The molecular control and evolution of bacterial shape

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Overview

Introduction to bacteria

Spatial organization of intracellular components

Part i

Molecular control of cell shape in bacteria

Part ii (brief)

Evolution of bacterial cell shape

Part iii (brief)

Shape vs hydrodynamics at low Re

Conclusion and outlook

Overview

Introduction to bacteria

Spatial organization of intracellular components

Spatial organization: three fundamentals

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ii. How is organization established (mol. level)?

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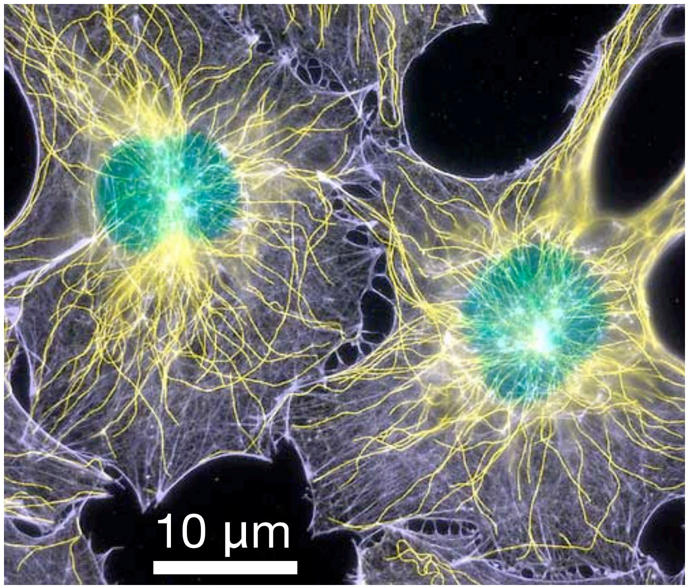
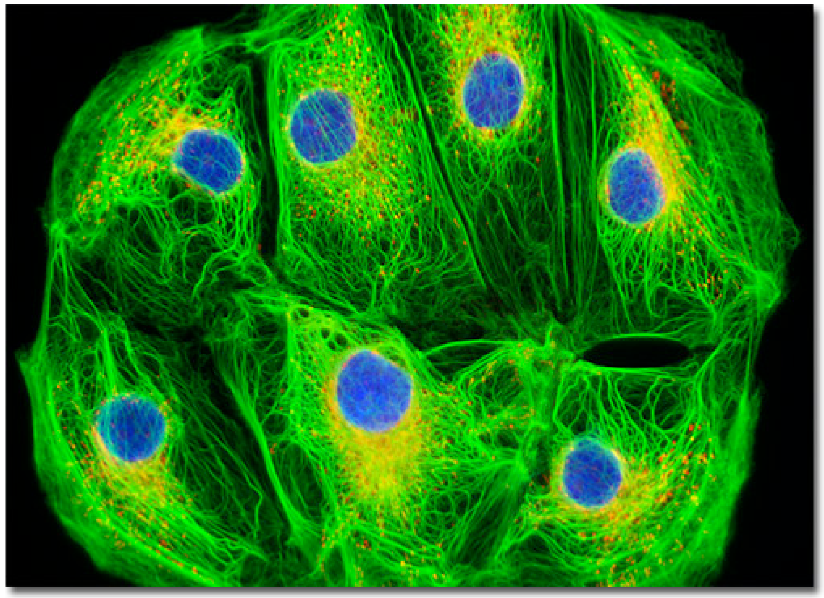


Torsten Wittman, UCSF



Nikon

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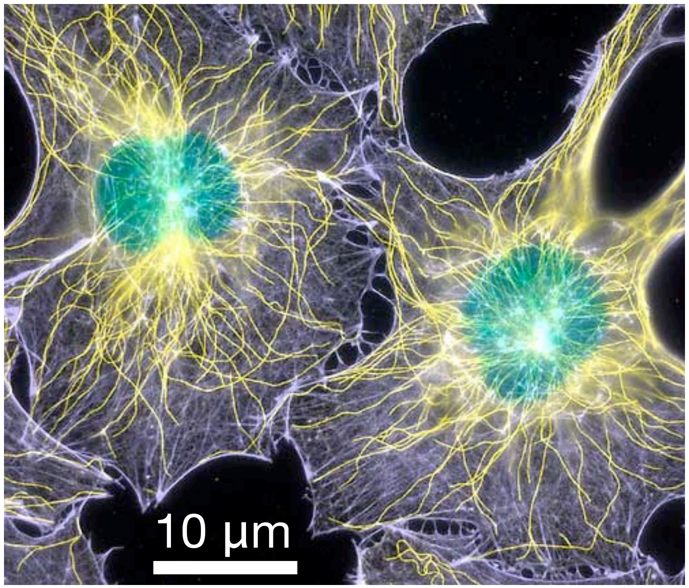

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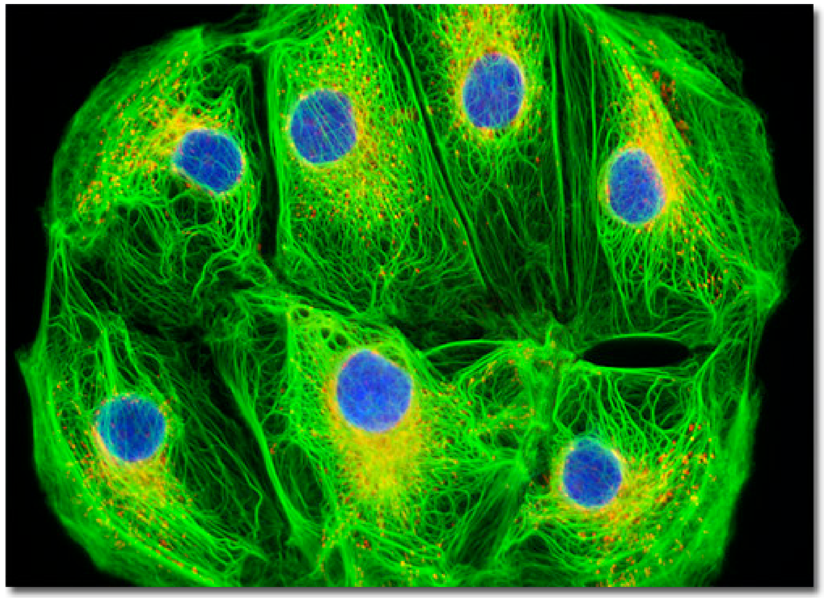

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Our understanding in eukaryotic cells is <u>emerging</u>

Spatial organization in bacteria

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Our understanding is just beginning to emerge

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Bacteria are small; tools are still not available

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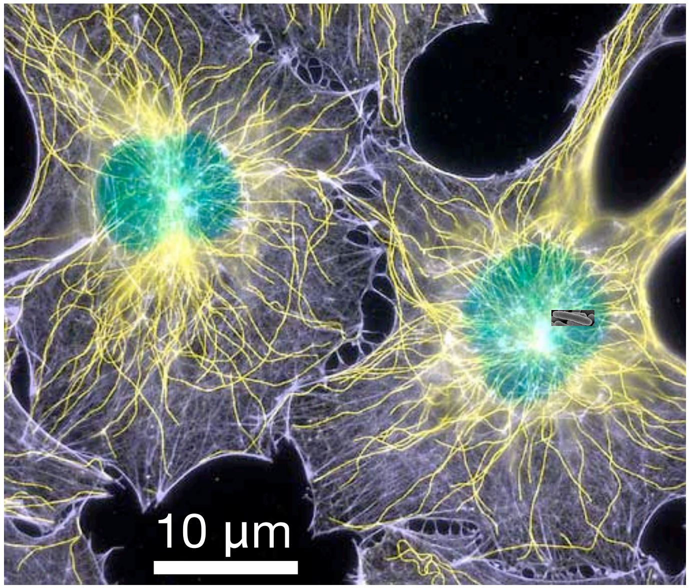

Swiss 3T3 fibroblast

Spatial organization in bacteria

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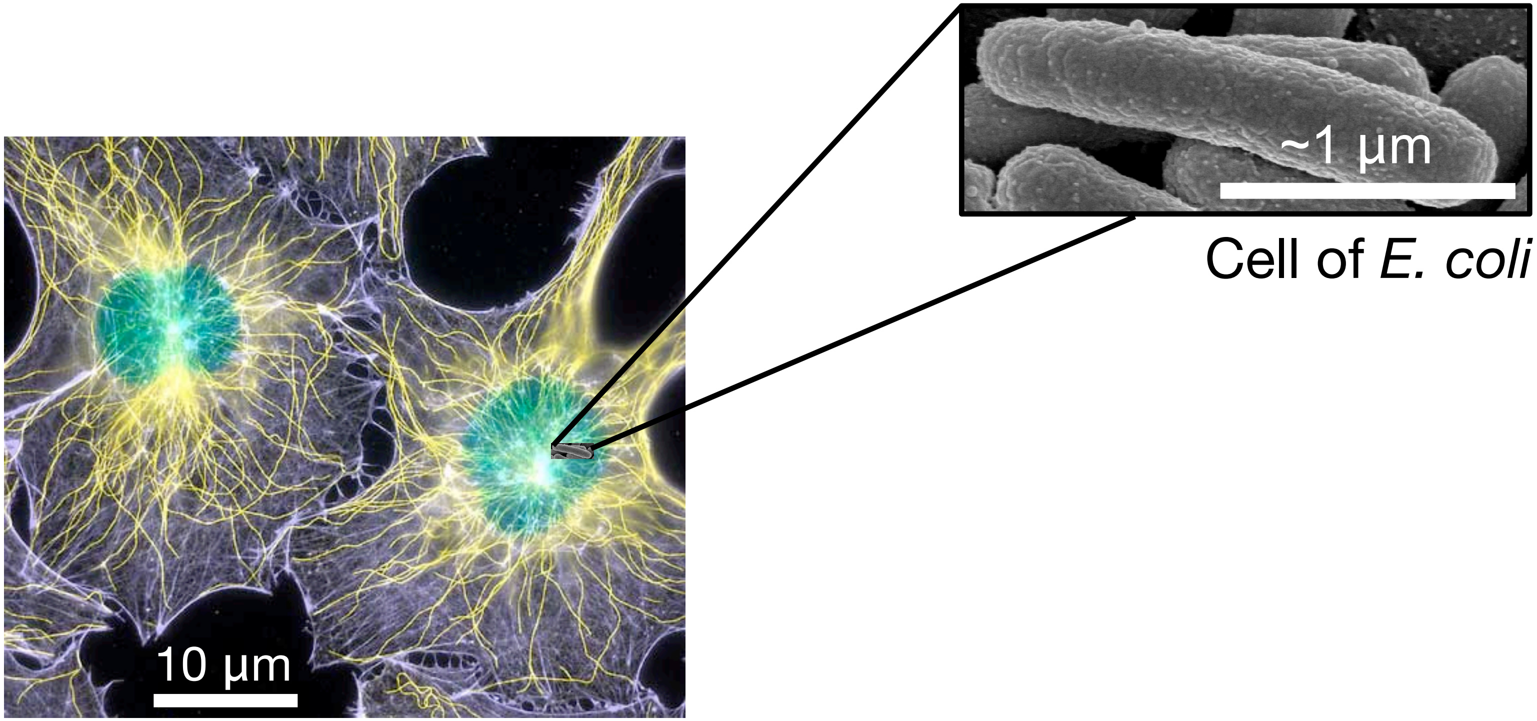

Cell of *E. coli*

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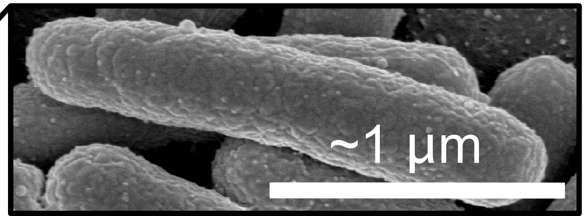

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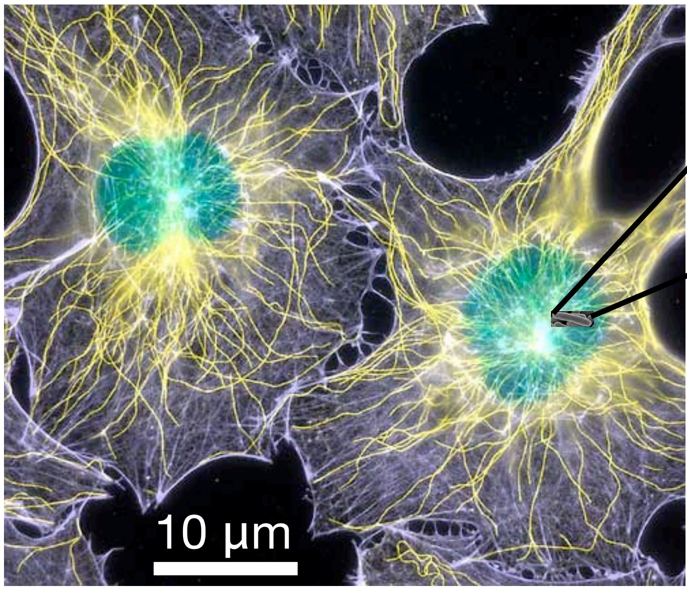


Swiss 3T3 fibroblast

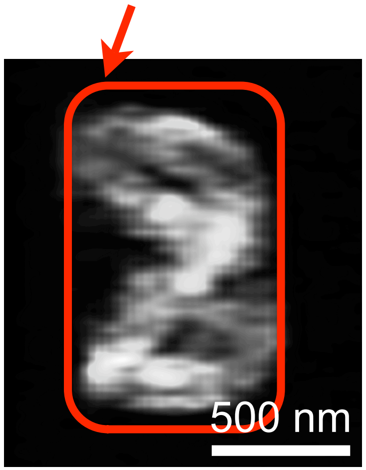
$$d = 0.61\ \lambda/N.A.$$

NA_{max} (100x)=1.49

λ_{min}=365 nm

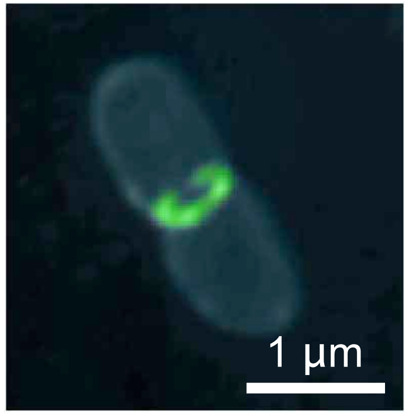
d_{max}>150 nm

Intracellular organization in bacteria

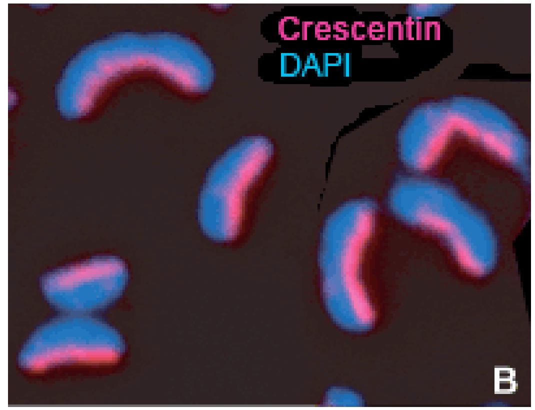


YFP-MreB chimera expressed in *E. coli* (Weibel)

ParM chimera in vitro (Mullins and Weibel)



GFP-FtsZ chimera expressed in *E. coli* (Beckwith)



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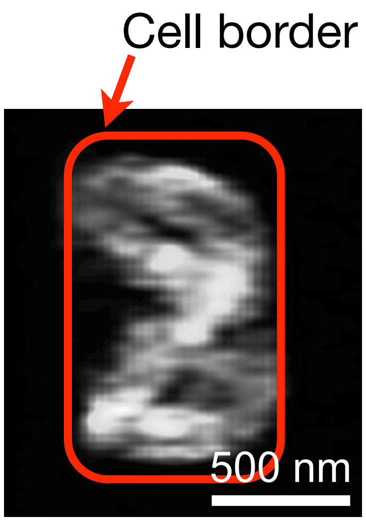

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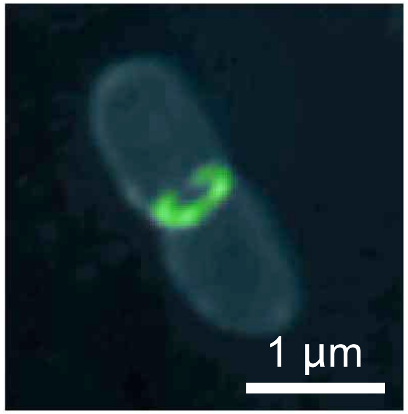

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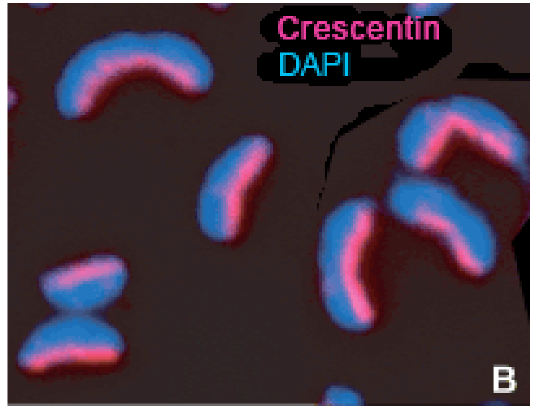

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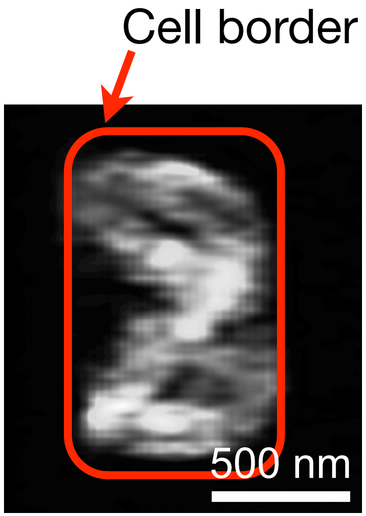
Intracellular organization in bacteria



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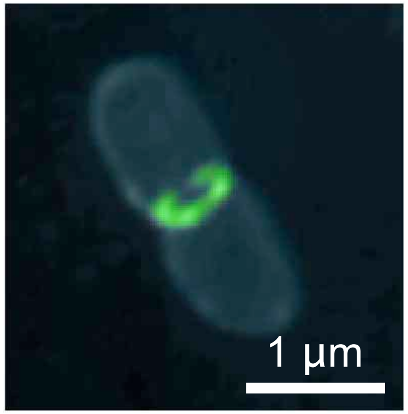

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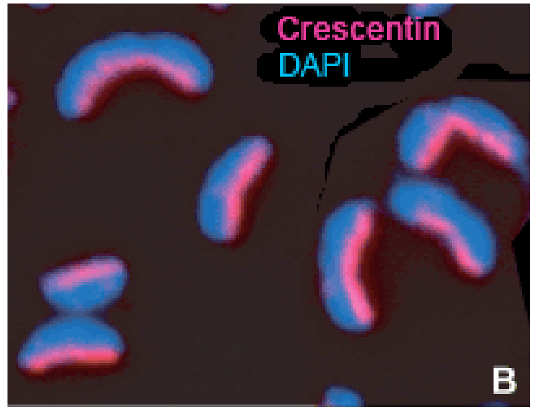

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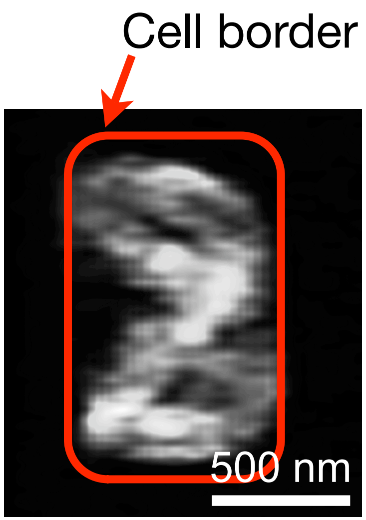
Actin homologs

Tubulin homolog

Intracellular organization in bacteria



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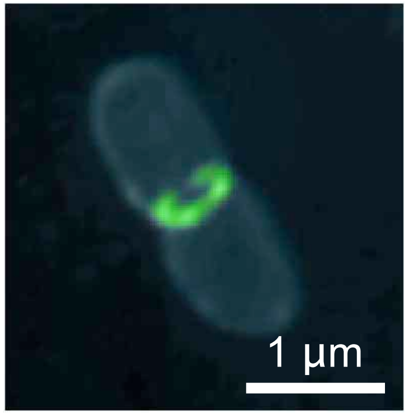

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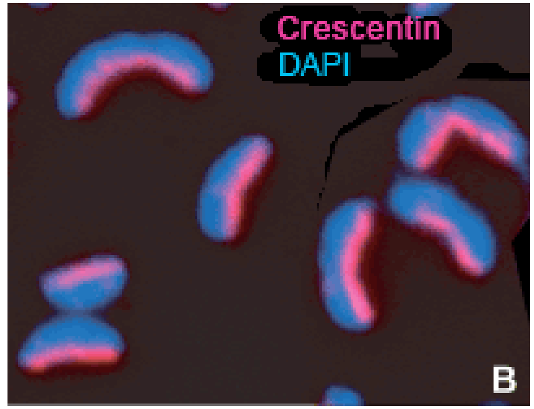

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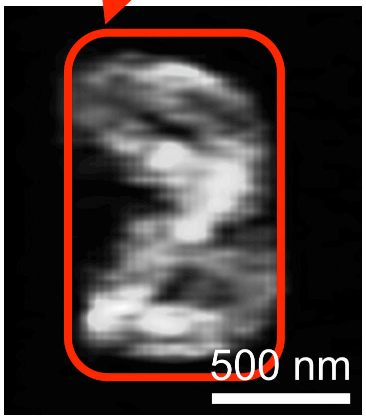
Tubulin homolog

Intermediate filament homolog

Intracellular organization in bacteria

Cell border



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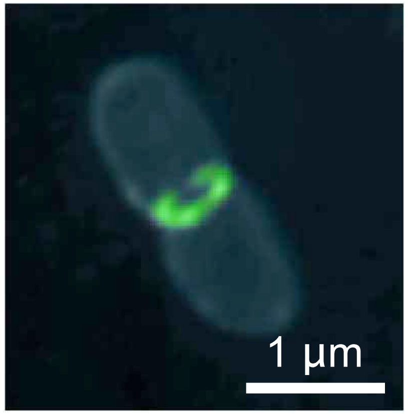

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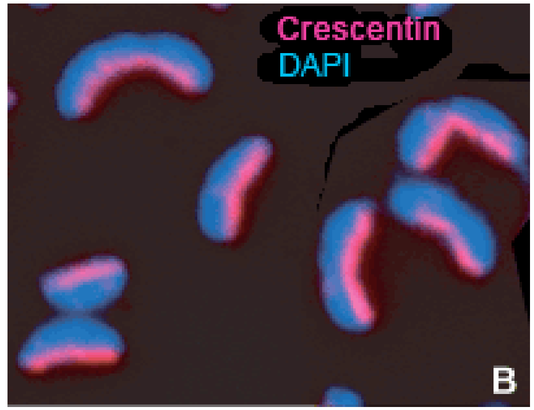

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Actin homologs

Tubulin homolog

Intermediate filament homolog

Bacteria 'share' the major classes of cytoskeletal proteins found in eukaryotes

Role of the bacterial cytoskeleton

Role of the bacterial cytoskeleton

Organization

- Plays a role in cell shape

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Dynamics

- Function?

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Evolution

- Unknown?

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We want to understand:

- The molecular basis for cell shape
- The evolution of bacterial cell shape

Bacterial cell shape: an esoteric area?

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Fundamental biological question

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Improves our systems level understanding of bacteria/microbes (recall: microbes account for ~50% of biomass on planet)

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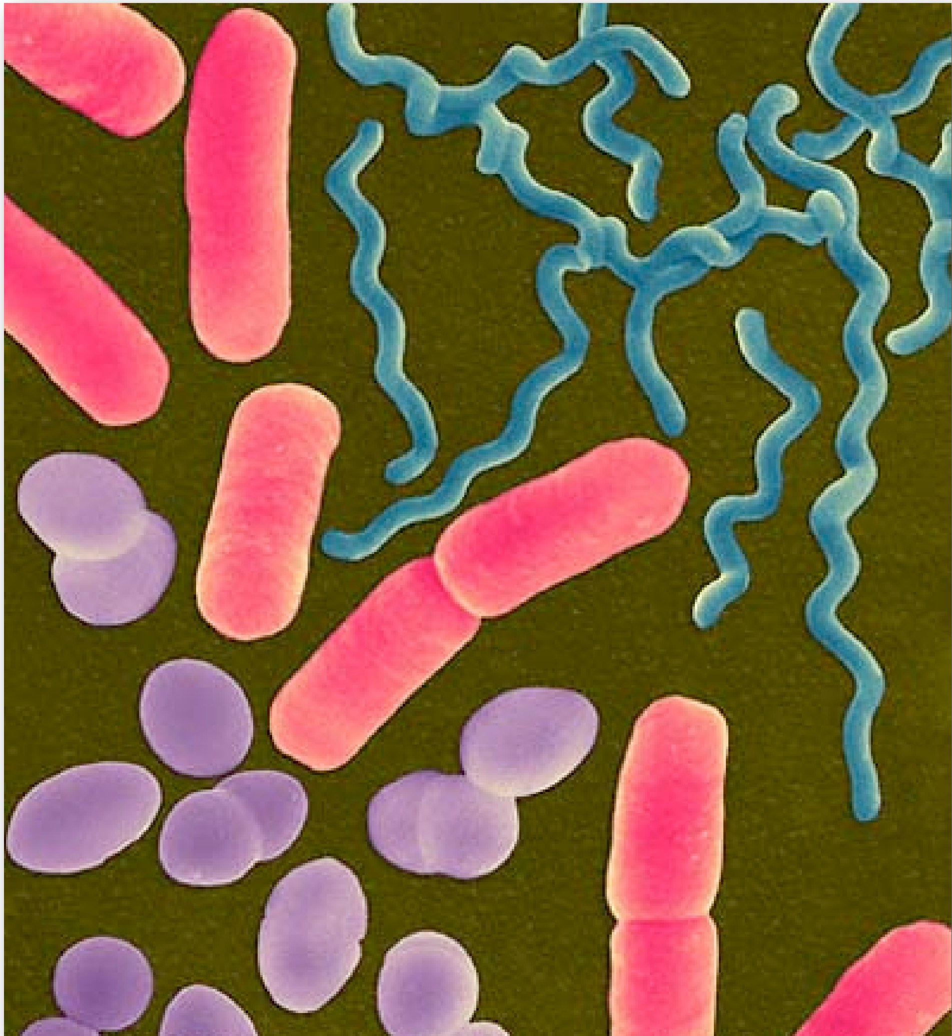
Applications to pathogenesis and infectious diseases

Overview

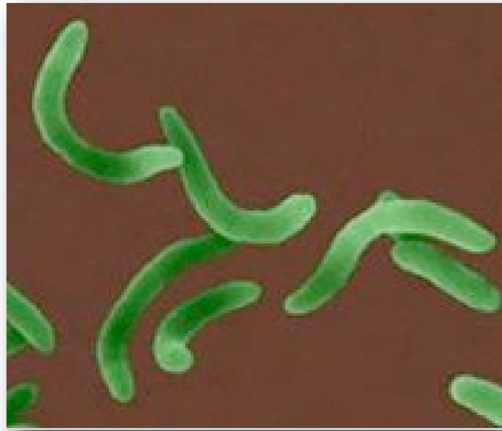
Part i

Molecular control of cell shape in bacteria

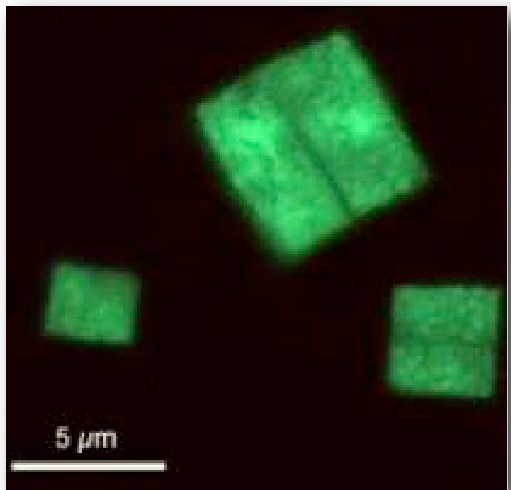
Bacterial cell shape: some examples



cocci; bacilli; spirillium

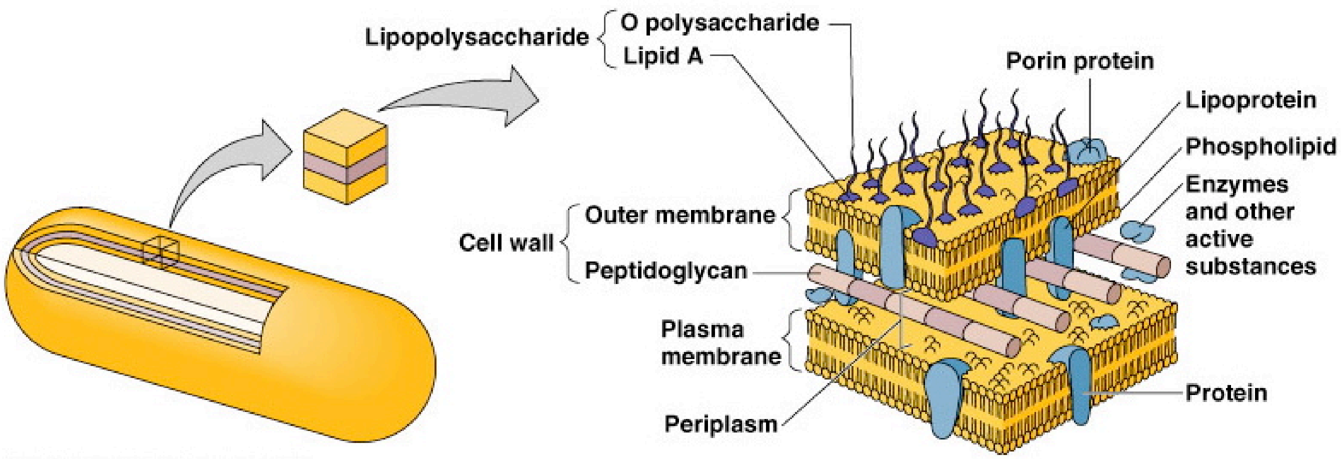


crescents



flat, square plates

Bacterial cell shape: structure of the cell wall



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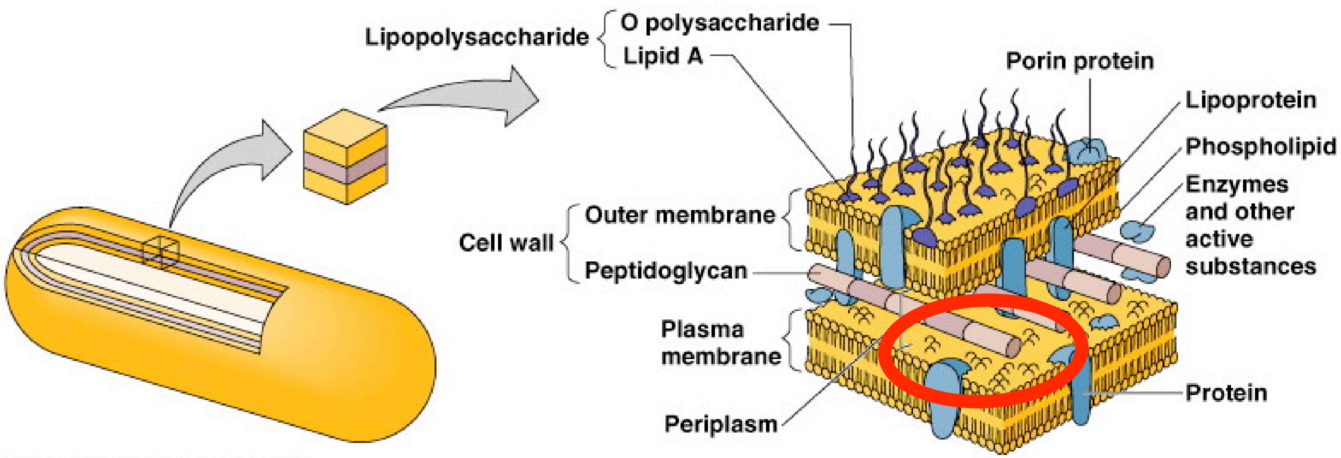

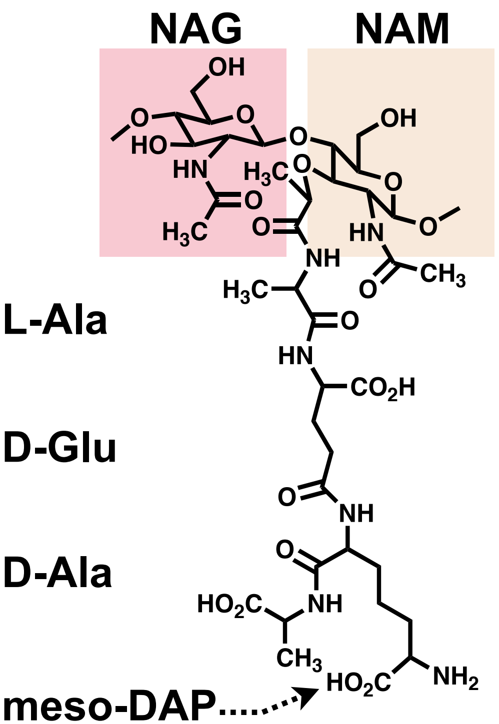

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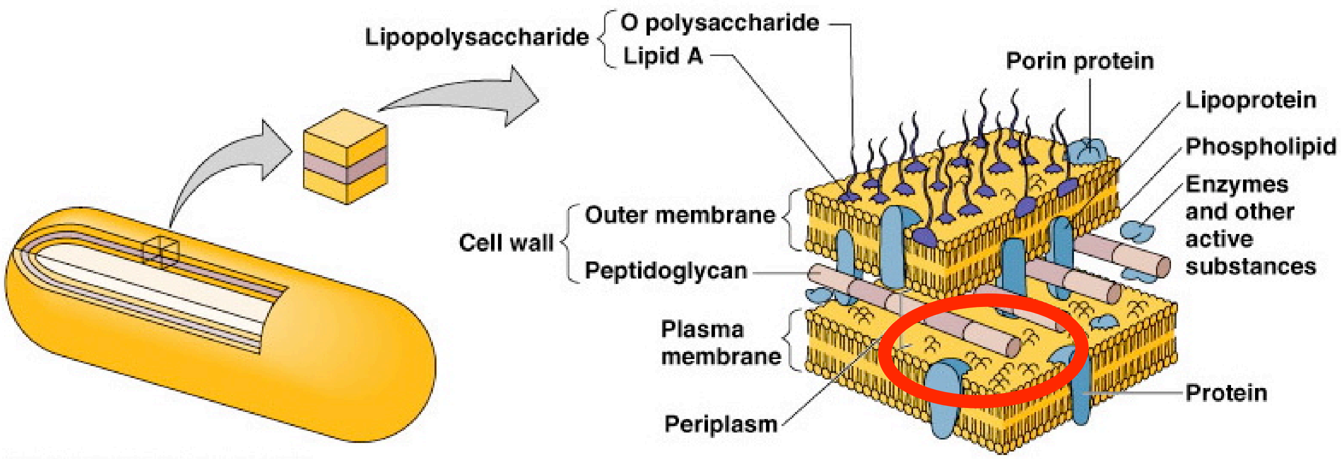

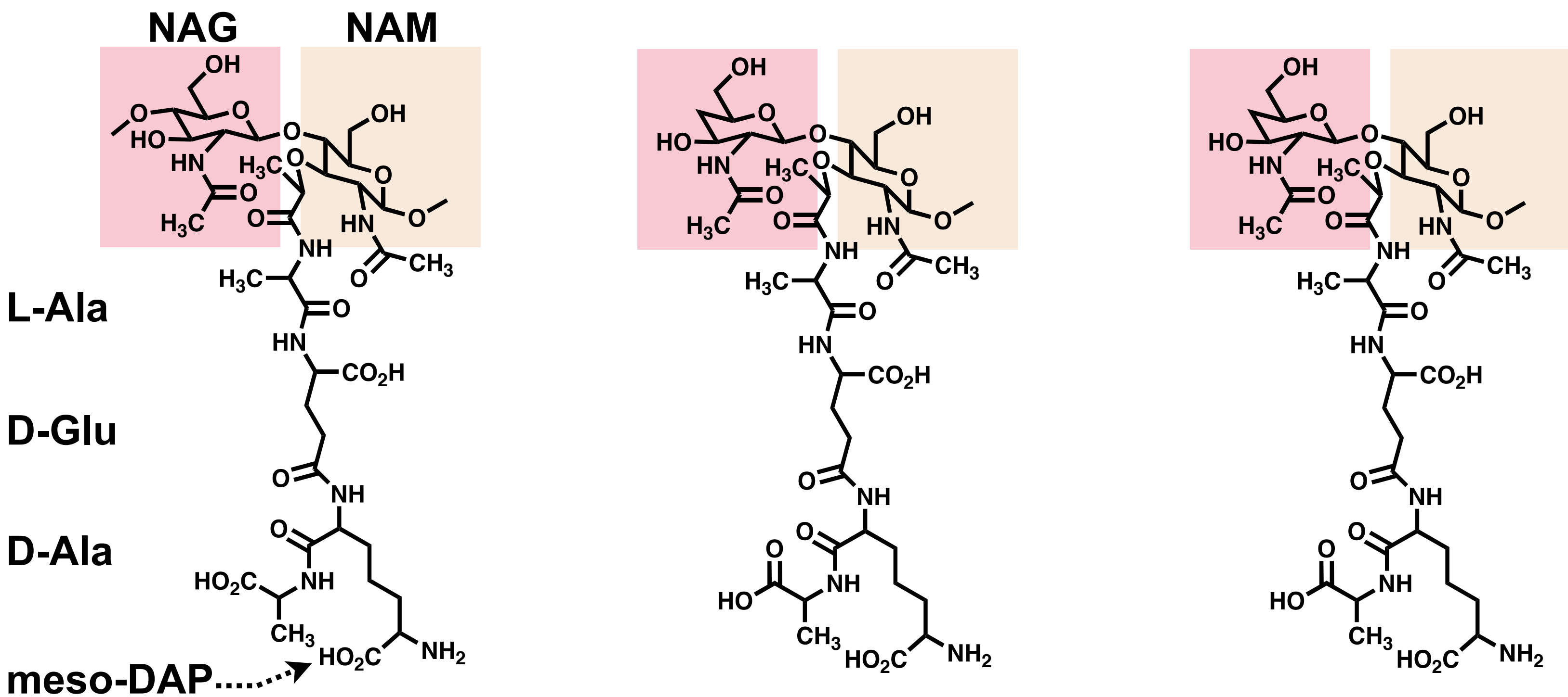

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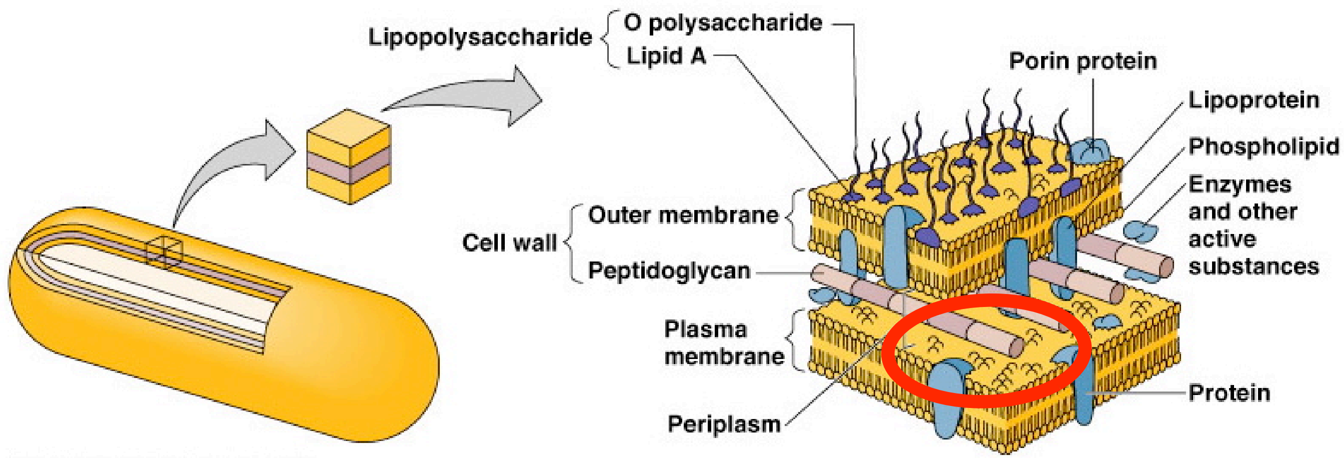

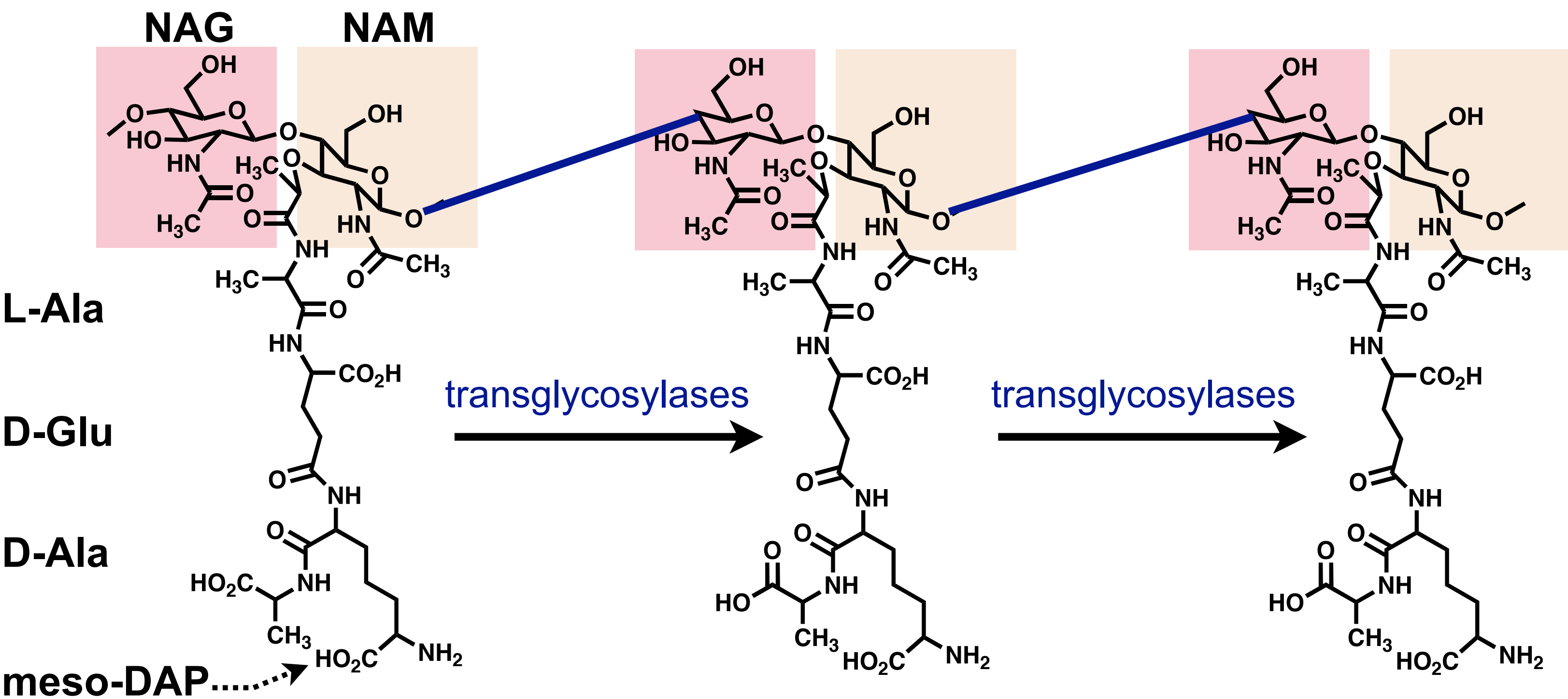

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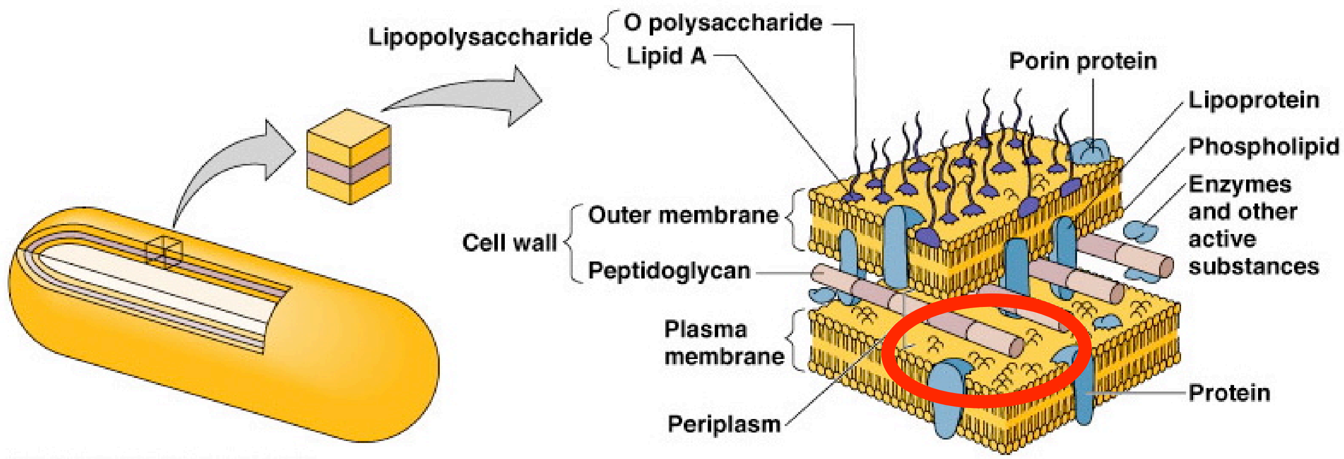
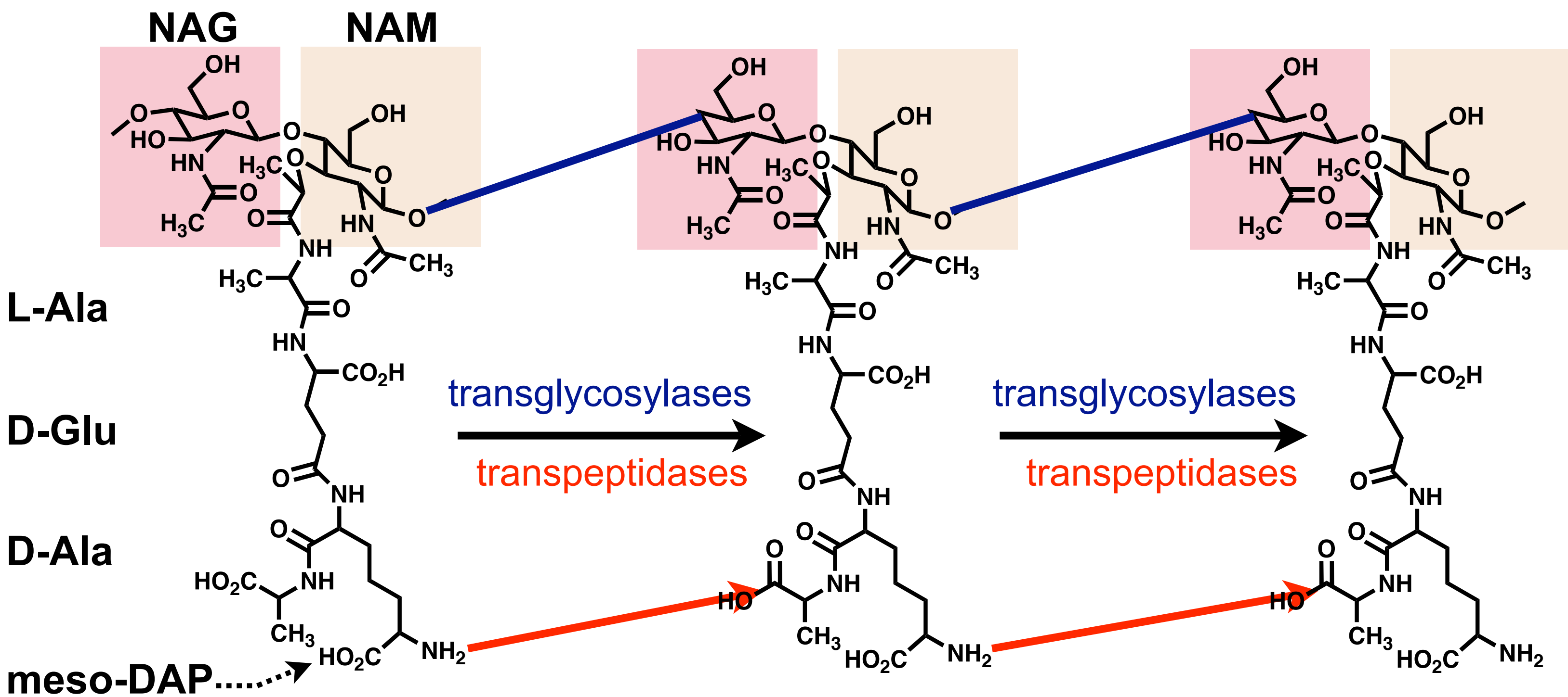
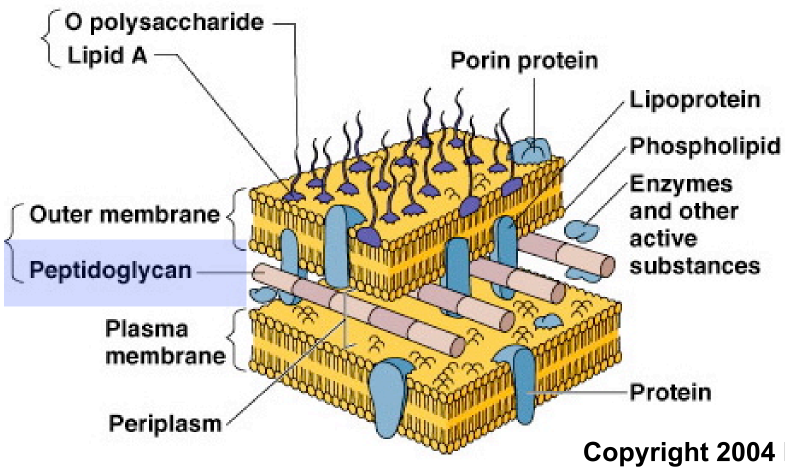




Structure of peptidoglycan



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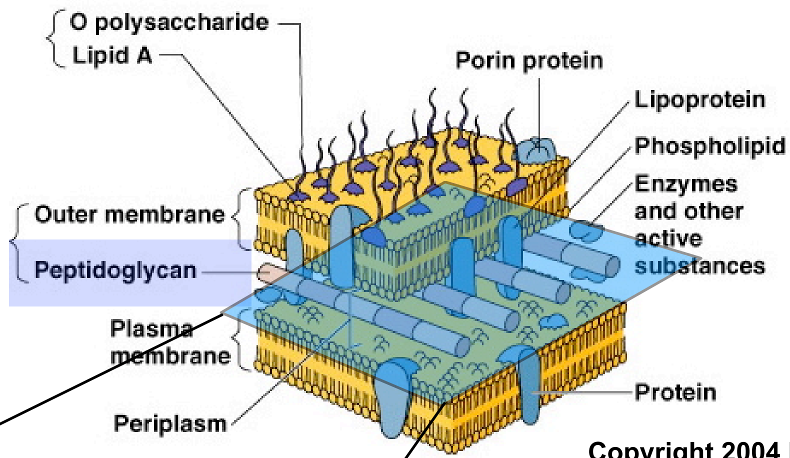

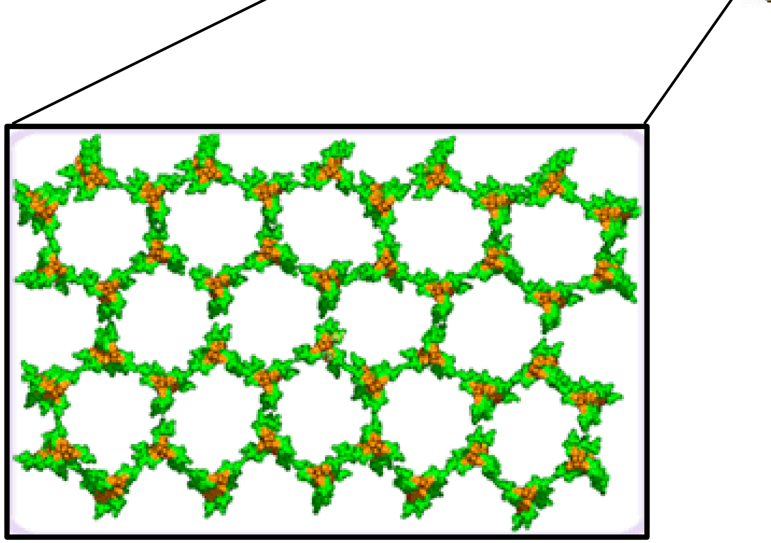

Mobashery, ND

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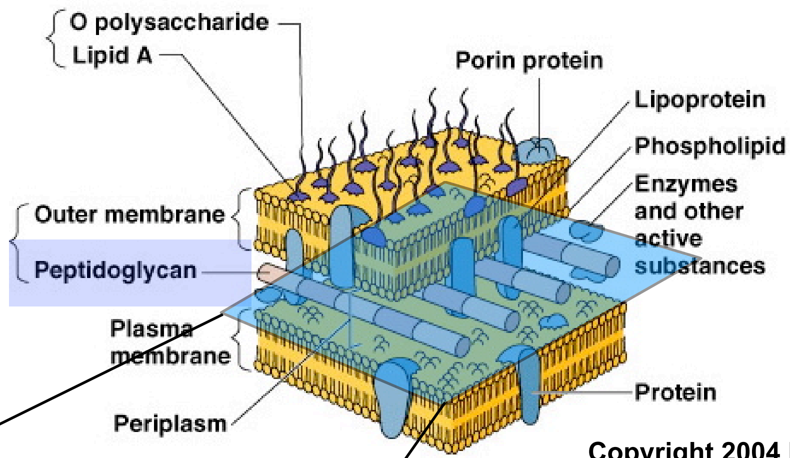

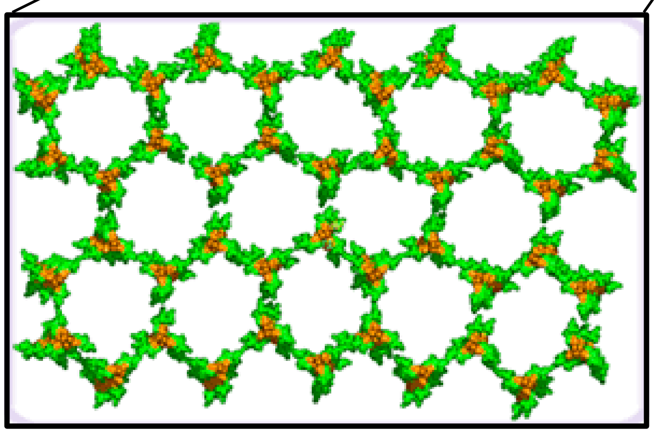

Mobashery, ND

Peptidoglycan (PG):

- Cross-linked polysaccharide
- Single molecule (~100,000 kD)
- ~20 nm thick (Gram-negative)
- Mechanical properties of cell

Mechanical properties of the PG

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Isolate PG from cells (Triton X-100)
Measure properties of intact PG with a scanning probe

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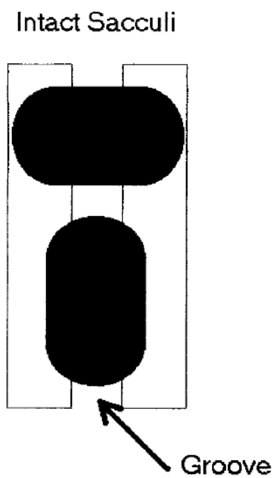

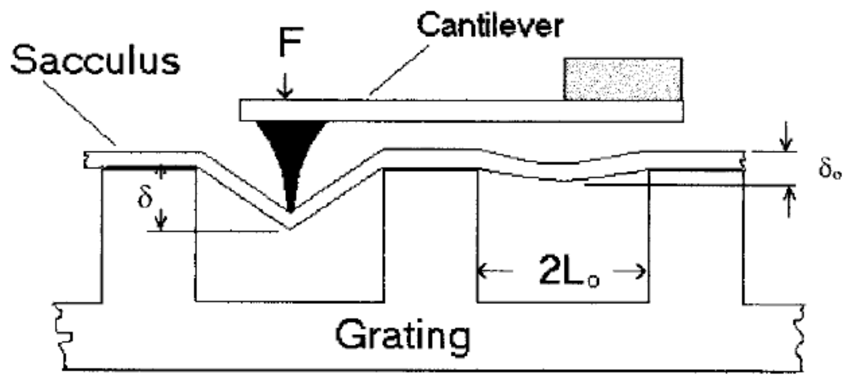

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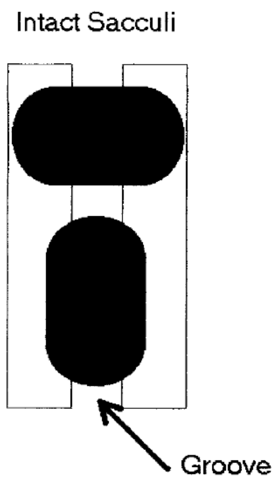
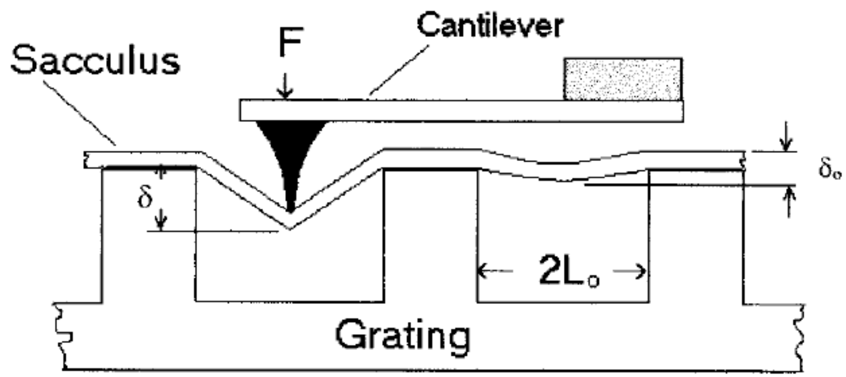




Properties of the PG:

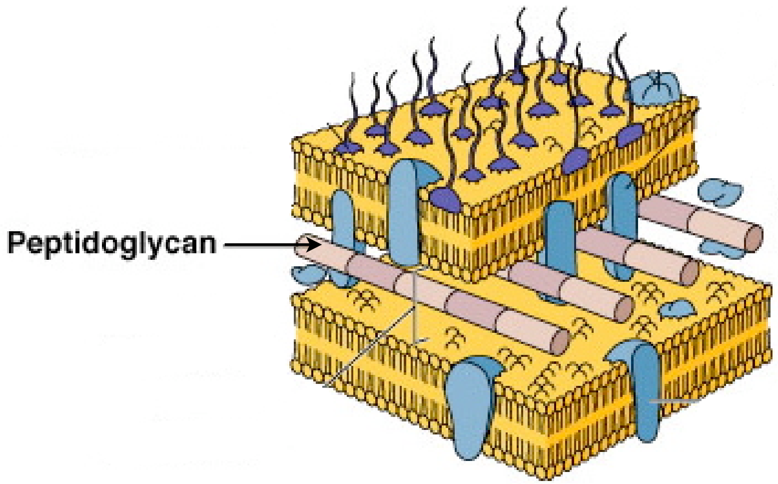
Perfectly elastic (no hysteresis)

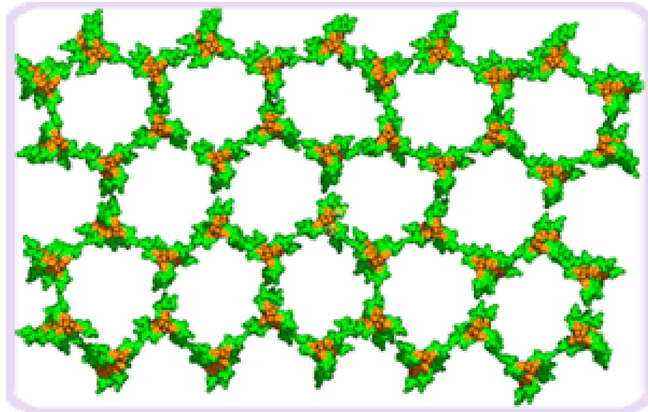
$\gamma_{peptidoglycan}$: 2.5×10^7 N/m^2

(γ_{latex}: ~2.5×10^7 N/m^2)

T. Beveridge (1999) *J. Bacteriology*, **181**, 6865

Cell shape is controlled at the level of the PG



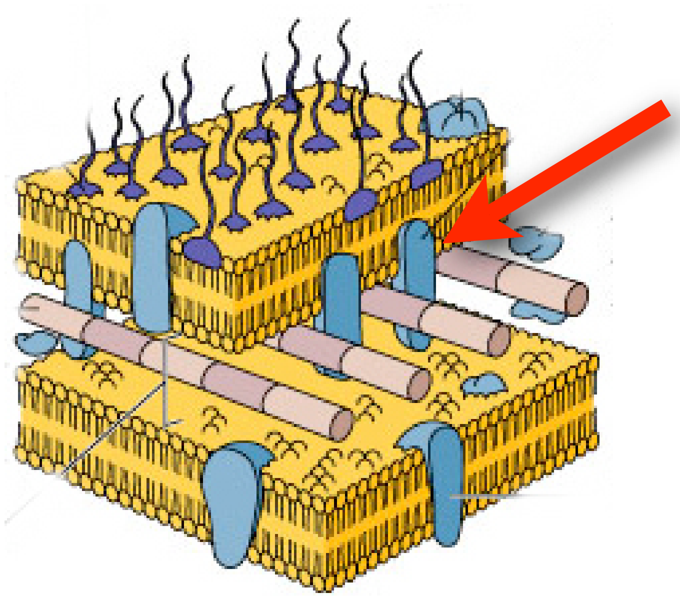


Two plausible mechanisms:

1. PG is reinforced with mechanical ‘struts’
   (molded and held in place)

2. PG is synthesized in a specific orientation
   (sculpted into a specific shape during synthesis)

Does the orientation of the PG control shape?



Penicillin binding protein (PBP)

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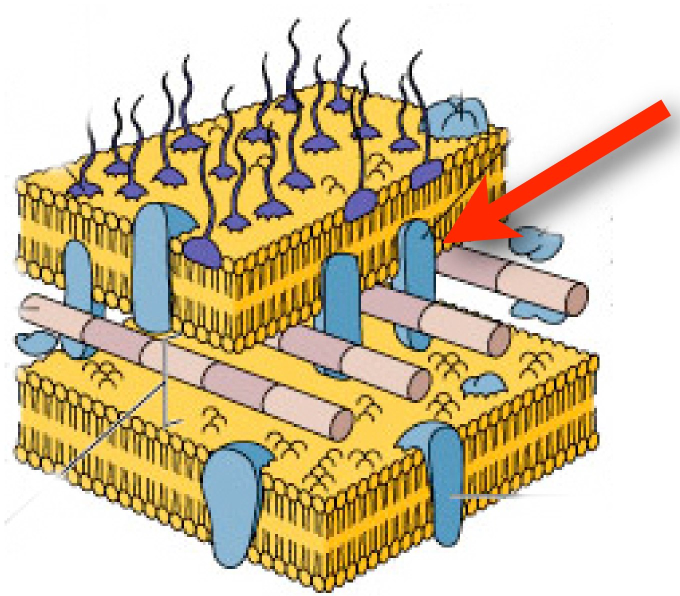

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12 known PBPs in *E. coli*

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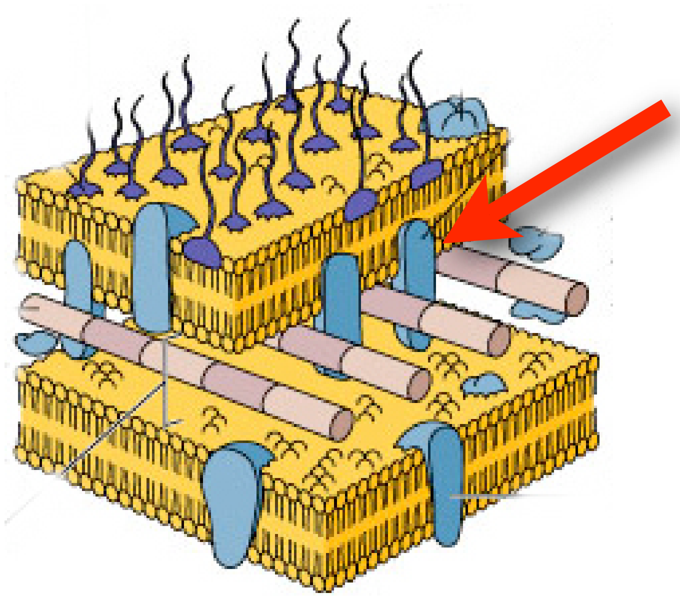

Penicillin binding protein (PBP)

12 known PBPs in *E. coli*

PBPs play a key role in:

- Elongation of cell wall (PBP2)
- Division/septation (PBP3)
- Cell shape?

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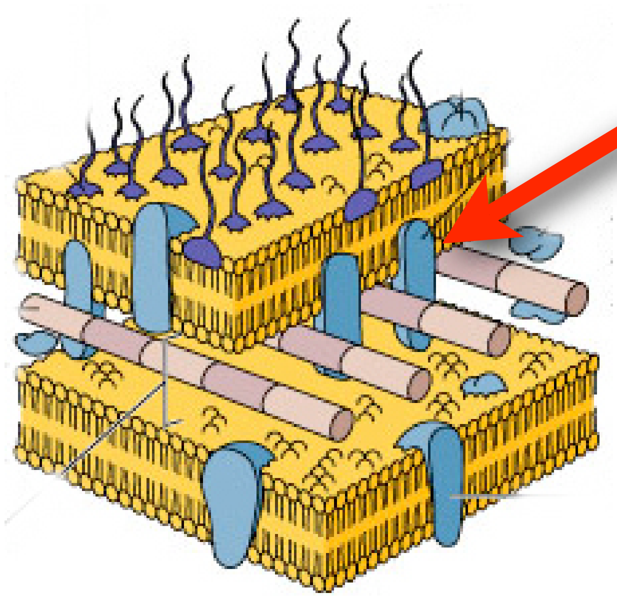

Penicillin binding protein (PBP)

12 known PBPs in *E. coli*

PBPs play a key role in:

- Elongation of cell wall (PBP2) → PG with κ_{low}
- Division/septation (PBP3) → PG with κ_{high}
- Cell shape?

Are PBPs moved/localized on a filament?

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PBP2 (synthesis of cylindrical walls) interacts with MreB

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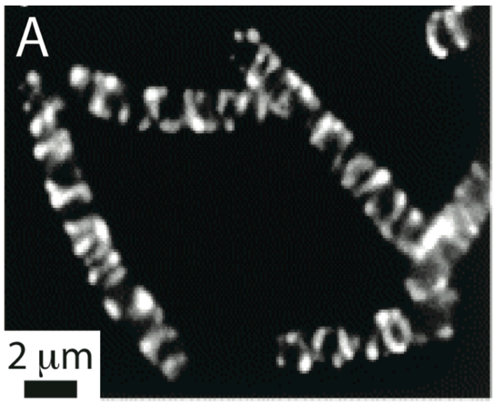
PBP2 (synthesis of cylindrical walls) interacts with MreB

MreB: a homolog of actin that forms helical (?) filament(s) in rod-shaped cells



A GFP-MreB chimera expressed in *B. subtilis* (J. Errington et al.)

A mesoscale model; an elastic spring inserted in a flexible plastic tube (Weibel)

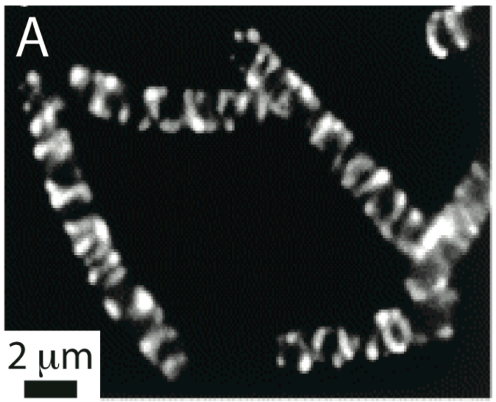
Are PBPs moved/localized on a filament?

PBP2 (synthesis of cylindrical walls) interacts with MreB

MreB: a homolog of actin that forms helical (?) filament(s) in rod-shaped cells

MreB may play a key role in controlling:

- *Spatial organization within the cell*
- *Cell shape*



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We are systematically studying proteins (and protein interactions) that play a role in controlling cell shape

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We want to understand how perturbations in protein levels and organization influence cell shape

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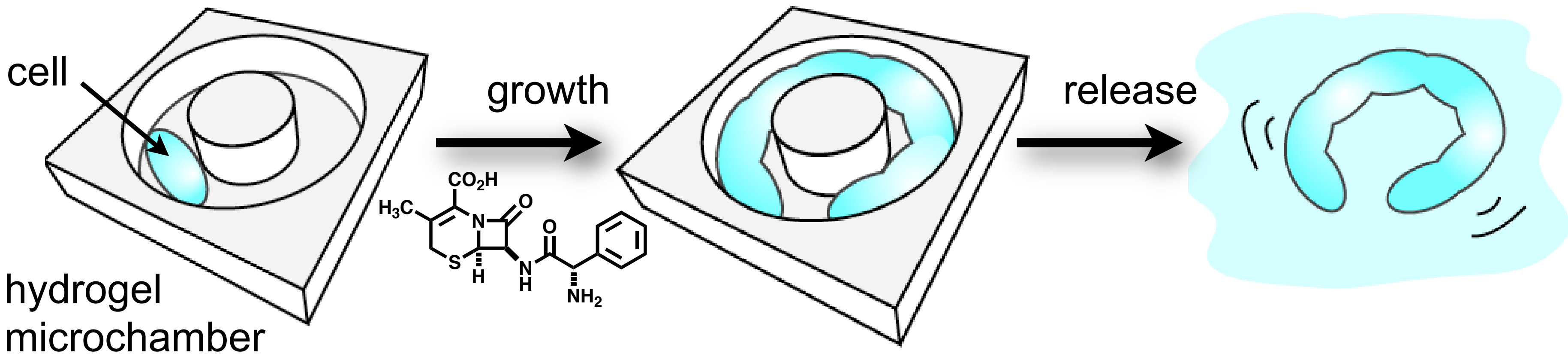
To make this approach possible we have developed a technique for manipulating cell shape

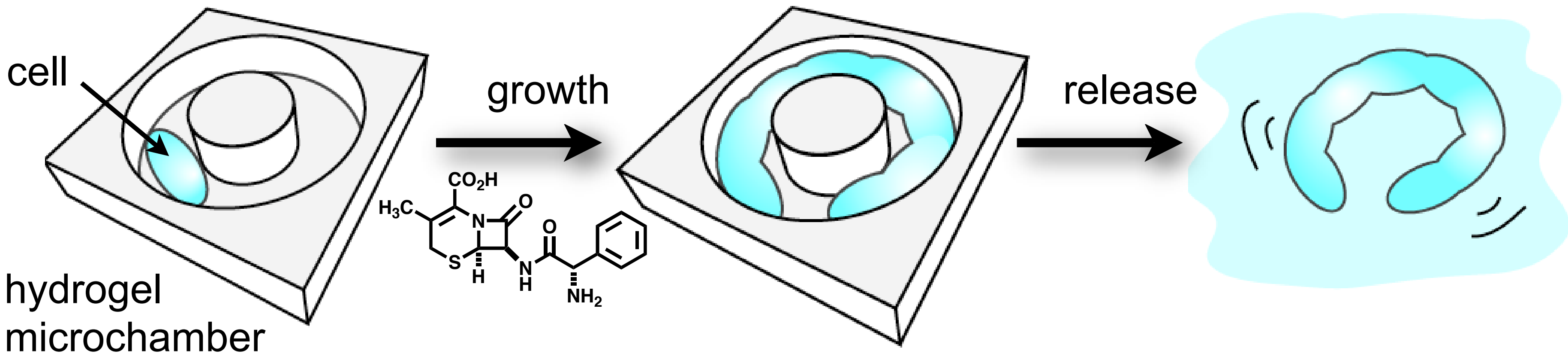
Controlling cell shape

Materials-based approach: (for example, rod-to-crescent)



Controlling cell shape

Materials-based approach: (for example, rod-to-crescent)



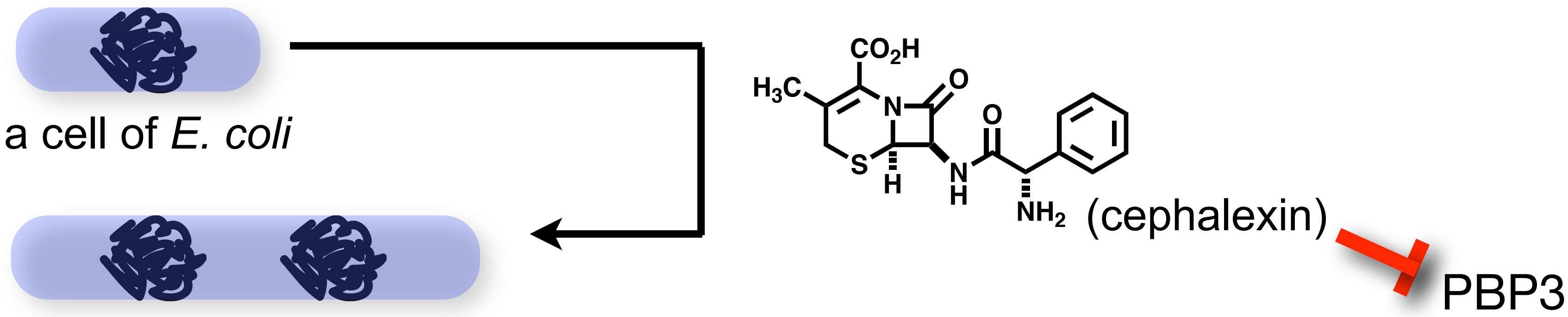
Steps:

1. 'Customize' microchambers
2. Seed cells
3. Grow into filaments (antibiotic or P_{arab}-FtsZ)
4. Release by removing 'ceiling'

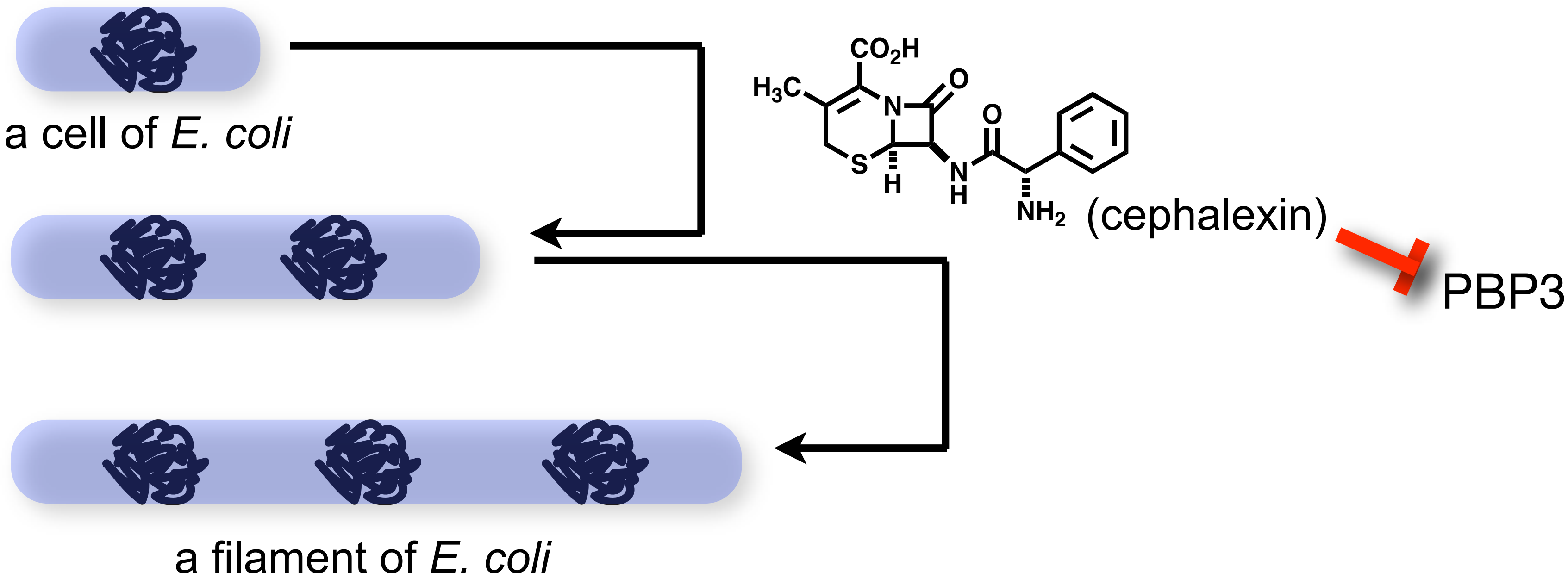
Filamentous cells of E. coli (filaments)

a cell of *E. coli*

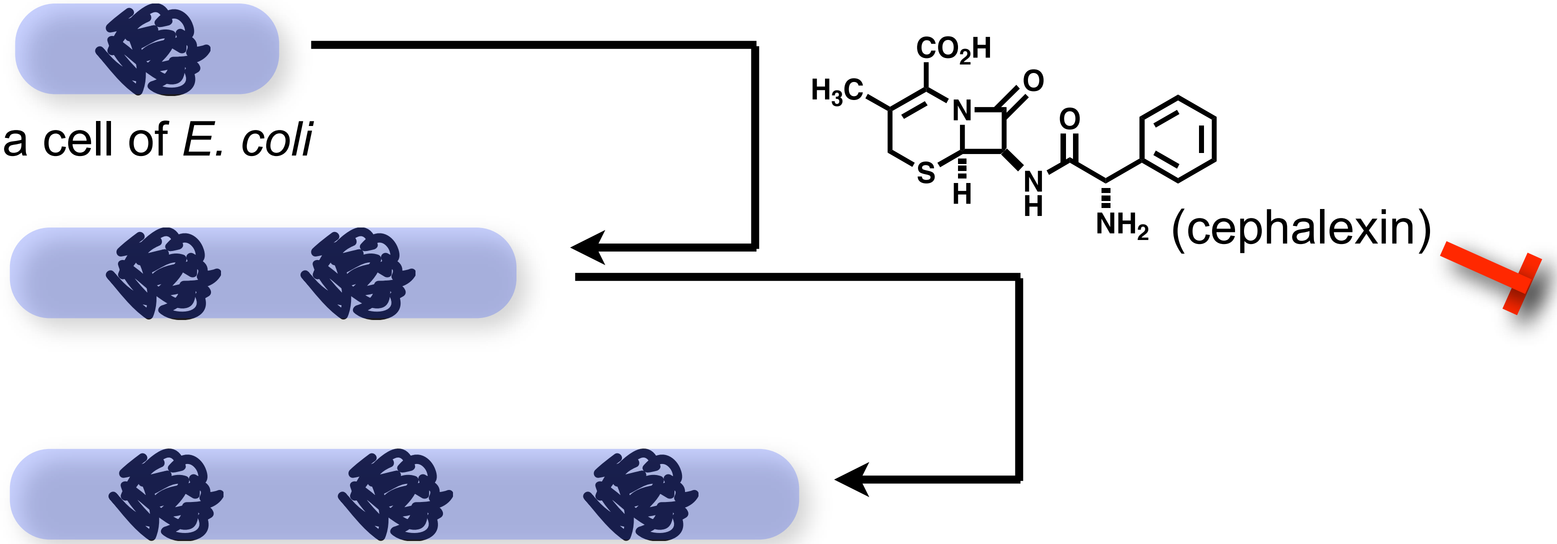
Filamentous cells of E. coli (filaments)



Filamentous cells of E. coli (filaments)



Filamentous cells of E. coli (filaments)



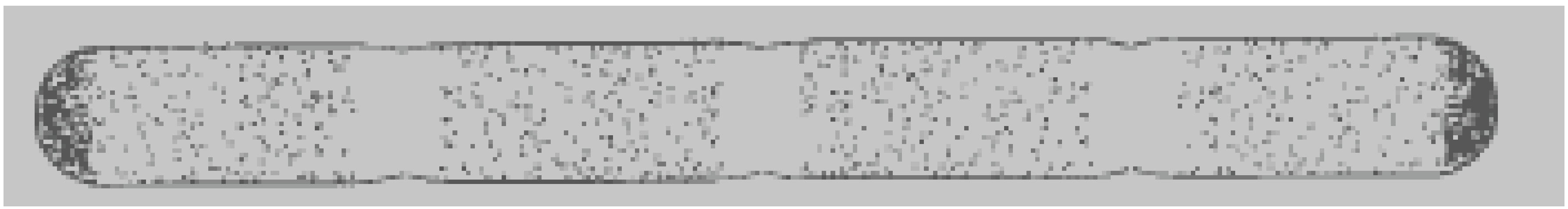
a cell of *E. coli*

(cephalexin)

PBP3

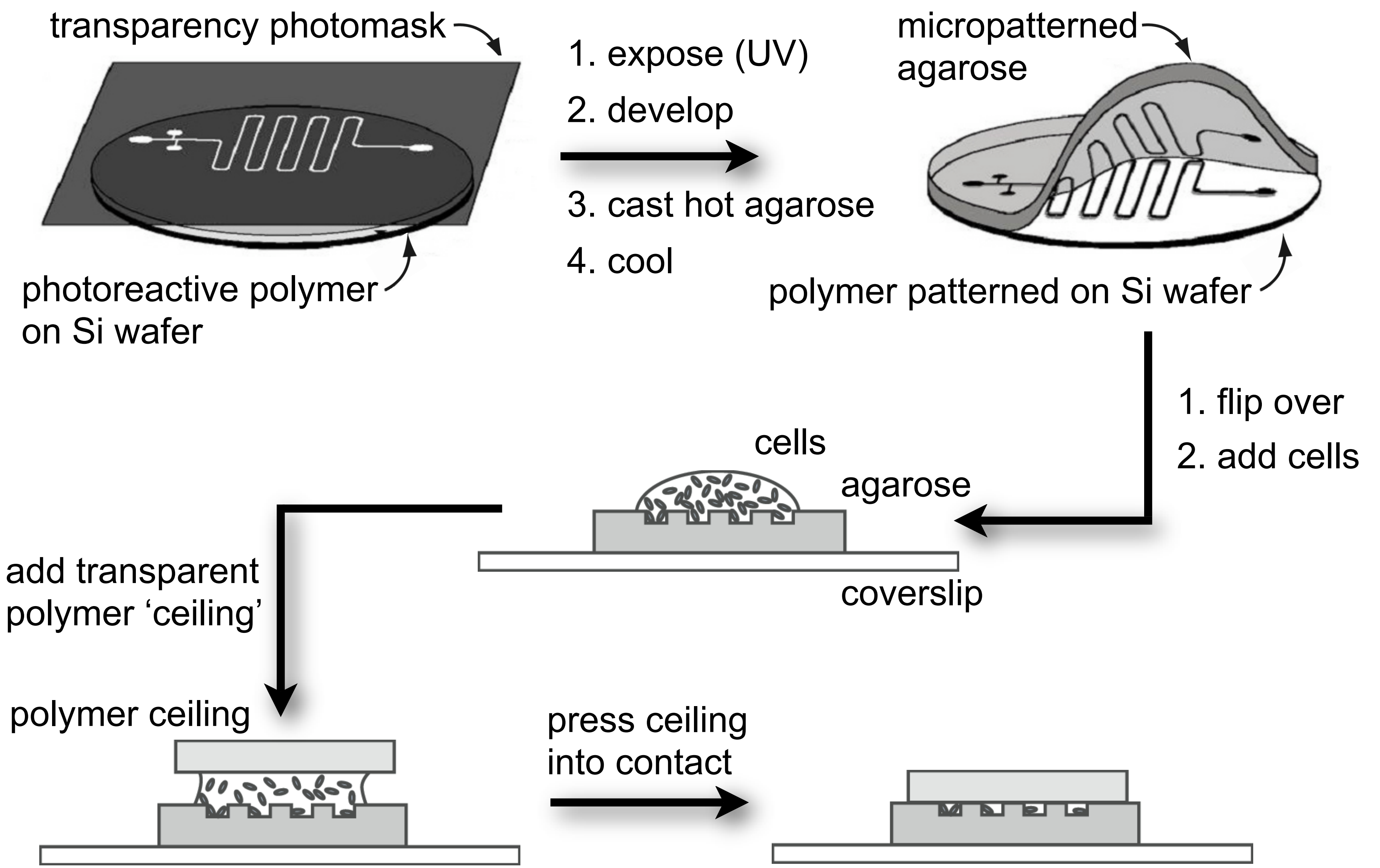
a filament of *E. coli*

Distribution of peptidoglycan from original cell (dark spots)

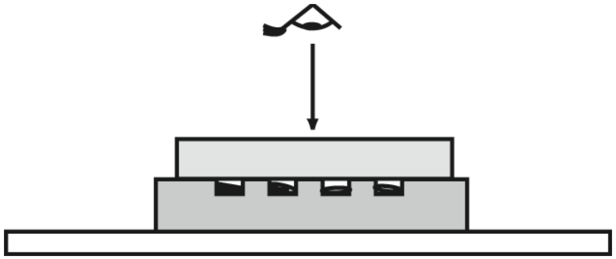


de Pedro et al. (1997) *J. Bacteriol.*, **179**, 2823

Fabricating molds for engineering cell shape



Cells of E. coli in hydrogel containers are motile and metabolically active



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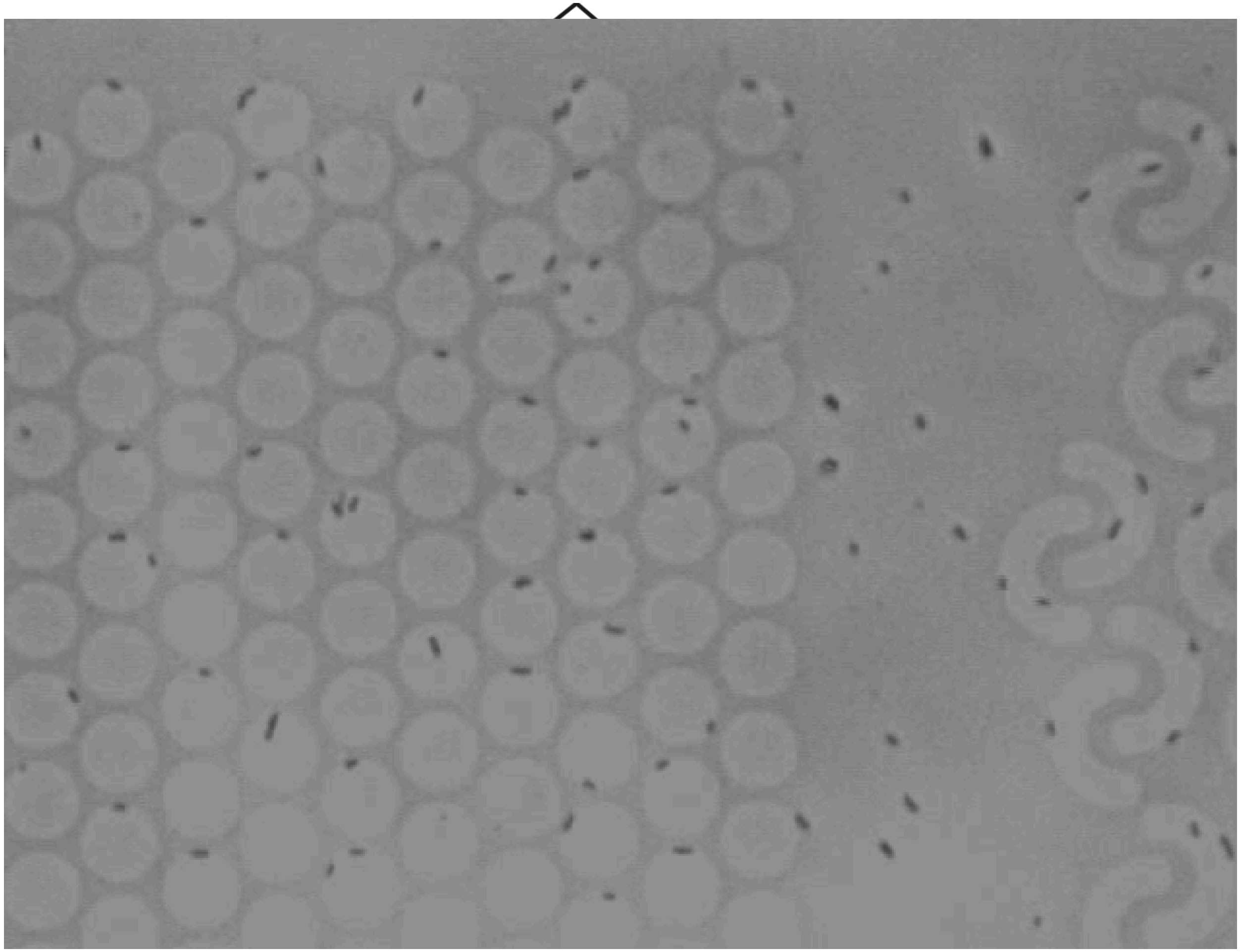

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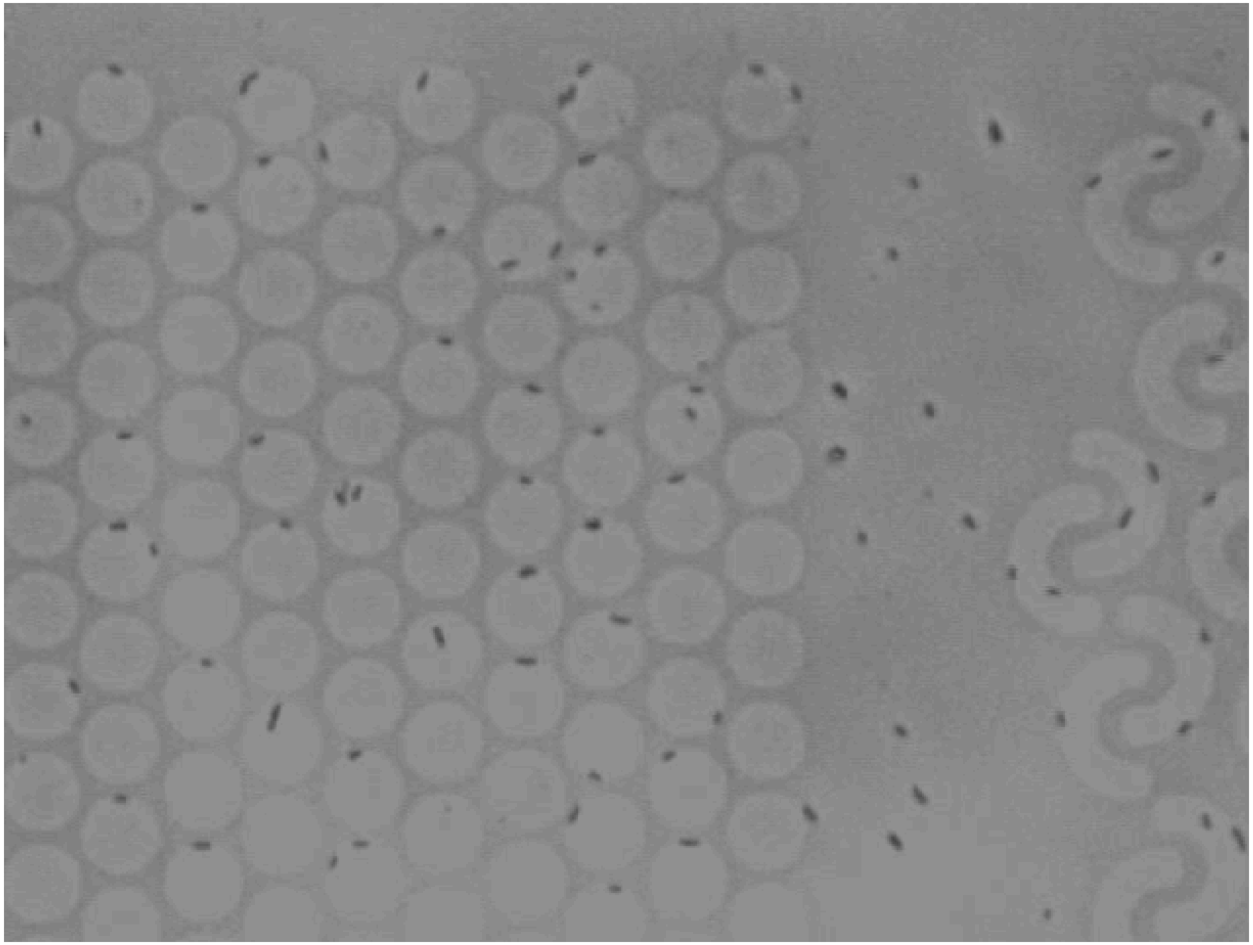

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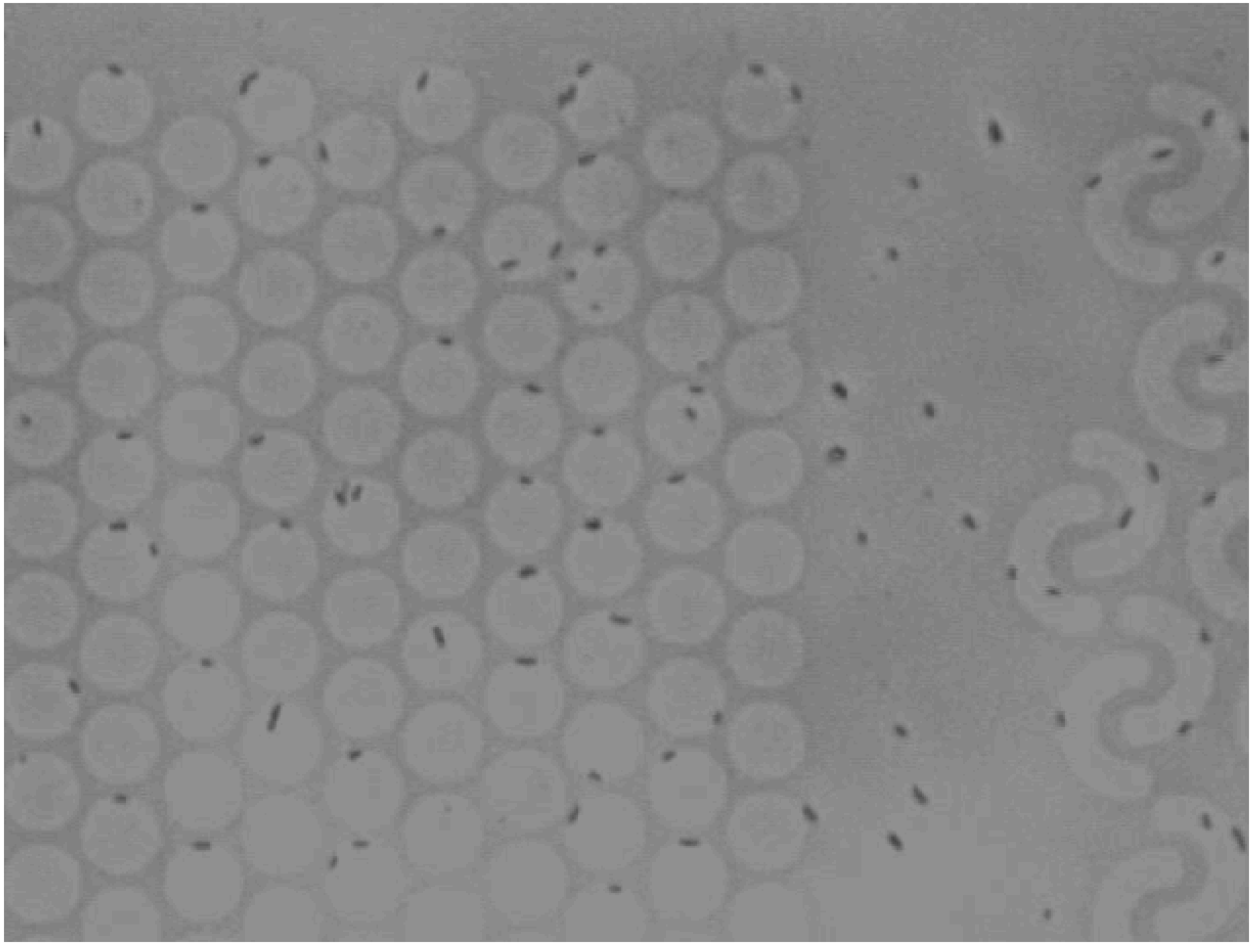
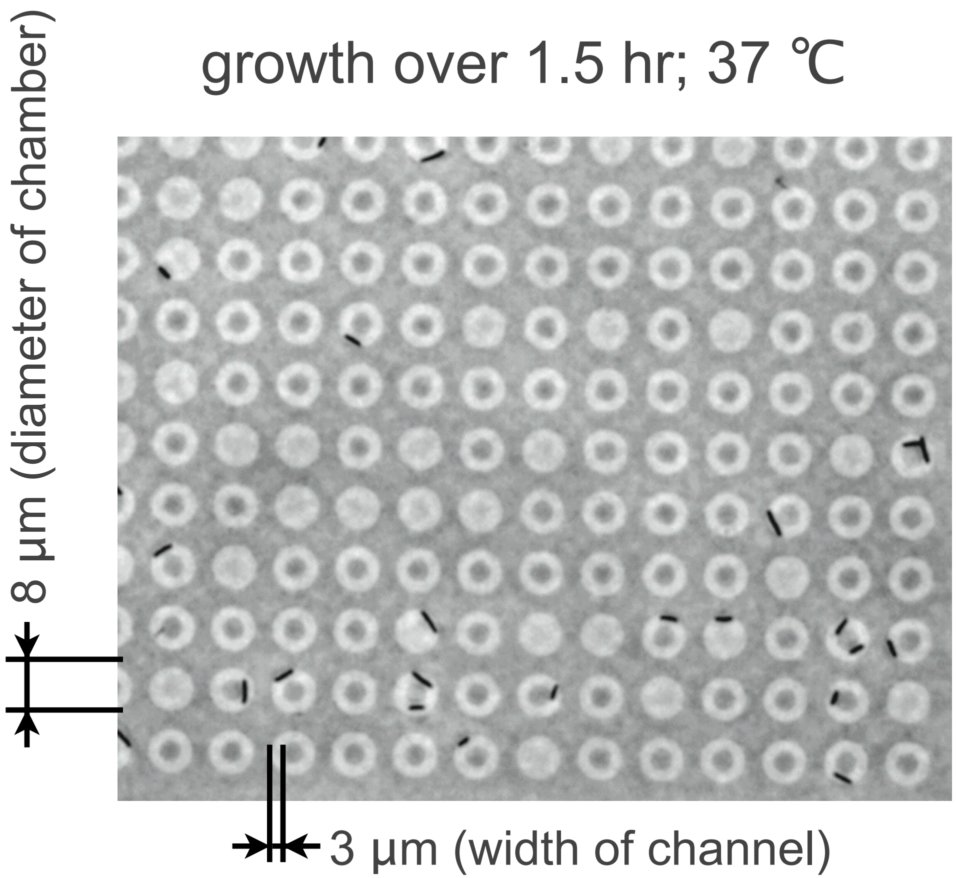
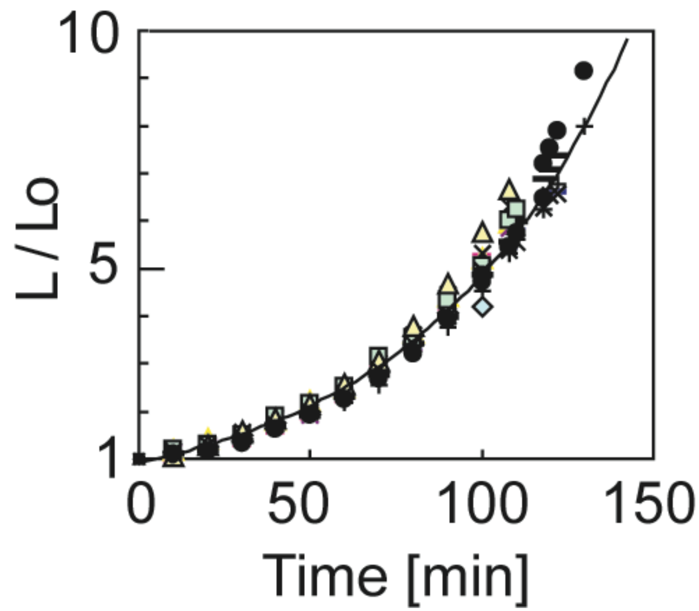


Hydrogel walls are ‘transparent’ to nutrients, ions, gas, metabolic waste

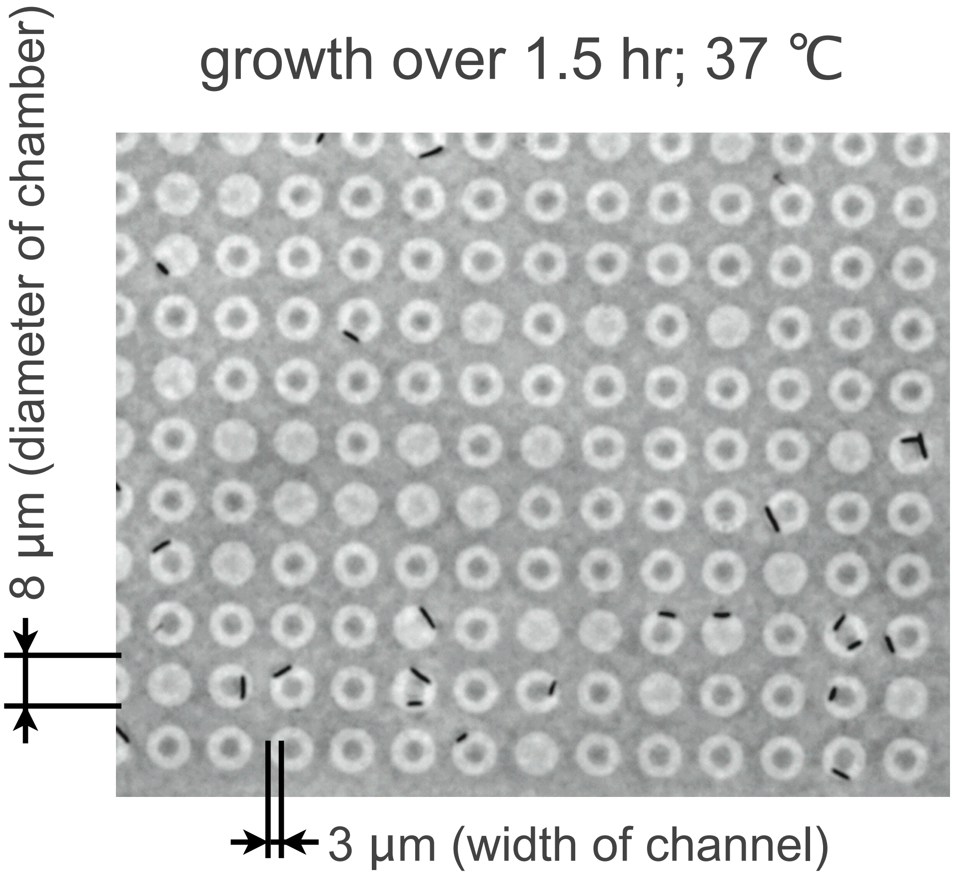
Growth of cells in microchambers

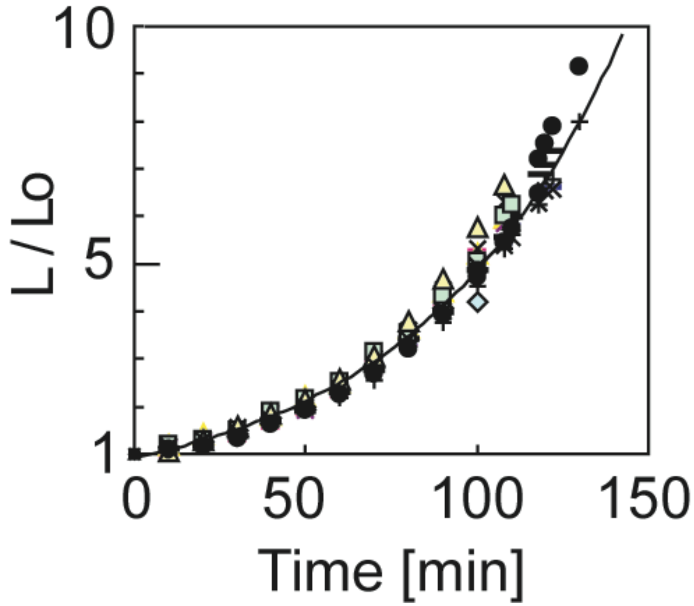




Growth of cells in microchambers

growth over 1.5 hr; 37 ℃



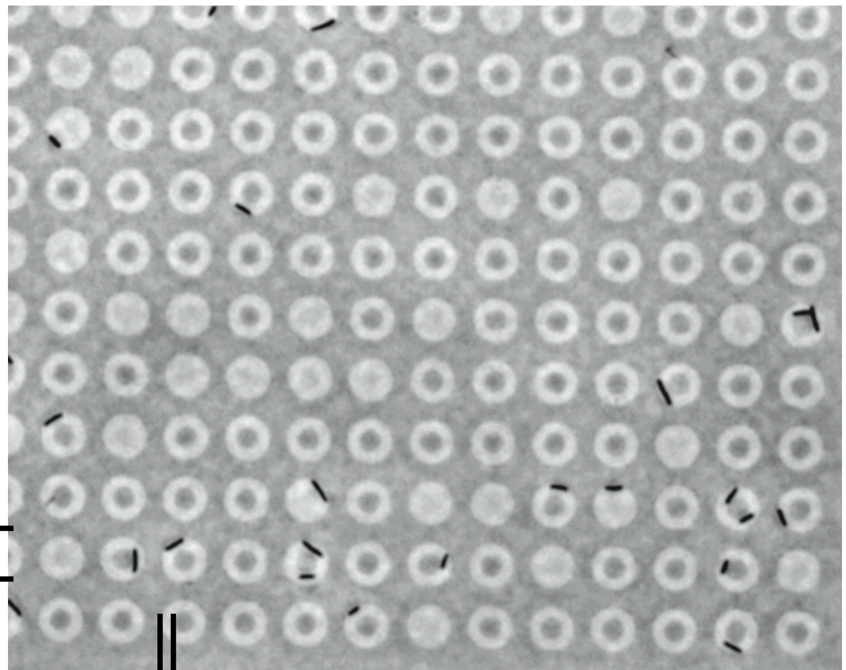


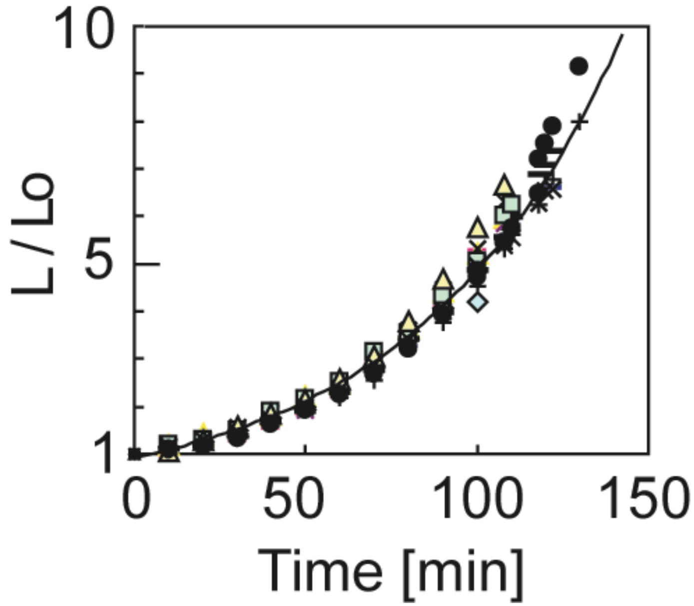
doubling time$_{mold}$: 41 ± 4 min

doubling time$_{soln}$: ~40 min

Growth of cells in microchambers

growth over 1.5 hr; 37 ℃





doubling time$_{mold}$: 41 ± 4 min

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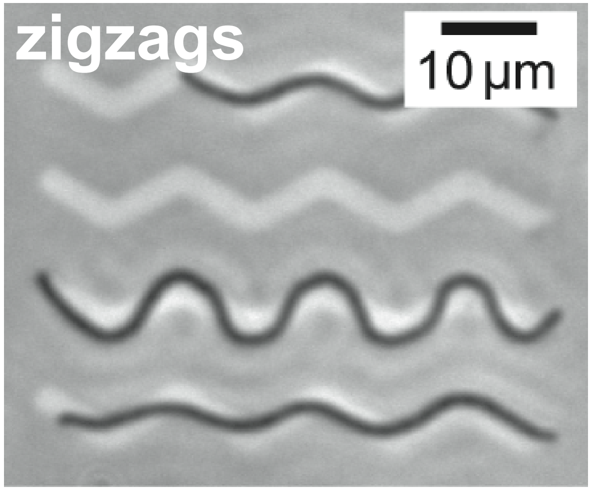
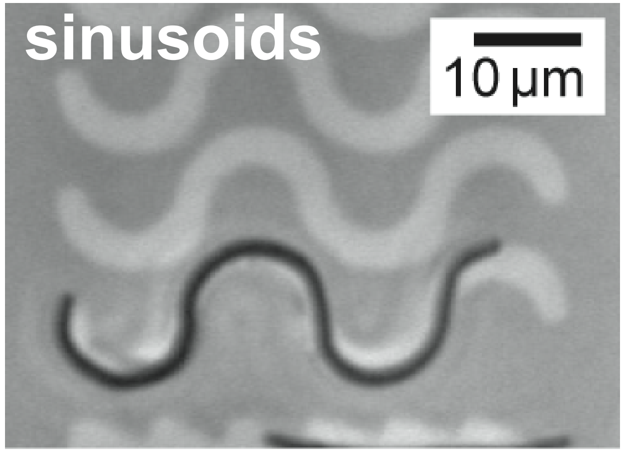
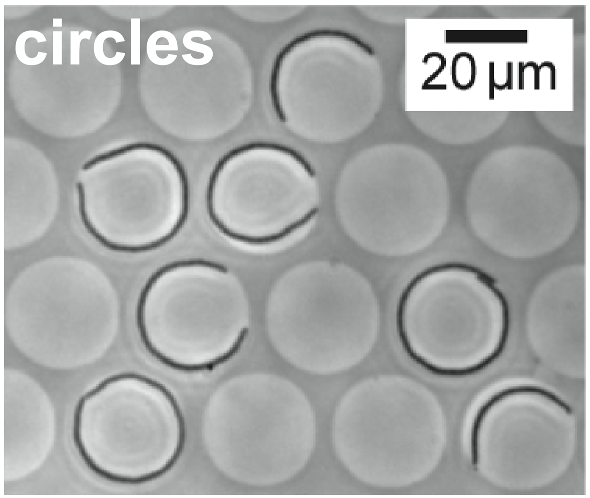
Cells retain all of the phenotypes we observe in liquid cultures

Geometry of walls controls cell shape







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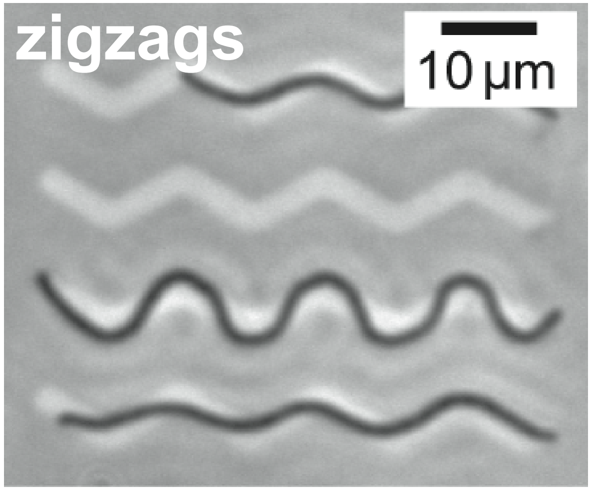
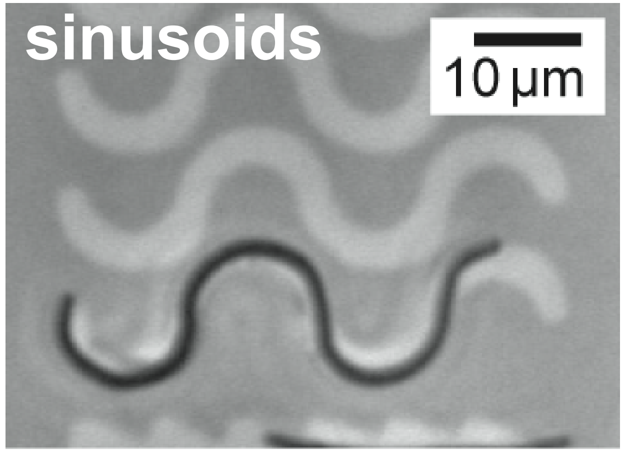
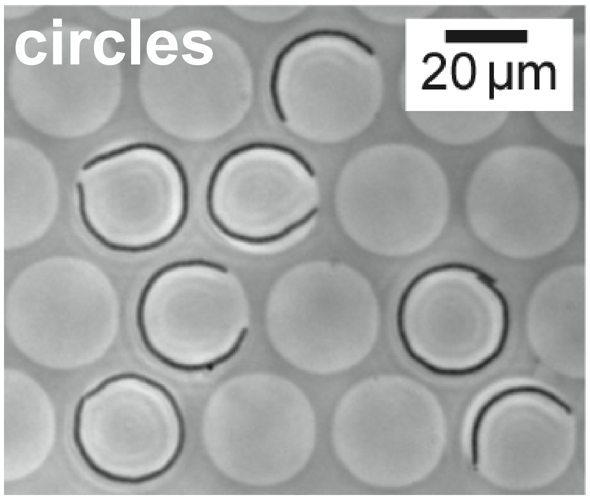
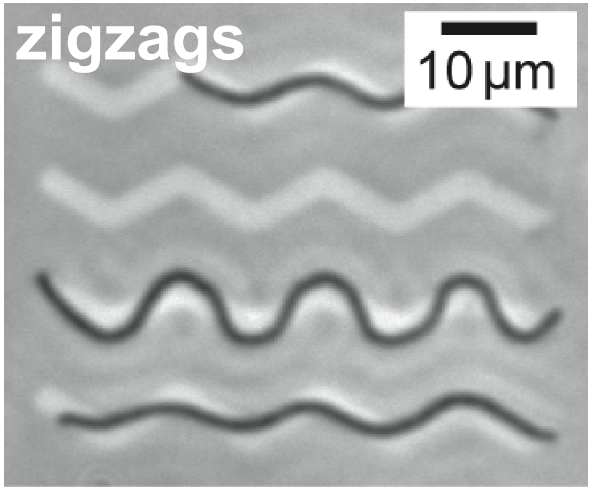


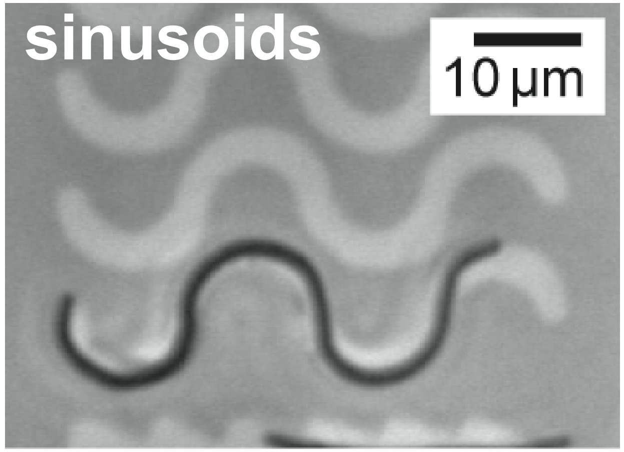


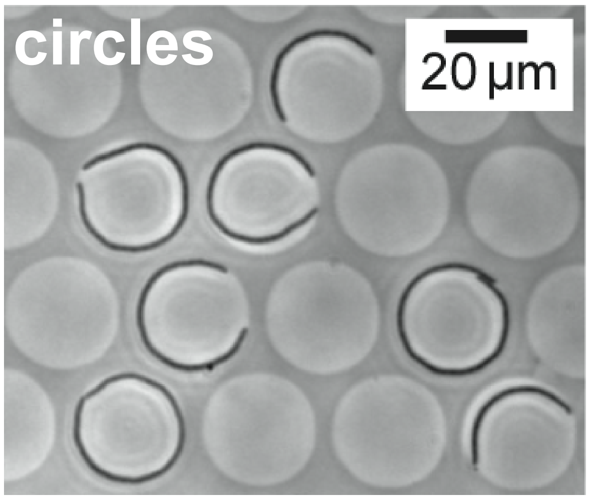


$\gamma_{\text{mold}} >> \gamma_{\text{cell}}$

Geometry of walls controls cell shape



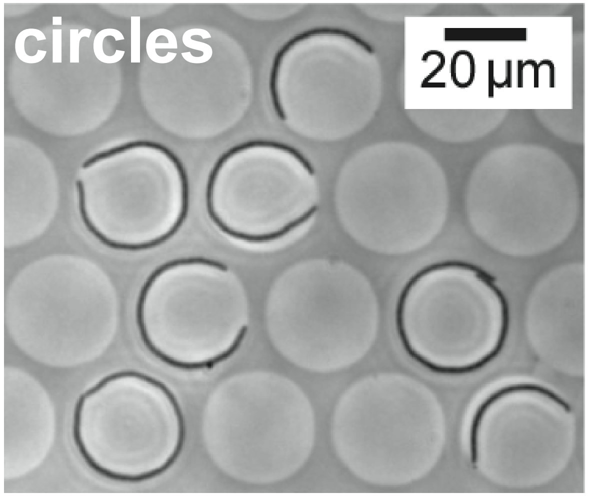




$\gamma_{mold} >> \gamma_{cell}$

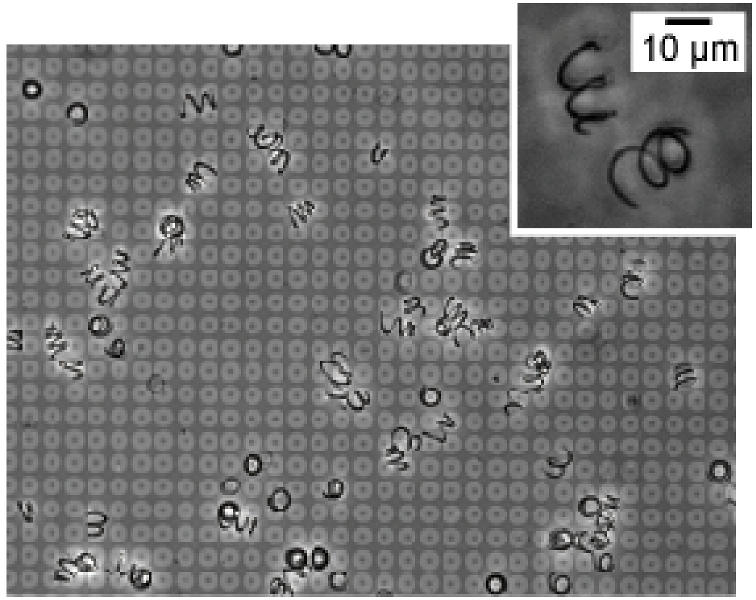
Cells appear to grow in an orientation that minimizes stress placed on the cell

Cells retain their shape after release



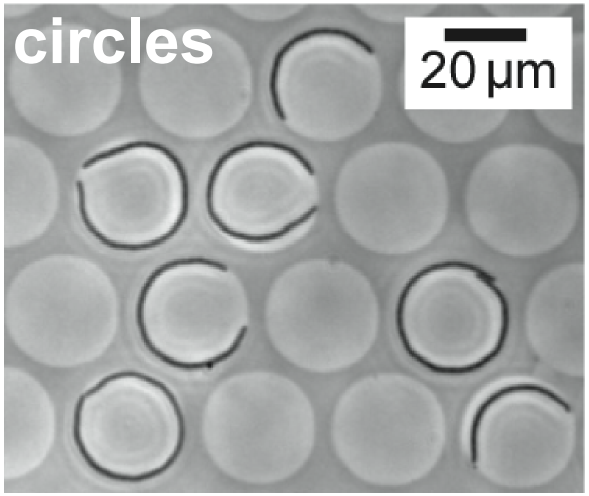
Grow cells of *E. coli*
(rods) in chambers

Release →

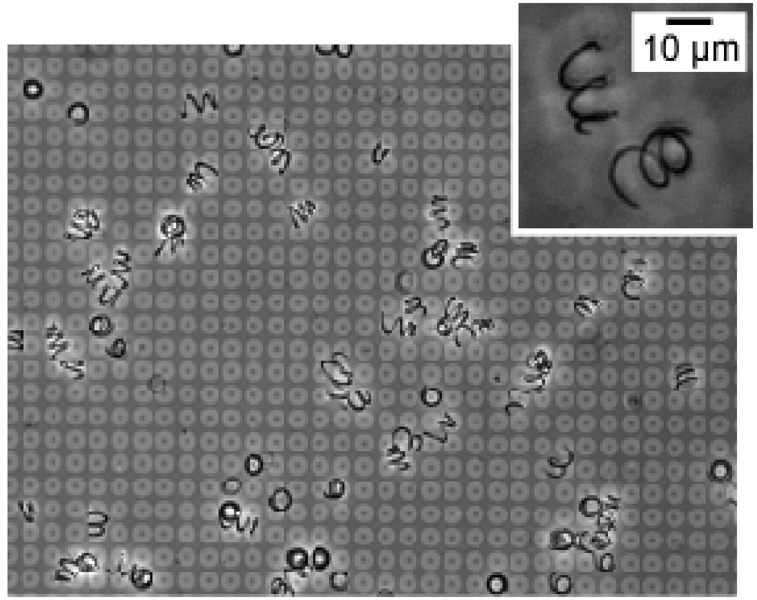


Cells in liquid
(with cephalexin)

Cells retain their shape after release



Release →



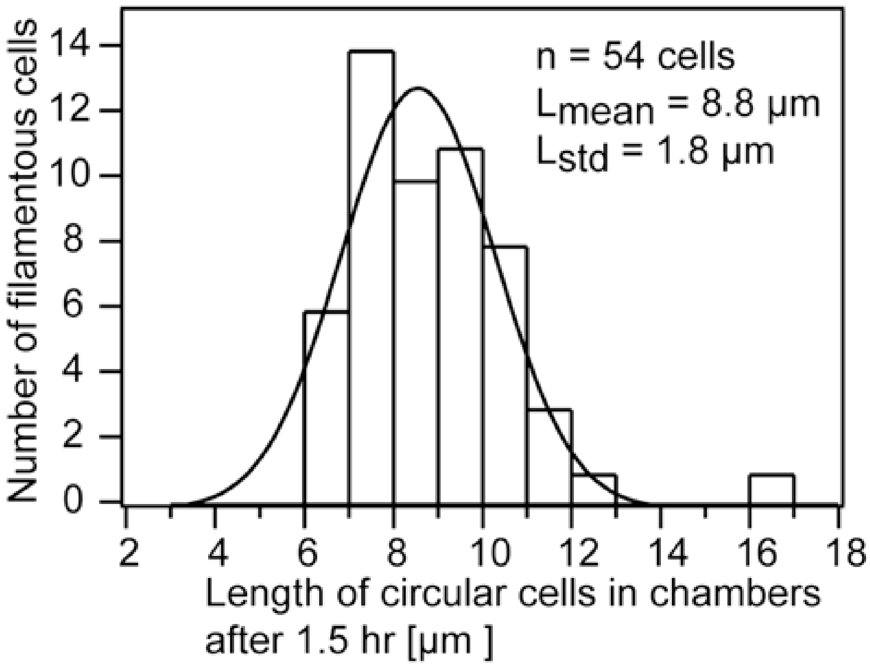
Grow cells of *E. coli* (rods) in chambers

Cells in liquid (with cephalexin)

Cell shape has been manipulated by the application of mechanical constraints

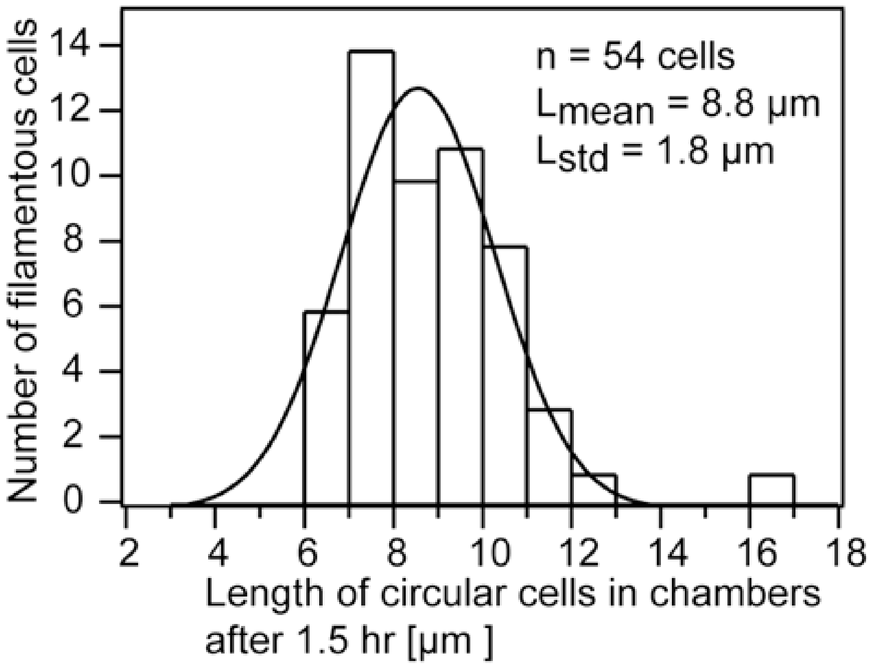
Hysteresis accompanies release of cells

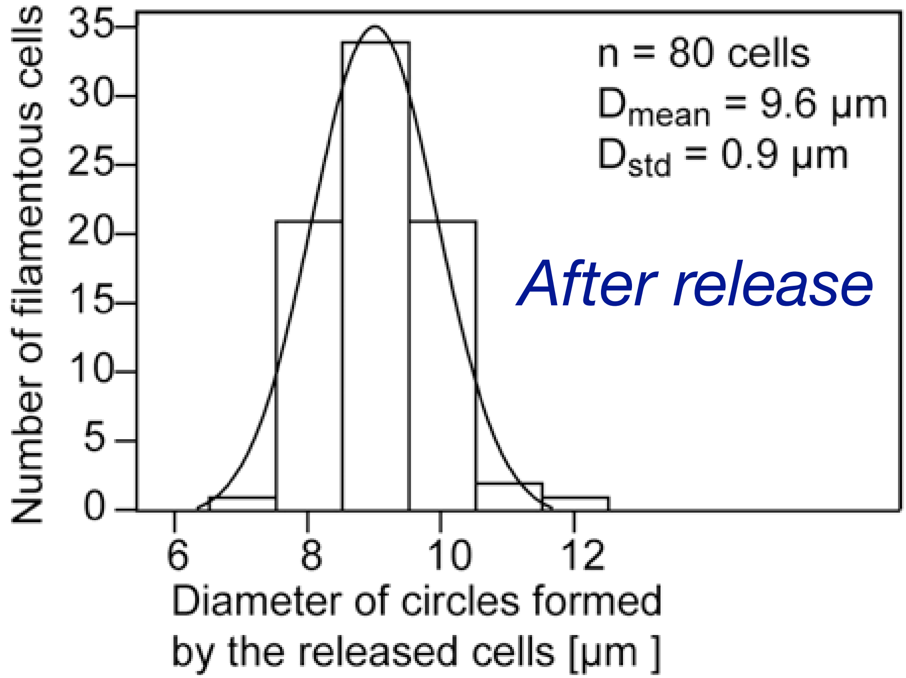
Dimensions of circular chambers: 8 μm diameter; 2.2 μm tall



Hysteresis accompanies release of cells

Dimensions of circular chambers: 8 µm diameter; 2.2 µm tall





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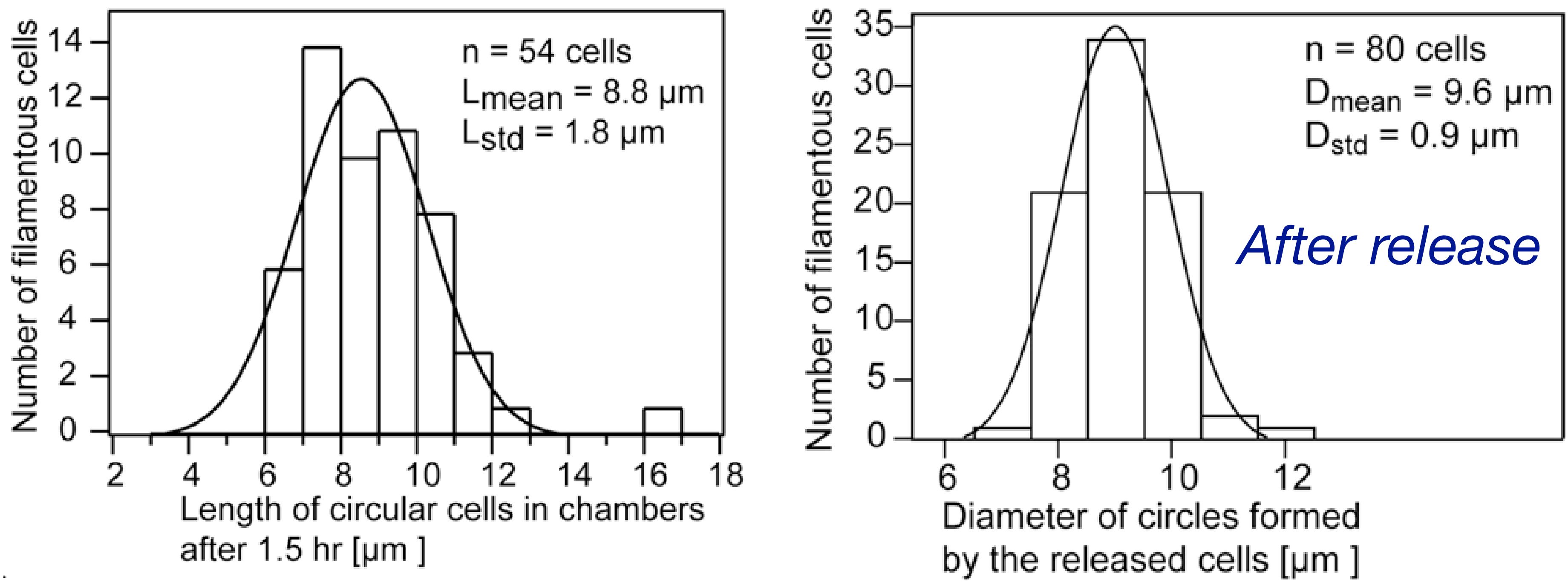
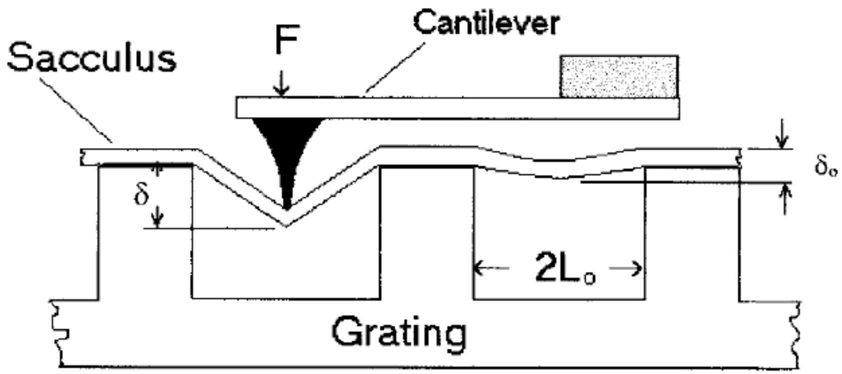


Hysteresis indicates strain on the cell wall

Hysteresis and calculation of γ_{cell}

Serial: measurement of γ_{cell}



Hysteresis and calculation of γ_{cell}

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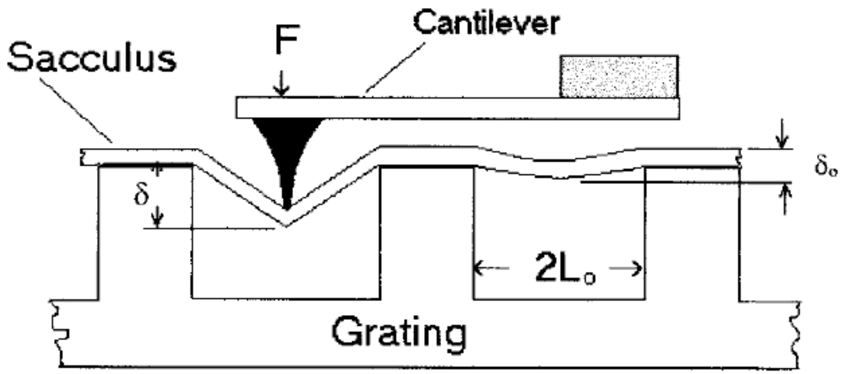
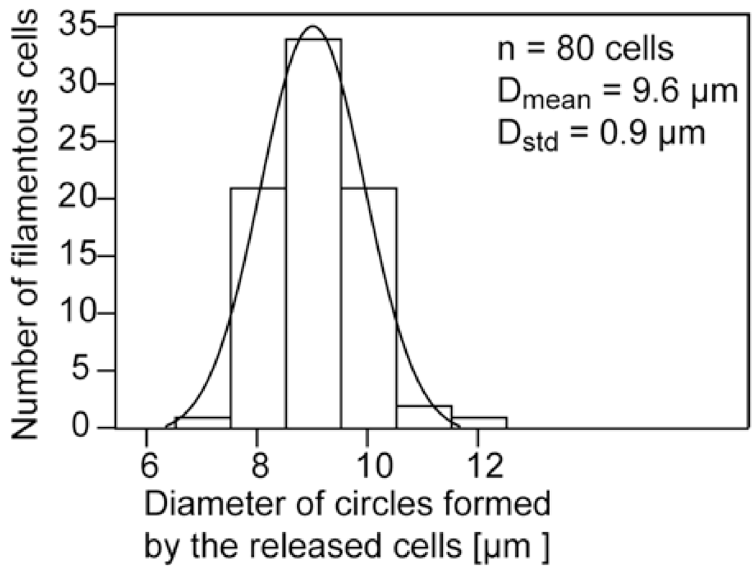
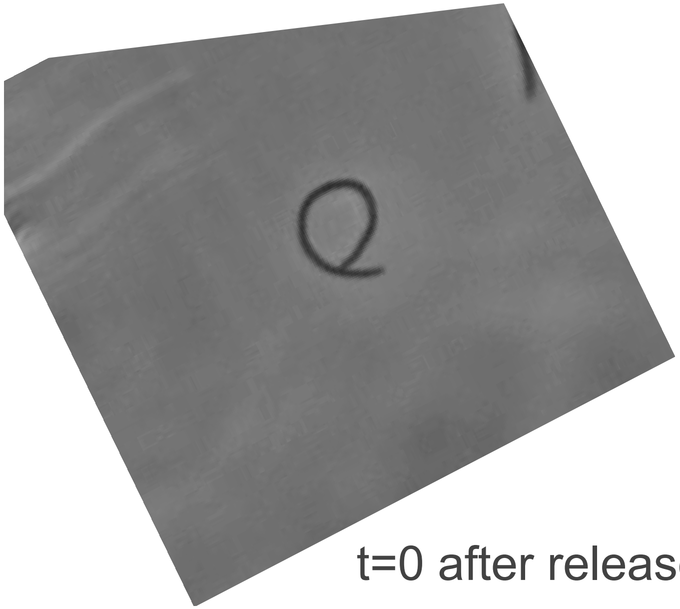


Parallel measurement of γ_{cell}

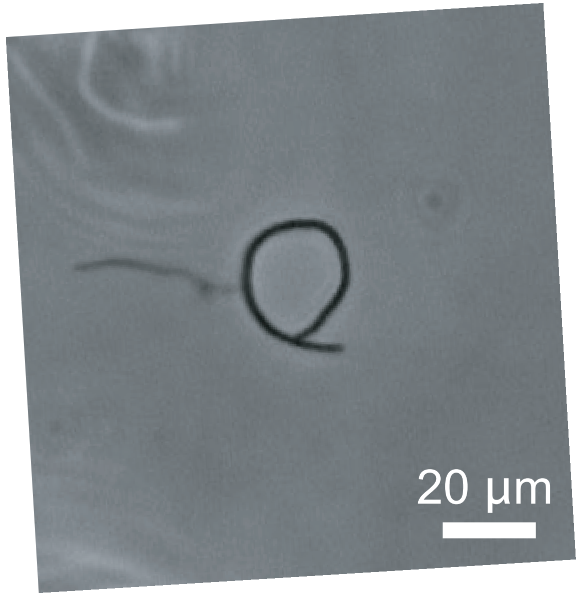
Calculate stress/strain curve using the 'relaxation' of shape after cell release



Engineered cells retain their shape during growth in liquid

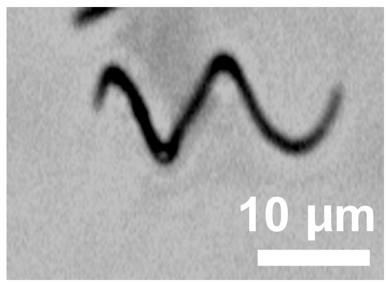


t=0 after release
d=14 μm



t=30 min
d=20 μm

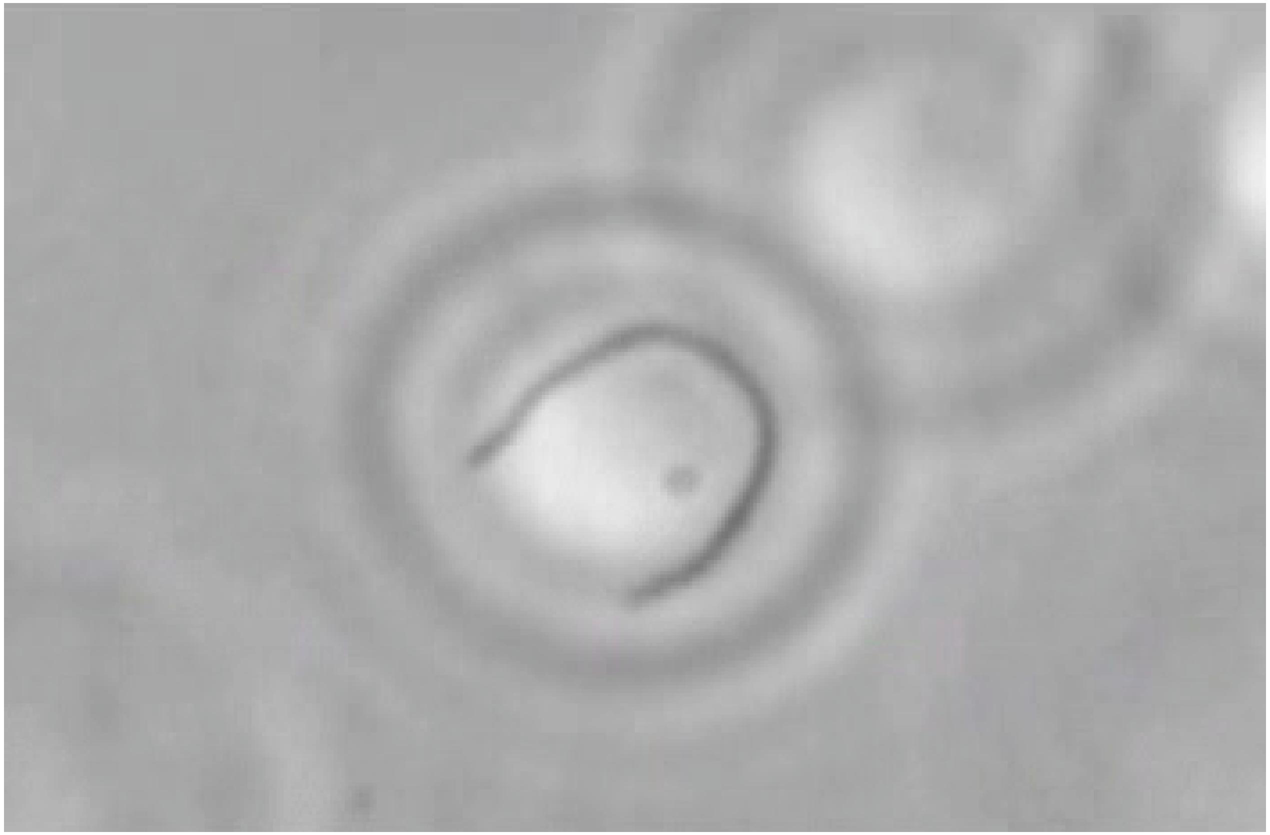
t=0 after release



t=45 min

Cells septate in the absence of cephalexin

Washing out cephalexin causes cells to septate



Motile cells retain their shape

Overview

Part ii (brief)

Evolution of bacterial cell shape

Part iii (brief)

Shape vs hydrodynamics at low Re

Evolution of bacterial shape

Why do cells have defined shapes?

Evolution of bacterial shape

Why do cells have defined shapes?

What is the evolutionary advantage of one shape over another? cocci vs. bacilli vs. spirillium and so on...

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Do we understand what bacteria really look like in their native habitats?

Evolution of bacterial shape

Why do cells have defined shapes?

What is the evolutionary advantage of one shape over another? cocci vs. bacilli vs. spirillium and so on...

Do we understand what bacteria really look like in their native habitats?

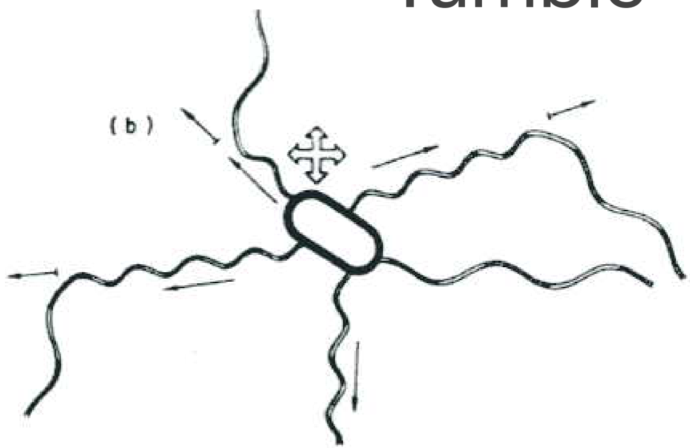
Bacterial motility is one interesting case to consider

E. coli motility requires the bundling of flagella

Run

Protonic Nanomachine Project
http://www.npn.jst.go.jp/index.html

Tumble



Bacterial motility and shape

E. coli (wild type) v_{trans}~10-20 μm sec^{-1}

Turner & Berg

Bacterial motility and shape

Crescents v_{trans}~3-4 µm sec^{-1}

Bacterial motility and shape

Crescents v_{trans}~3-4 μm sec^{-1}

Bacterial motility and shape

Spirals v_{trans}~0 µm sec^{-1}

Bacterial motility and shape

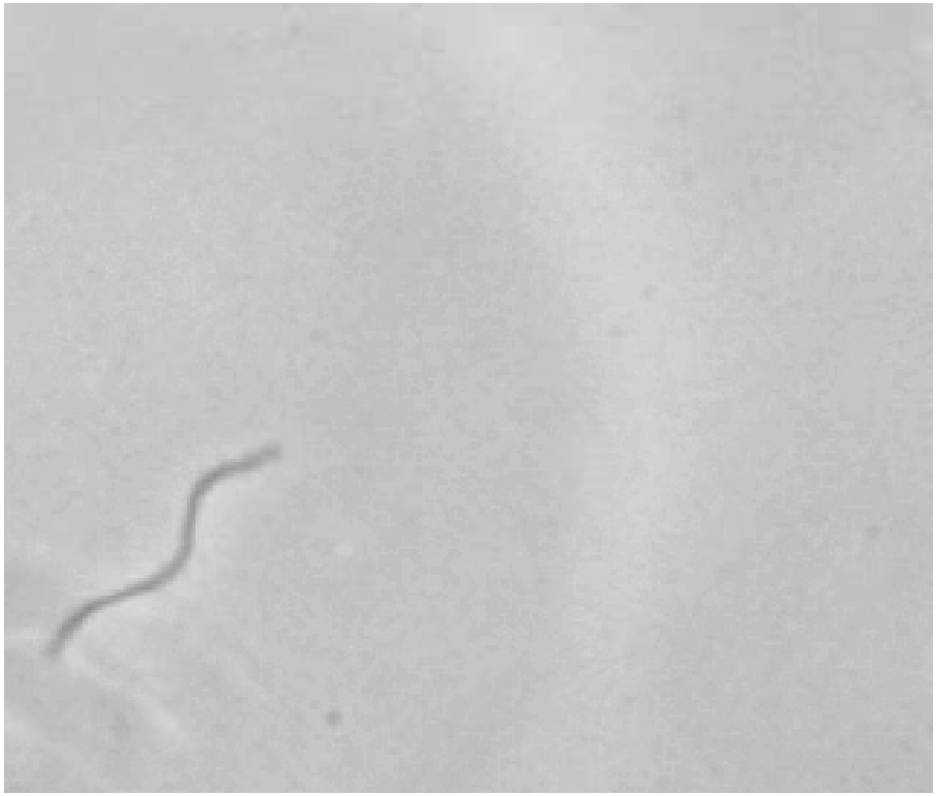
Spirals v_{trans}~0 µm sec^{-1}

Bacterial motility and shape

Extended spirals v_{trans} ~5 µm sec^{-1}

Bacterial motility and shape

Extended spirals v_{trans} ~5 µm sec^{-1}



Hydrodynamics on motility

Are we observing hydrodynamic effects on the cell body?

Hydrodynamics on motility

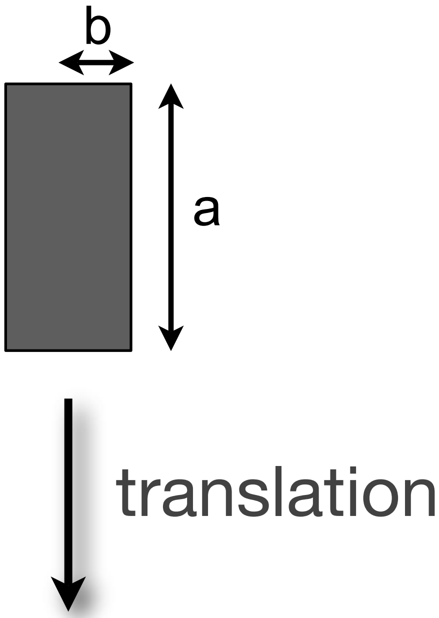
Are we observing hydrodynamic effects on the cell body?

Slender body theorum

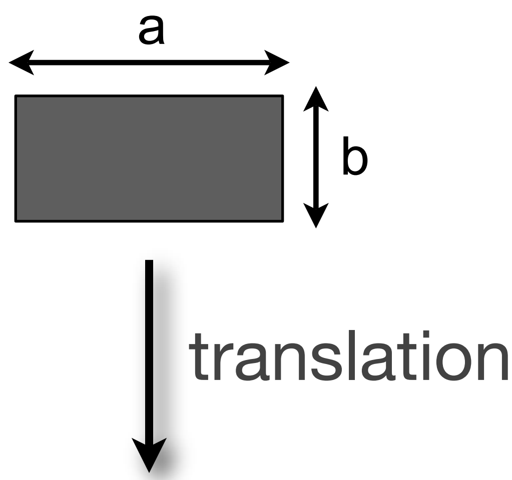
Hydrodynamics on motility

Are we observing hydrodynamic effects on the cell body?

Slender body theorum



$$f = \frac{4\pi\eta a}{\ln\frac{2a}{b} - \frac{1}{2}}$$

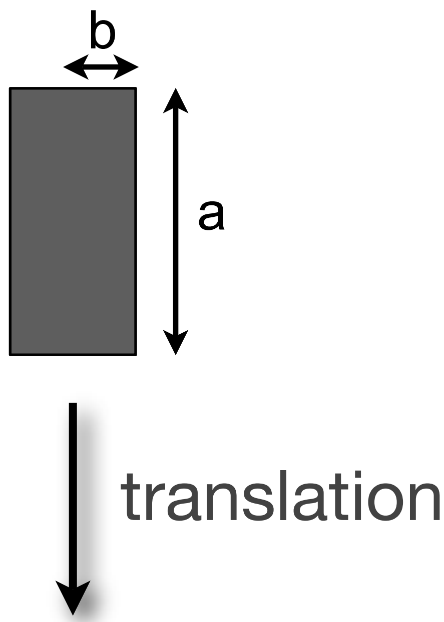


$$f = \frac{8\pi\eta a}{\ln\frac{2a}{b} + \frac{1}{2}}$$

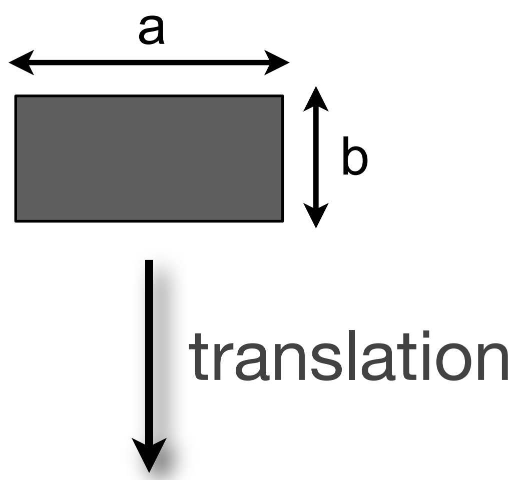
Hydrodynamics on motility

Are we observing hydrodynamic effects on the cell body?

Slender body theorum



$$f = \frac{4\pi\eta a}{\ln\frac{2a}{b} - \frac{1}{2}}$$



$$f = \frac{8\pi\eta a}{\ln\frac{2a}{b} + \frac{1}{2}}$$

Probably not

Hydrodynamics on motility

Are we observing hydrodynamic effects on the flagellum?

Bundling

Hydrodynamics on motility

Are we observing hydrodynamic effects on the flagellum?

Bundling

Probably

Hydrodynamics on motility

Are we observing hydrodynamic effects on the flagellum?

Bundling

Why have multiple flagella if curvature prevents them from bundling?

Probably

Hydrodynamics on motility

Are we observing hydrodynamic effects on the flagellum?

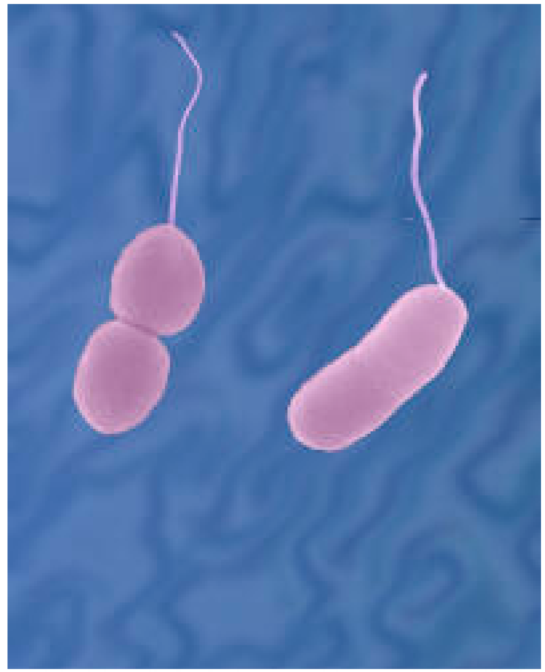
Bundling

Why have multiple flagella if curvature prevents them from bundling?

Flagella are ‘expensive’ from an energetic standpoint

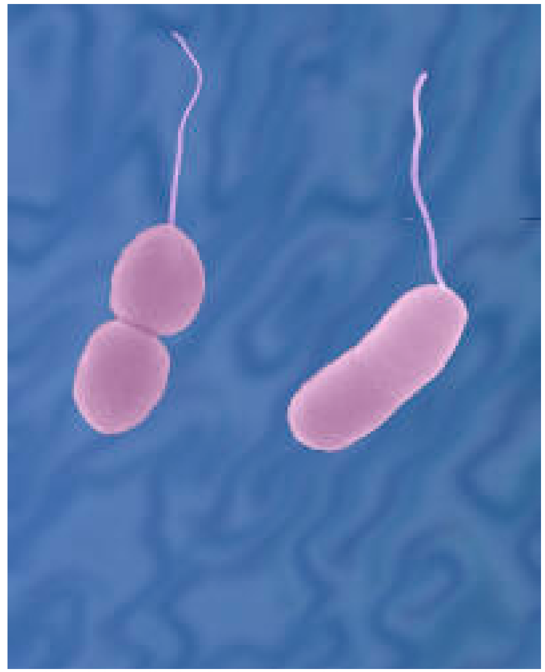
Probably

Have motile cells evolved shapes that maximize the bundling of flagella?



Have motile cells evolved shapes that maximize the bundling of flagella?

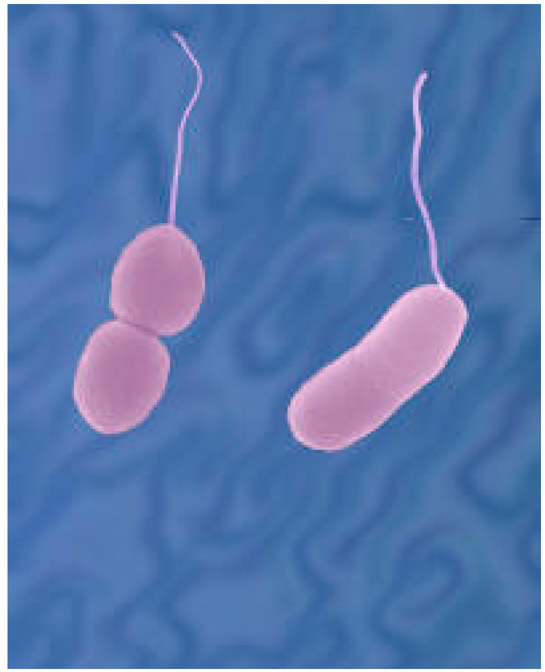
Vibrio have evolved into crescents. 21/24 species of Vibrio we have looked at have a crescent shape and a single, polar flagellum



Have motile cells evolved shapes that maximize the bundling of flagella?

Vibrio have evolved into crescents. 21/24 species of Vibrio we have looked at have a crescent shape and a single, polar flagellum

E. coli and other multi-flagellated strains of motile bacteria are rod-shaped



Conclusion

Relationship between intracellular organization and bacterial cell shape

Our approach is to develop new capabilities for manipulating and studying bacterial cells

New techniques for intracellular imaging

Theoretical models for studying the evolution of shape

Acknowledgements

Group

Matt Copeland
Hannah Tuson
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Joe Molenda
Charlie Burns
Sean McMaster

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