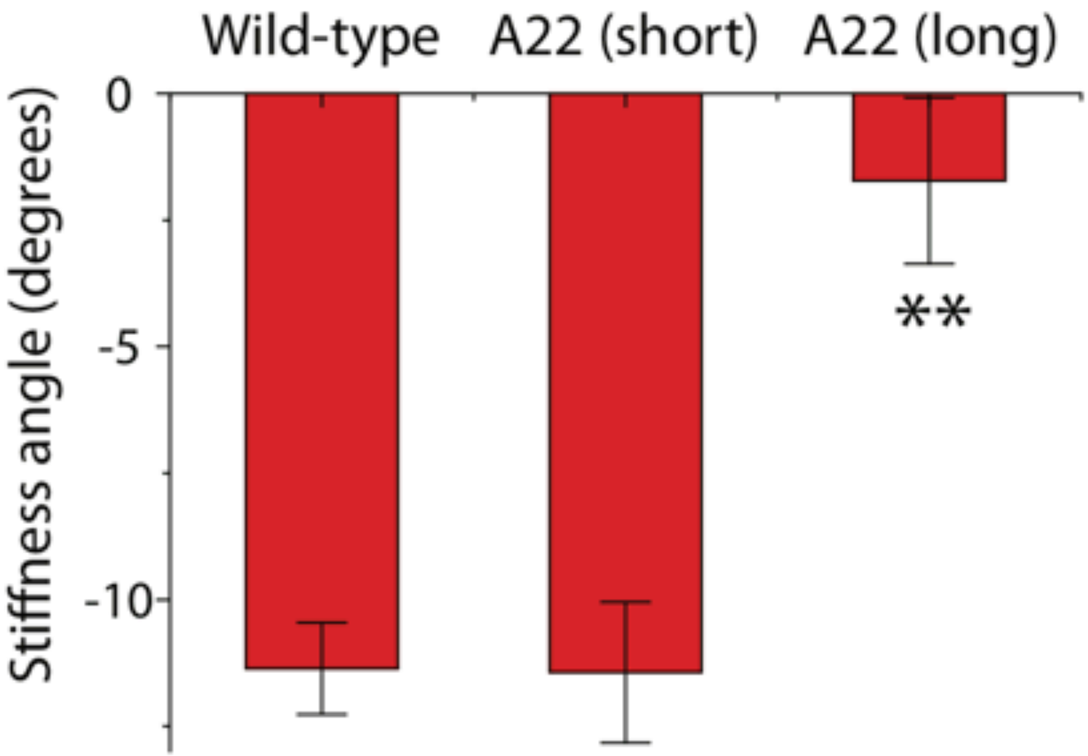
Osmotic shock



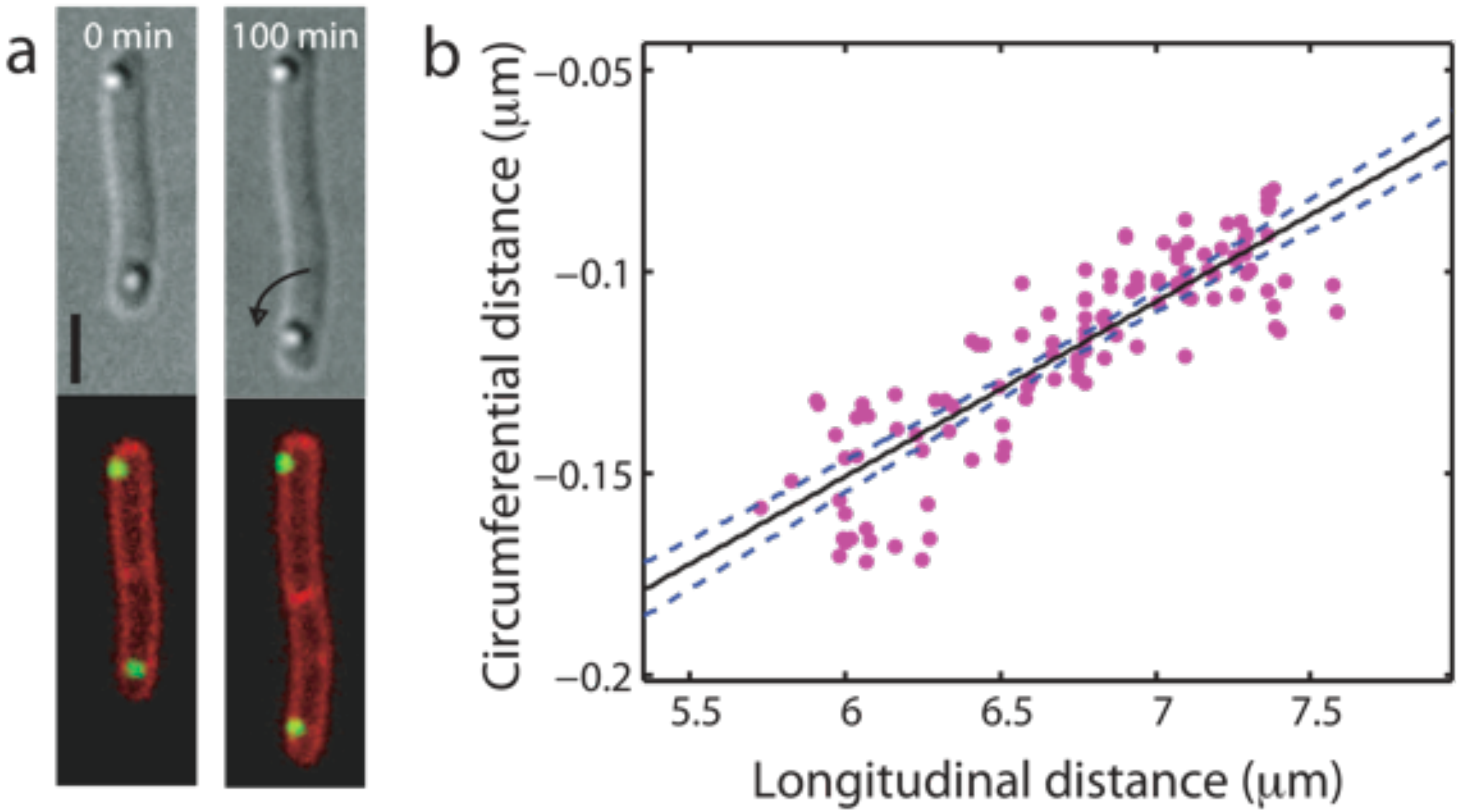
Osmotic shock



Osmotic shock



Twist in other species



B. Subtilis (**right-handed cytoskeleton**)
18±6 degrees/μm right-handed

Can we design the shape of bacteria?

Conclusions

- Cell shape maintenance relies on a combination of spatial patterning, biochemical regulation, and mechanical force
- Modeling can be used to interpret molecular mechanisms underlying cell growth
- Predictions about mechanisms for altering cellular dimensions

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