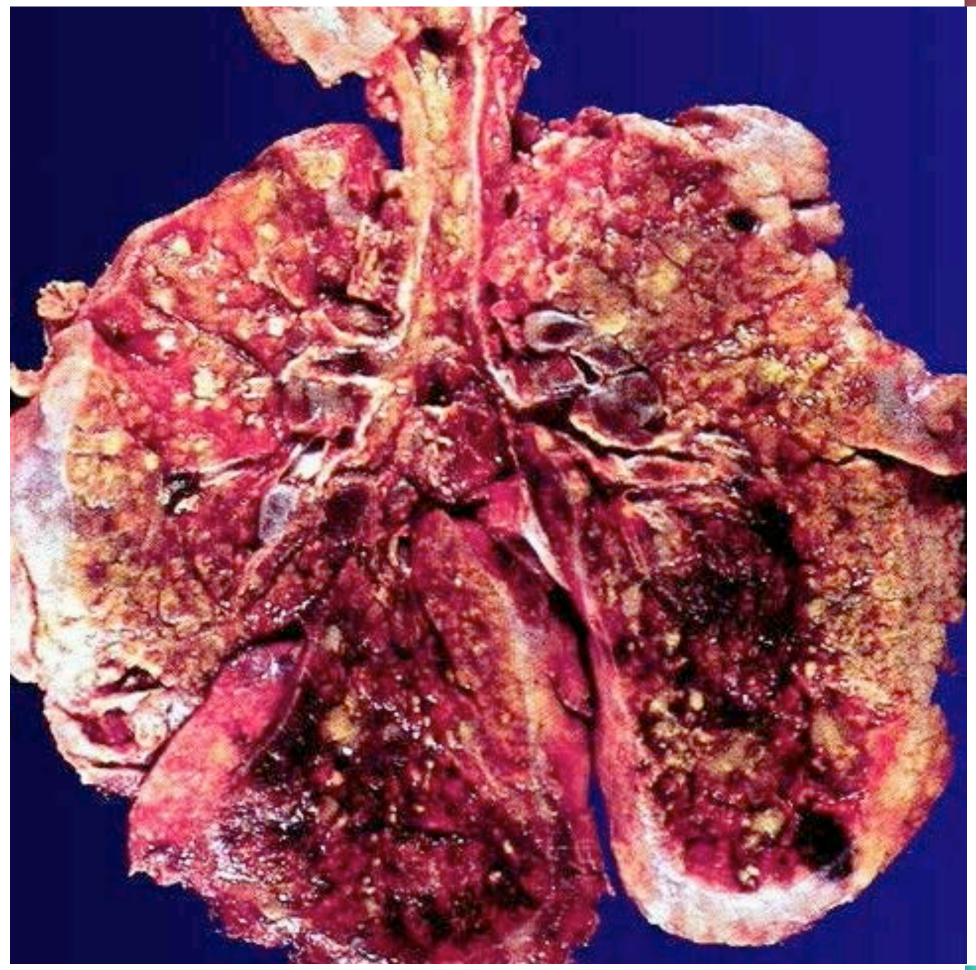
Mechanobiology of bacterial multicellularity

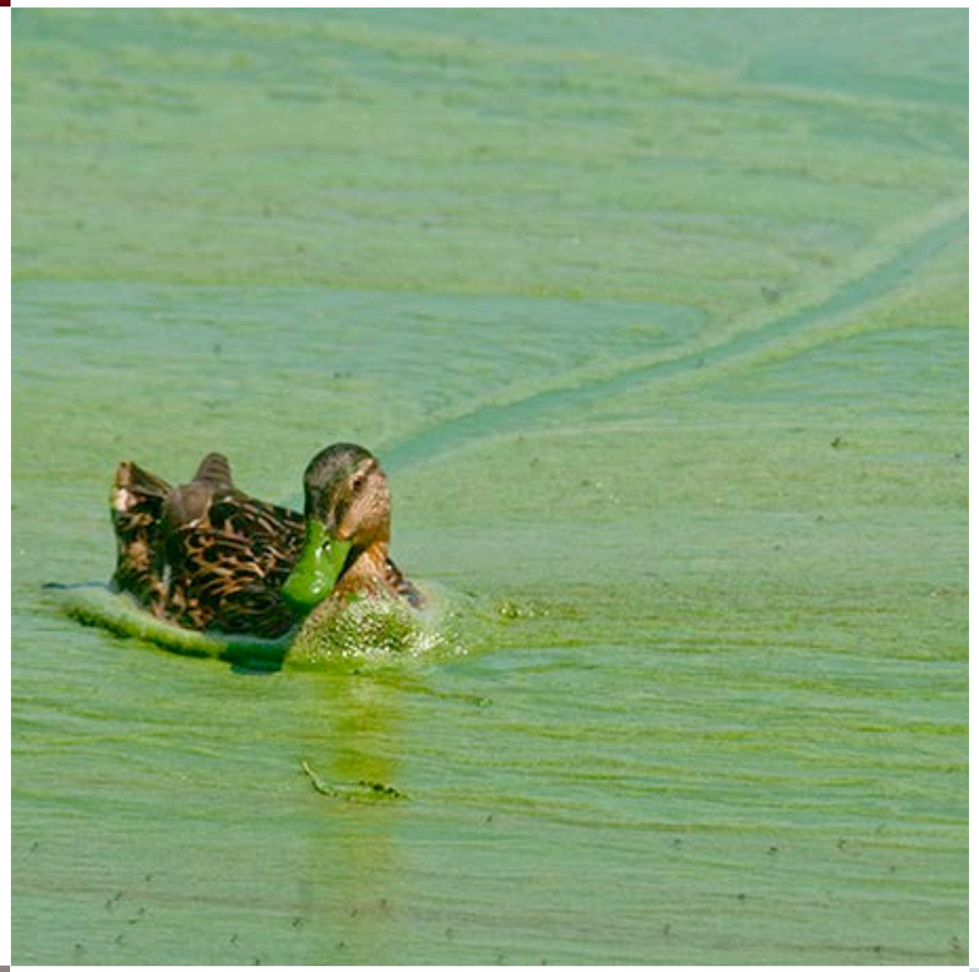
ME-480 Lecture 3

Lecture 3 outline

- Biofilms: what are they?
- The mechanics of biofilm formation
- Flow regulates biofilm architecture
- Biofilms generate force

Biofilms



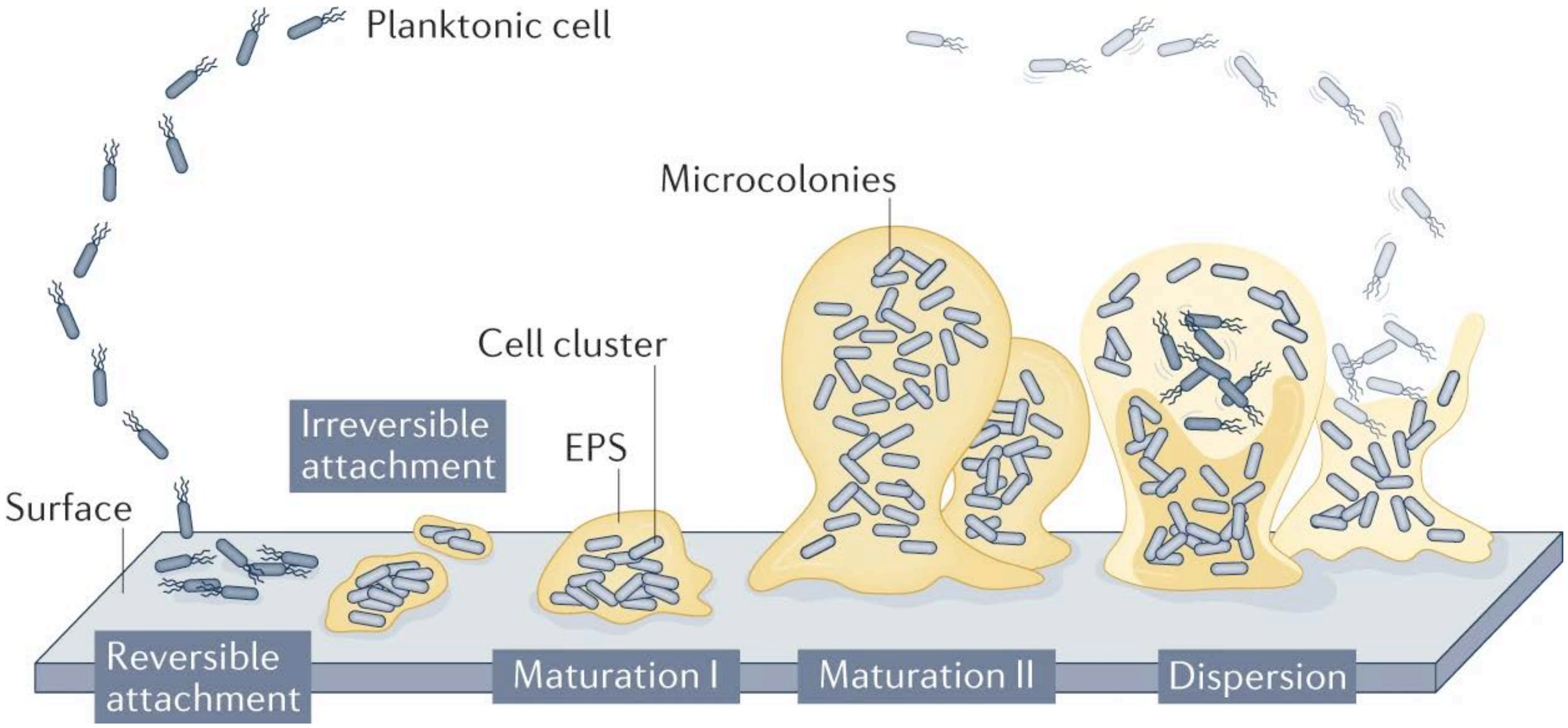


Images:
waterandhealth.org
www.eawag.ch
wikipedia.org
www.cs.montana.edu

Biofilms are ubiquitous

- Biofilms are the main form of life of terrestrial microbes: 90% of the biomass
- Biofilms contains thousands to billons of bacteria
- They are very resilient against external chemical and physical stresses.
- They’re a huge problem most of the time (in hospital and industry)

The biofilm lifecycle



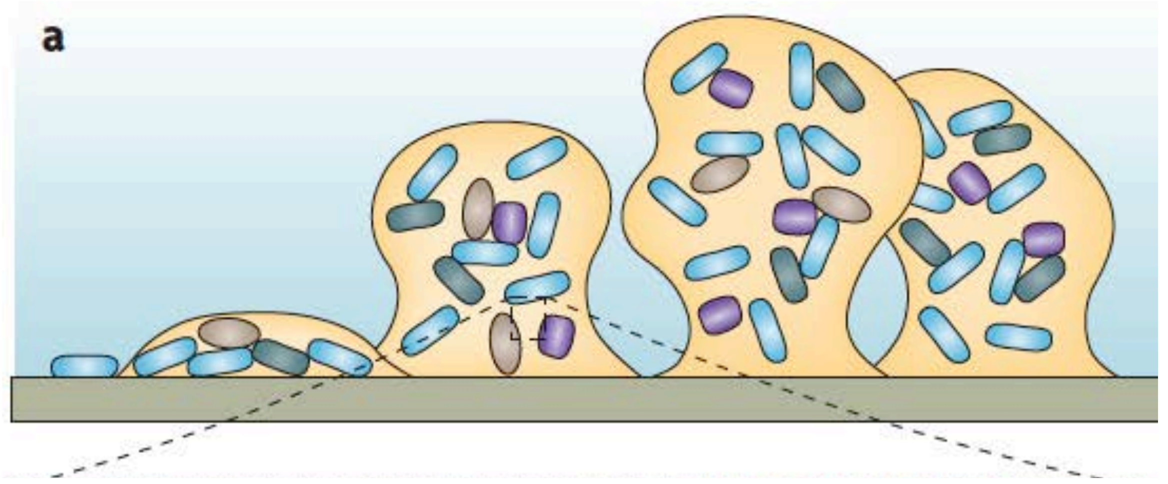
The classic 3-step model

Sauer, *Nat Rev Micro,* 2022

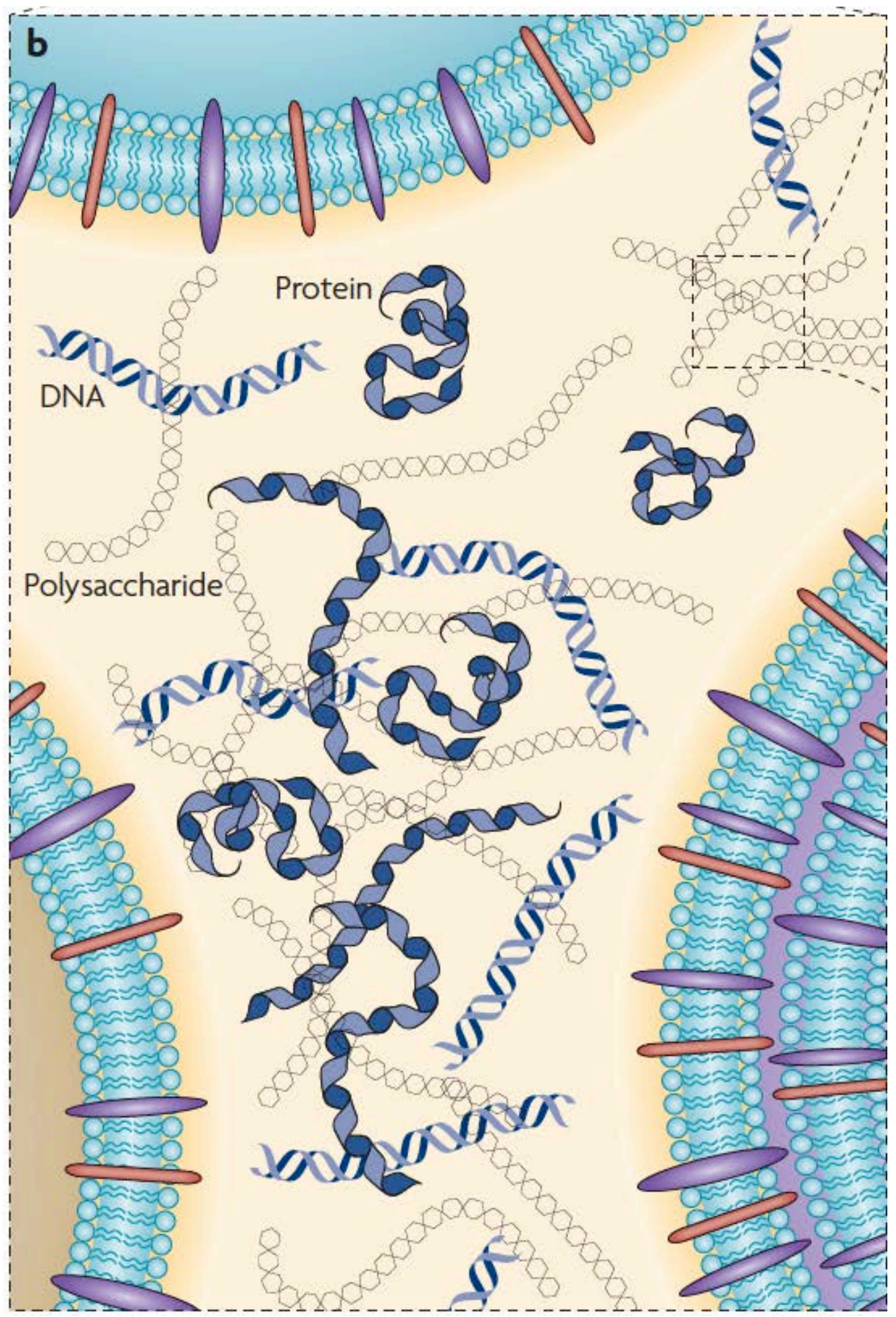
Zooming in on the biofilm matrix

the extracellular polymeric substance (EPS) matrix is a cement for biofilms

Main function: mechanically maintain the cohesion of the community

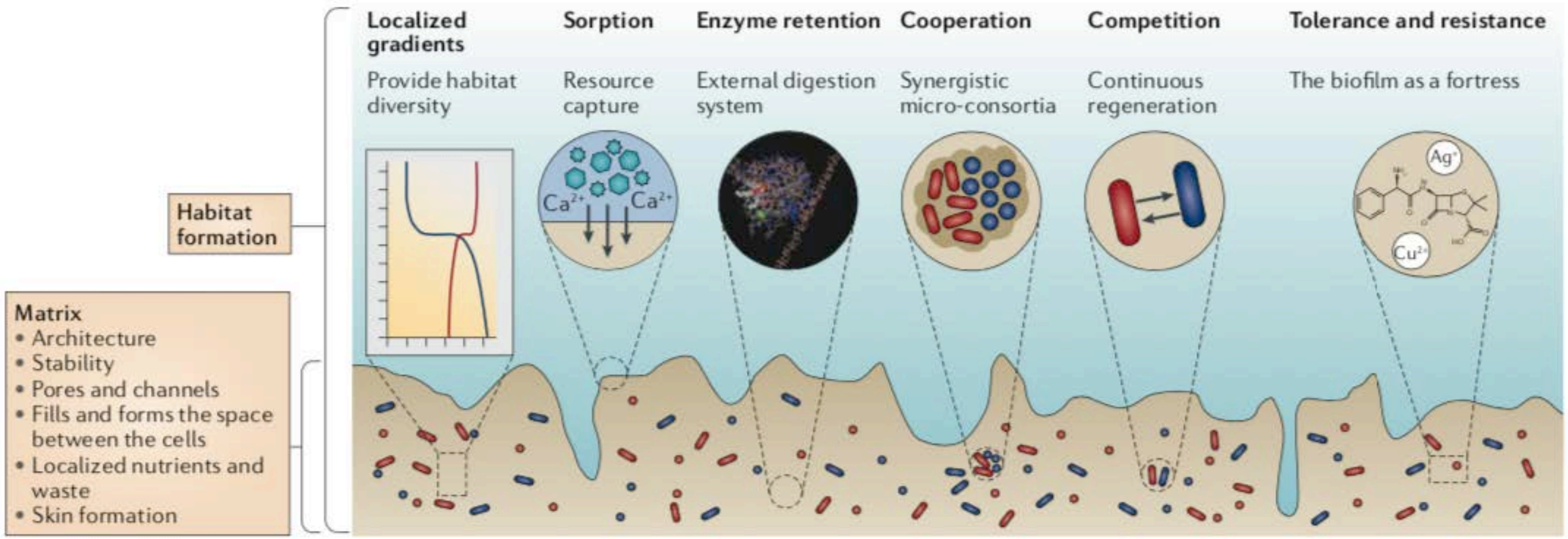


The properties of the matrix sets the architecture of the biofilm and the spatial organization of single cells



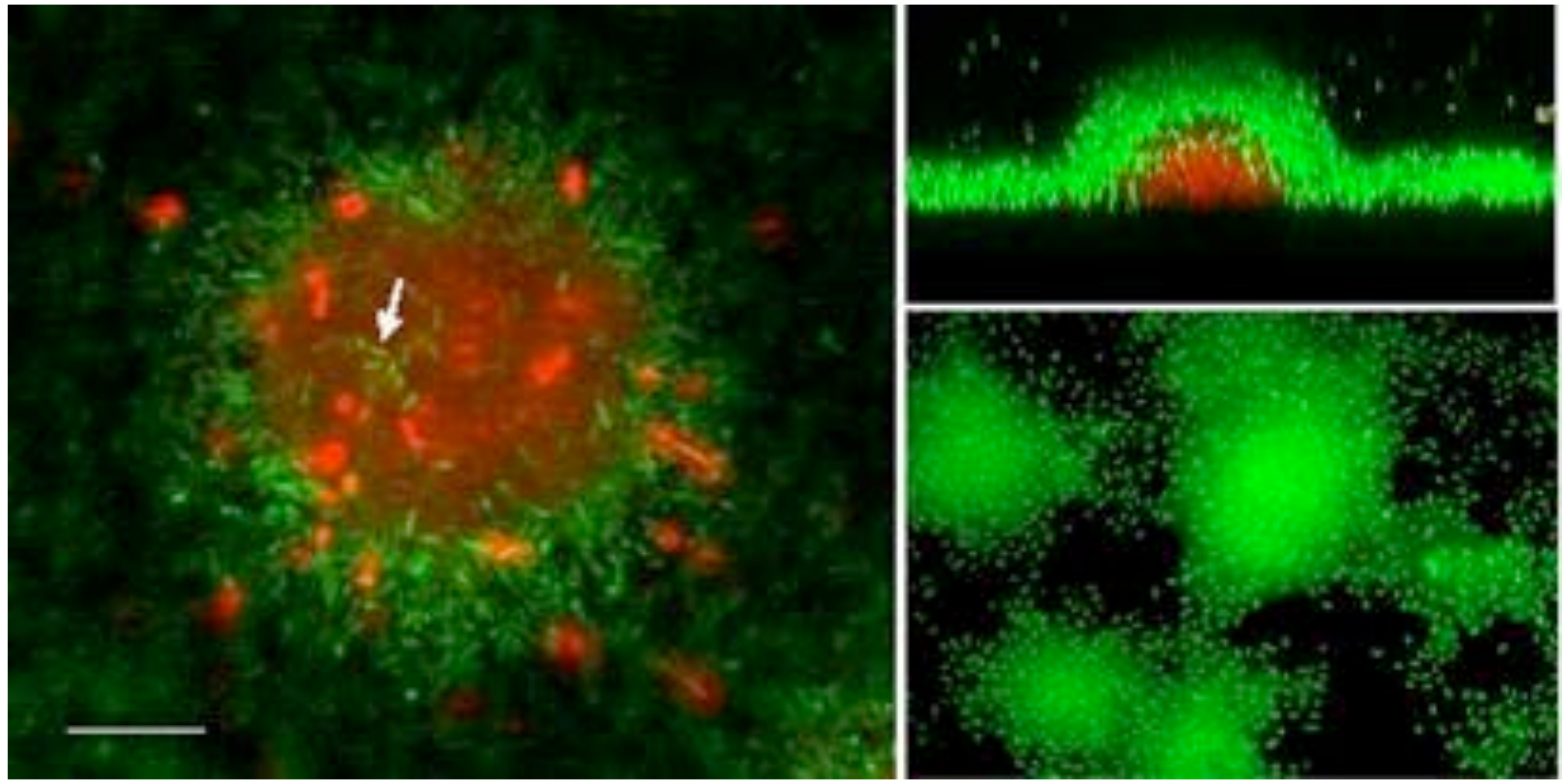
The advantages of living in a biofilm

the matrix has many beneficial physico-chemical properties

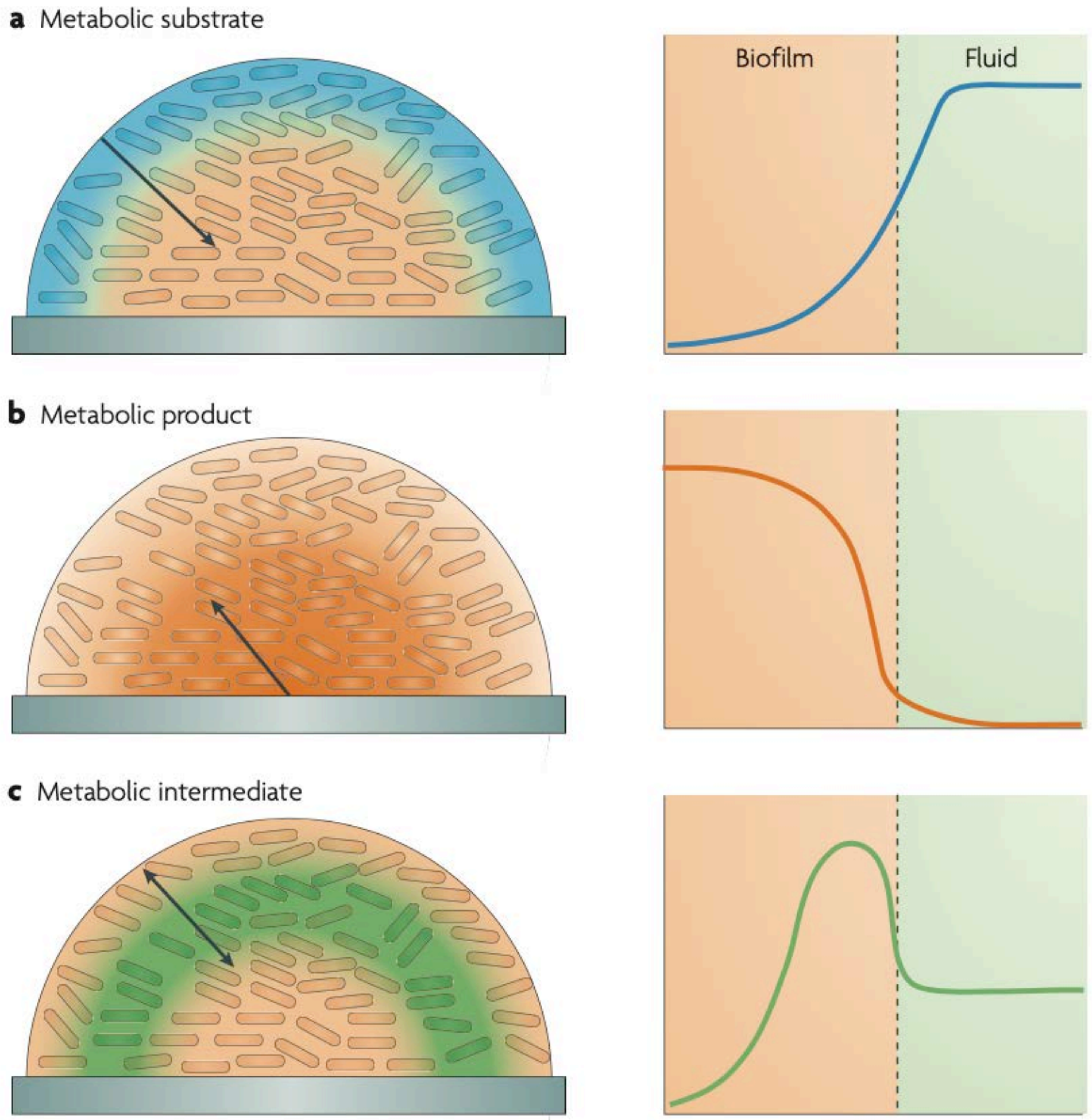


Cellular heterogeneity in biofilms

Molecular transport define cellular landscape



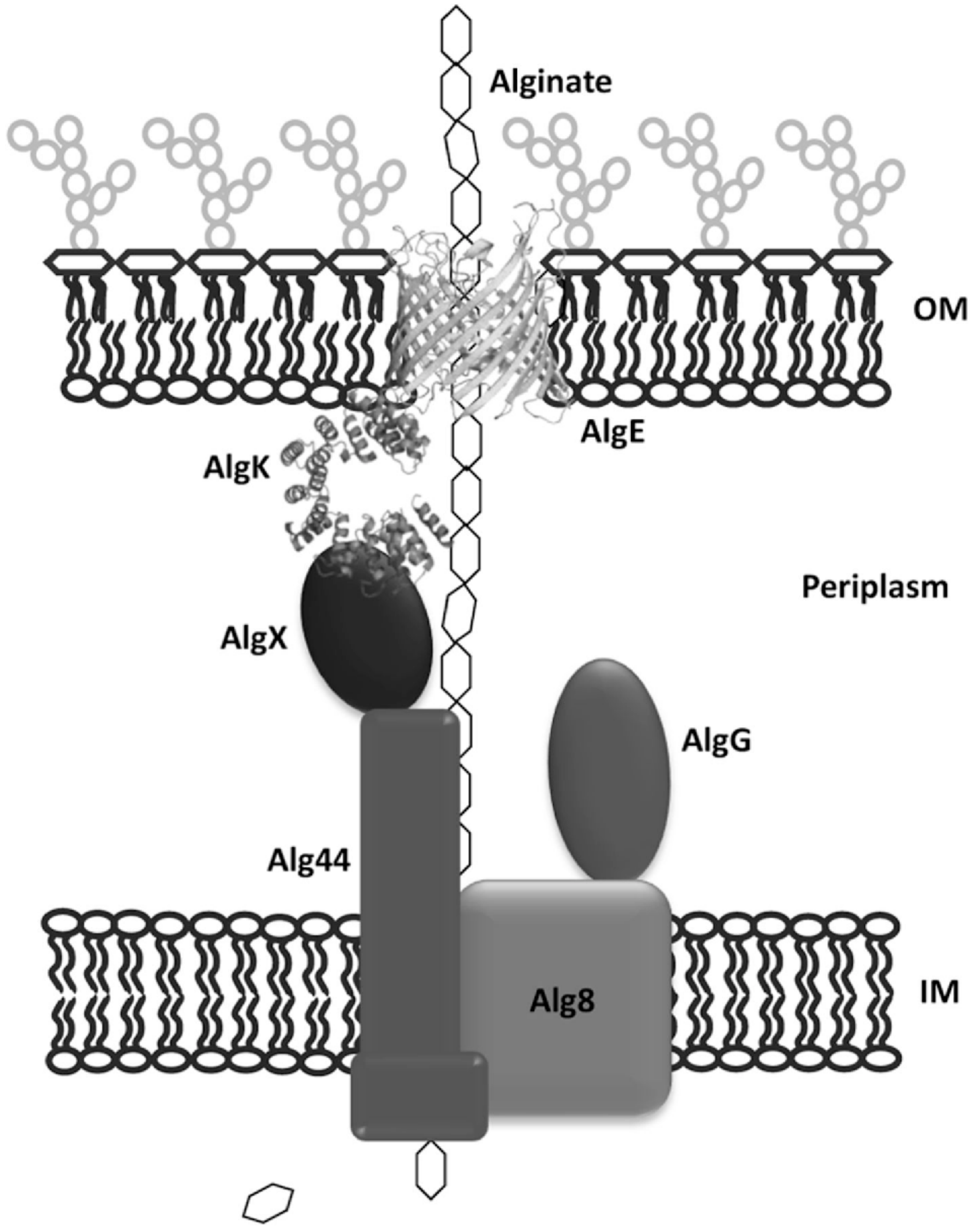
Live dead stain of a *Pseudomonas aeruginosa* biofilm

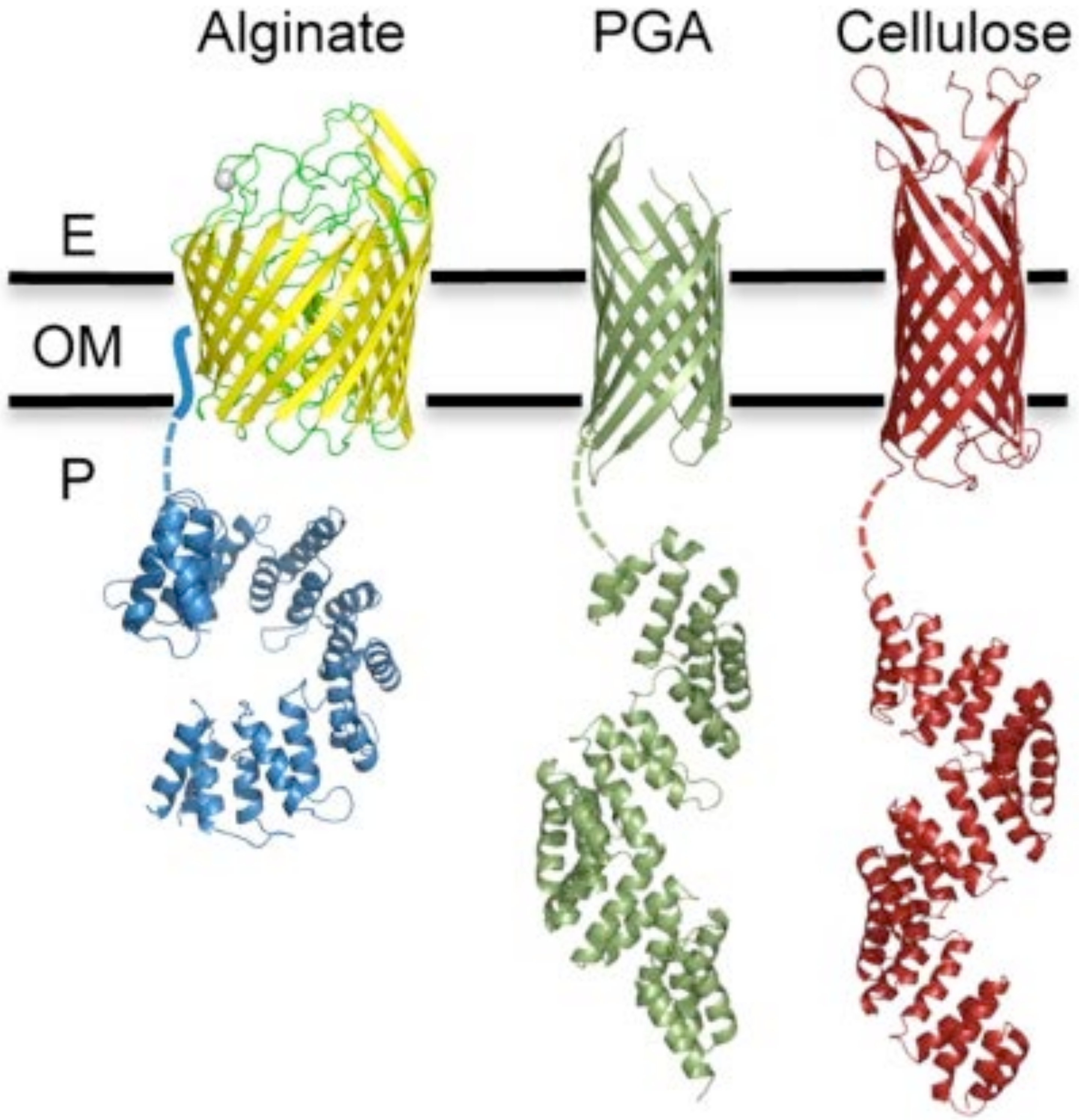


How do cells make EPS matrix?

Polysaccharide secretion systems

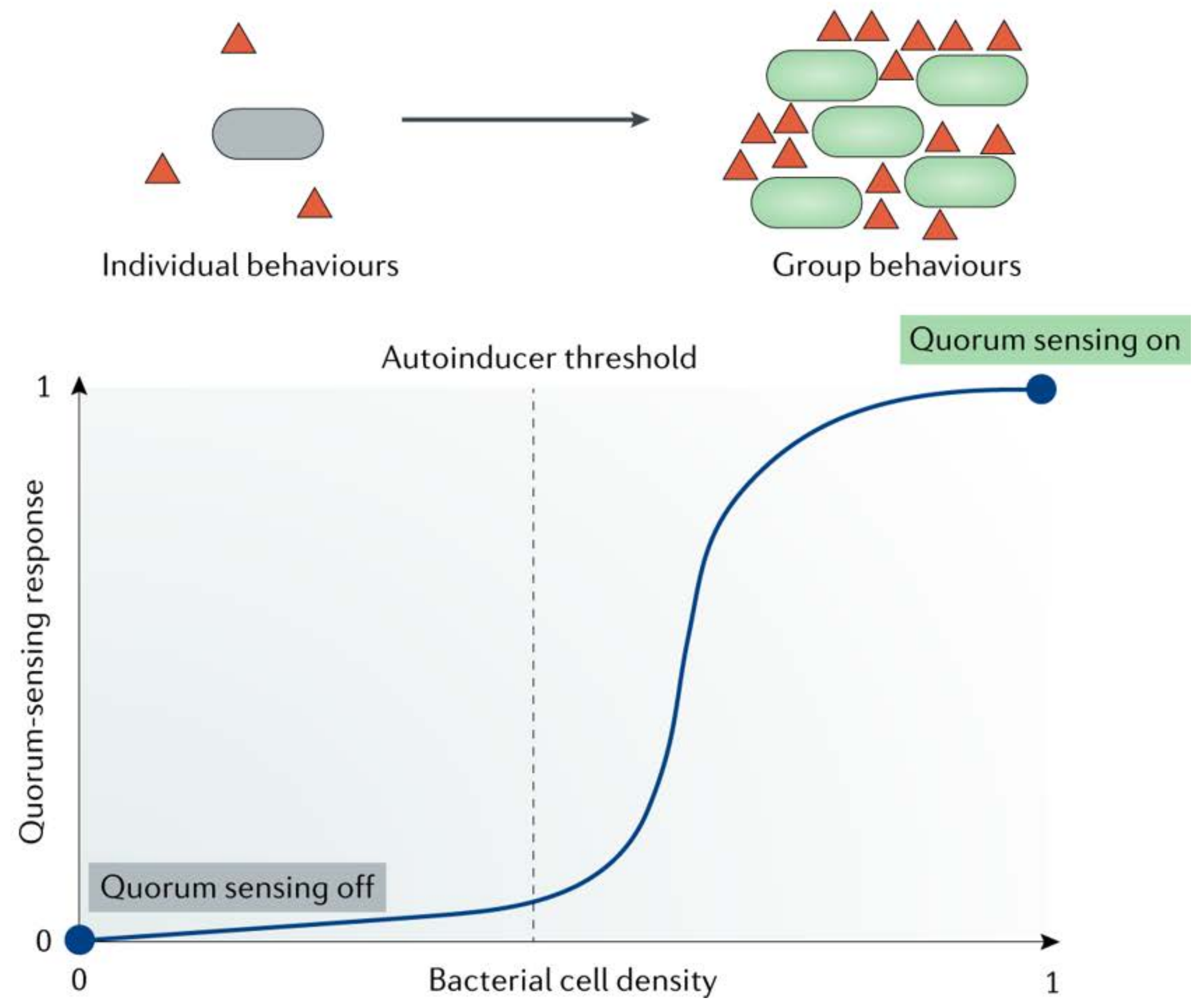
Alginate secretion system





Single cells synchronize to build biofilms

- Single cells secrete signaling molecule (autoinducer)
- Autoinducer level depend on bacterial density
- Single cells sense autoinducer levels
- Response is dependent on concentration

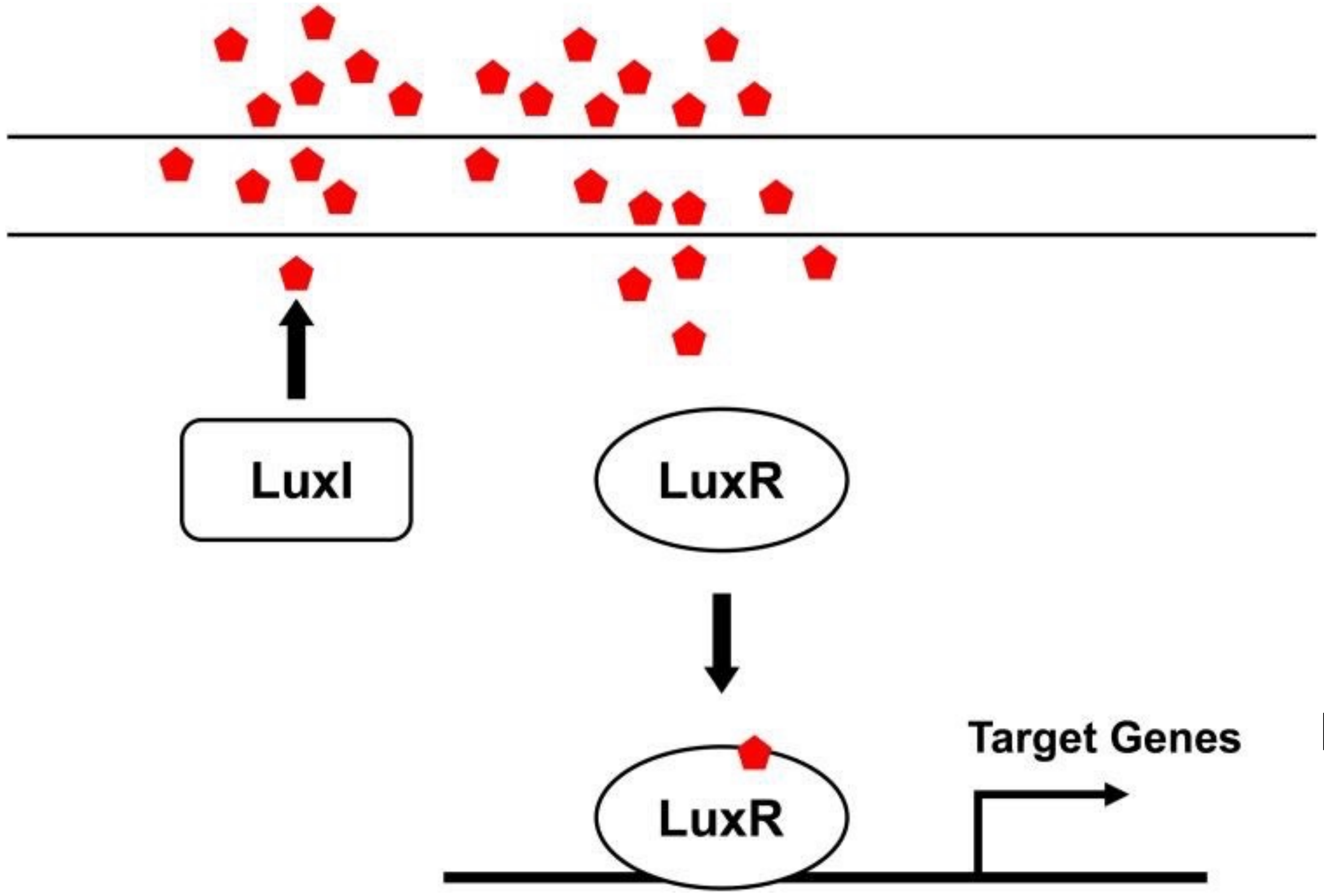


Quorum sensing regulates a breadth of collective phenotypes:

- virulence (patogenicity)
- light production
- biofilm formation

hawaiian bobtail squid
and *Vibrio harveyi* (camouflage)

Quorum sensing system



For example: biofilm matrix

Biofilm architecture

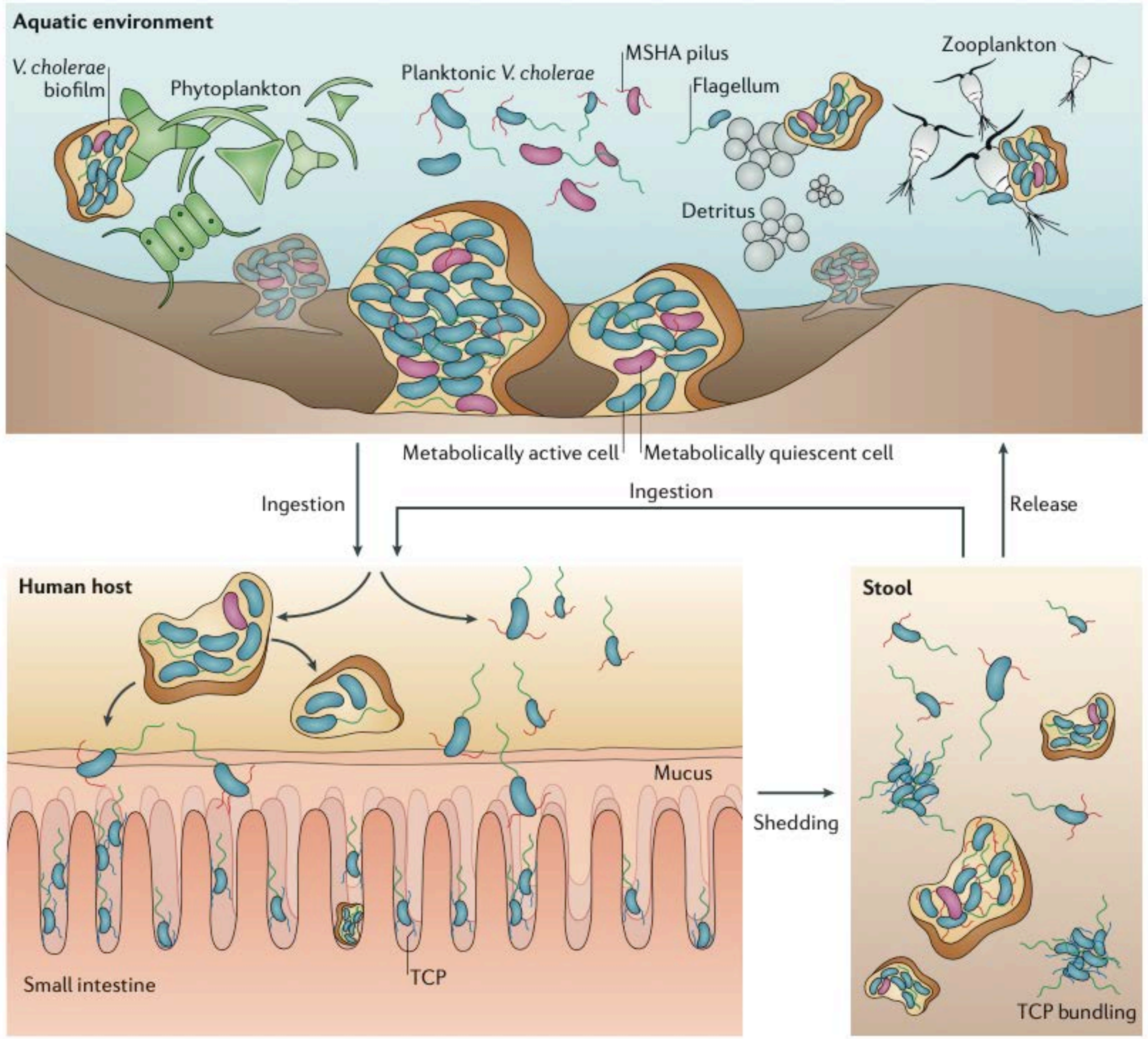
Do bacteria build their biofilms randomly?

- Do they know it’s time to form biofilm?
- Do they choose where and when to secrete matrix?
- Does the biofilm aquire a specific architecture?

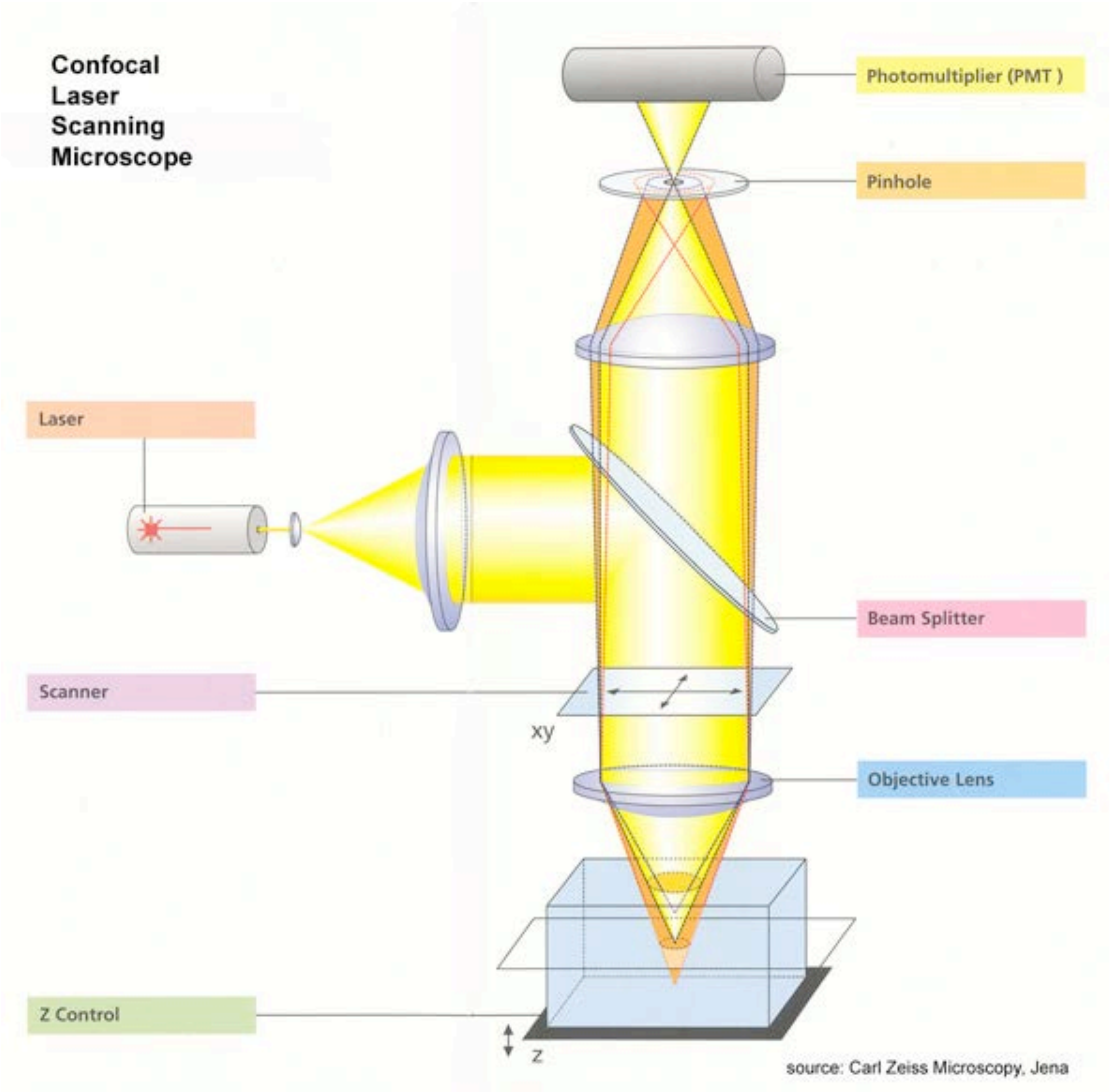
Vibrio cholerae as a model for biofilms

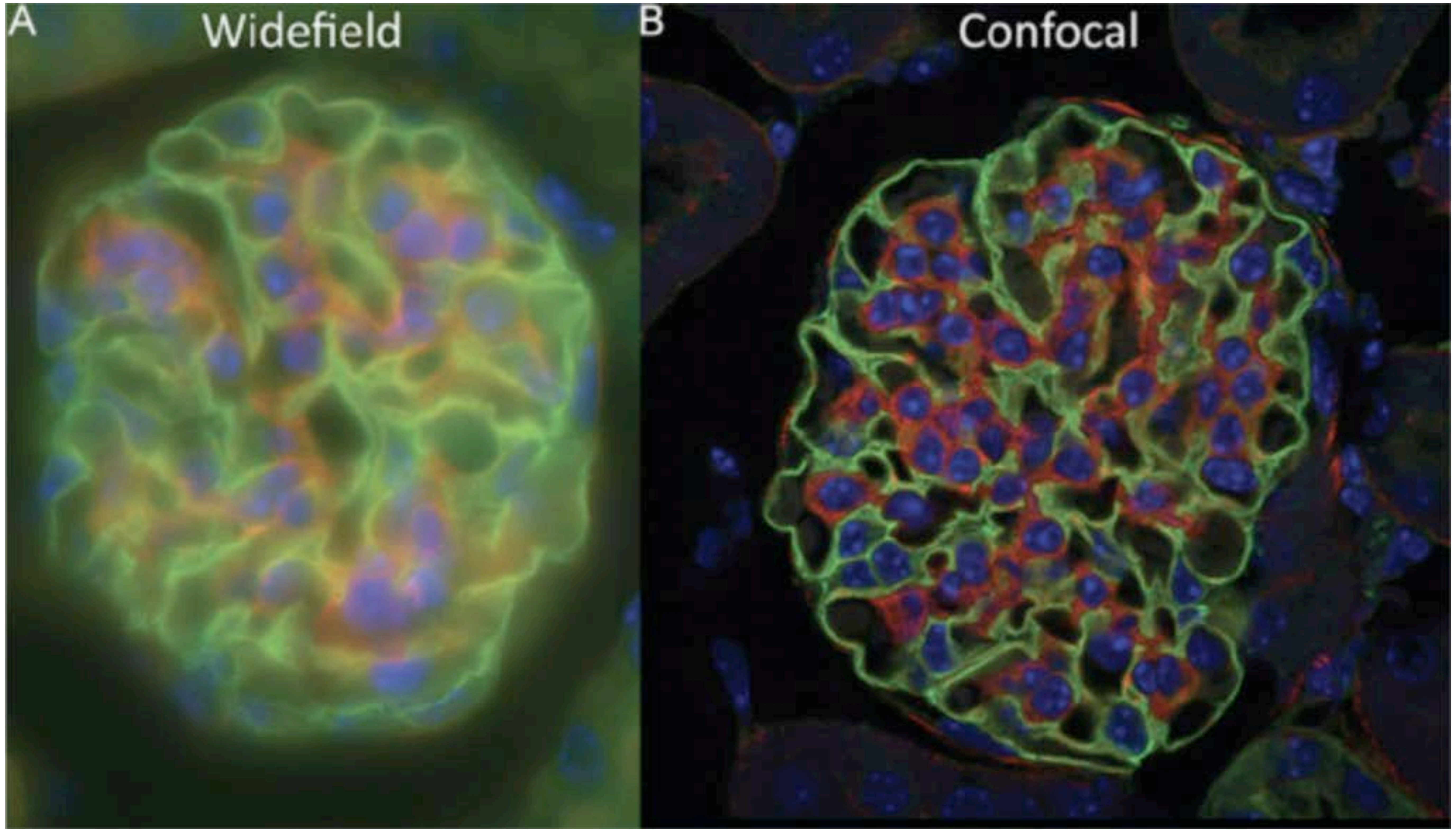
V. cholerae biofilms in the wild

Vibrio cholerae biofilms play a major role in pathogenicity



Imaging biofilms in 3D

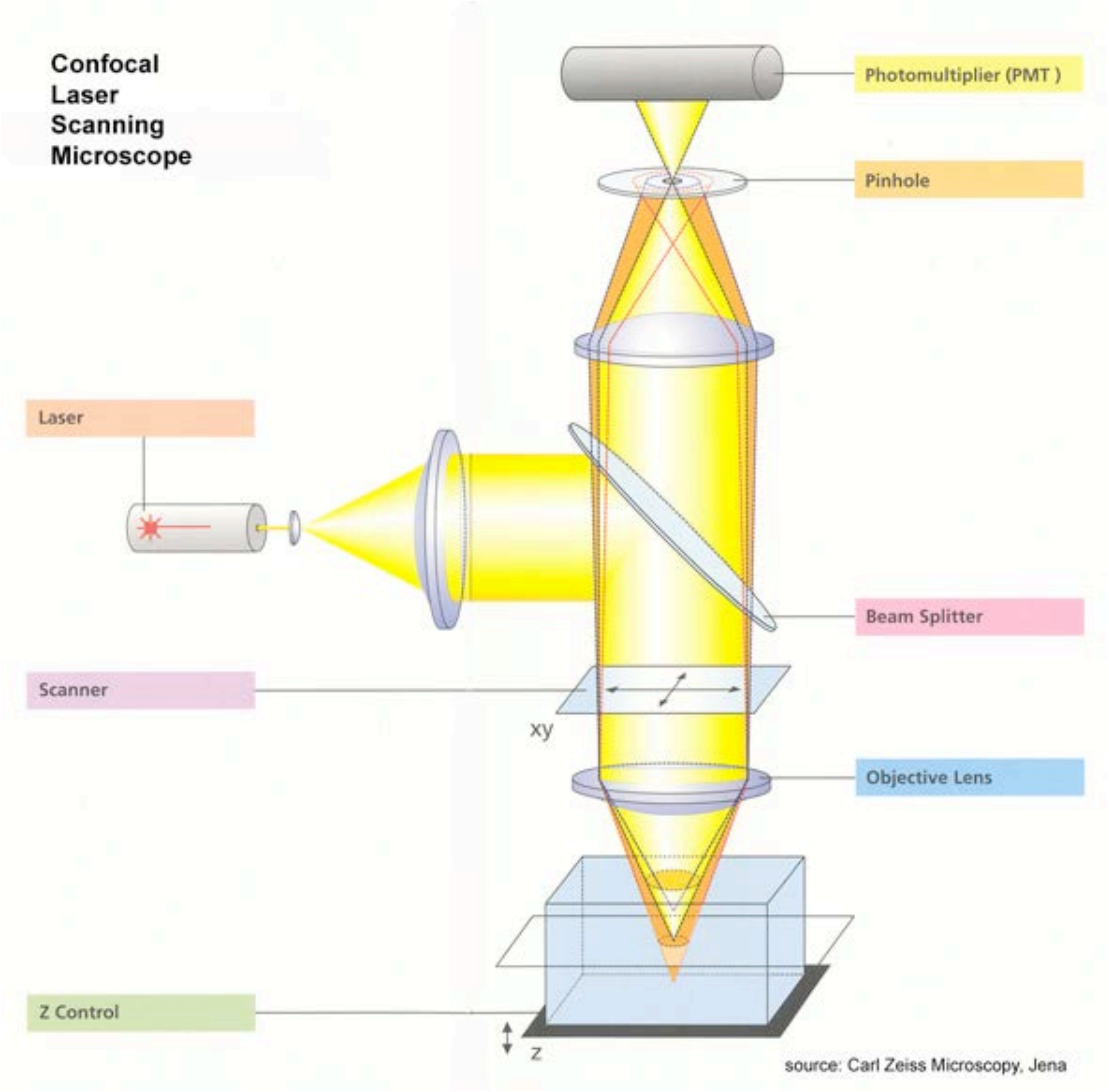




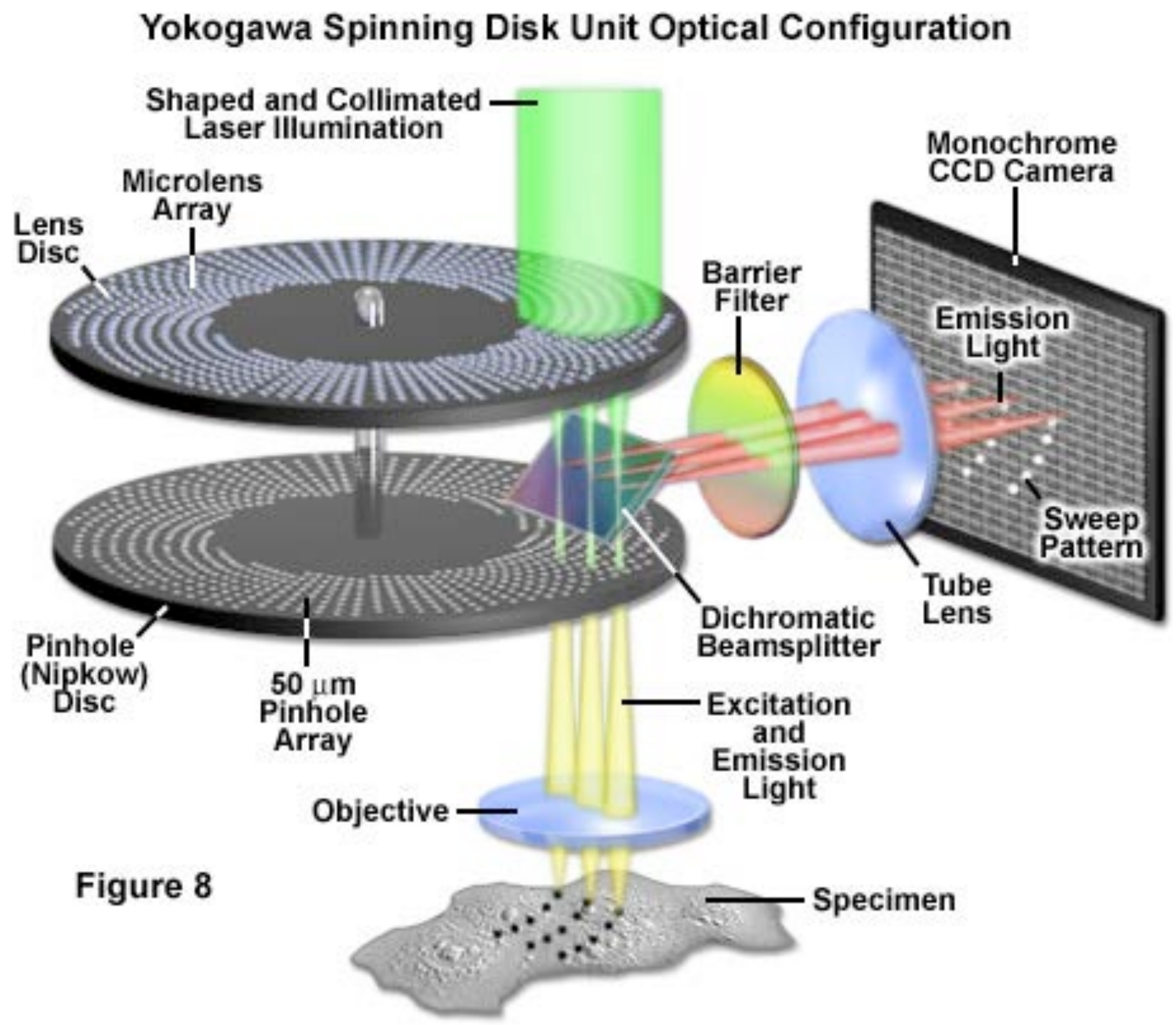
Confocal fluorescence microscopy

Imaging live biofilms in 3D

Icrease imaging speed: spinning disk



Confocal fluorescence microscopy



Spinning disk confocal microscopy

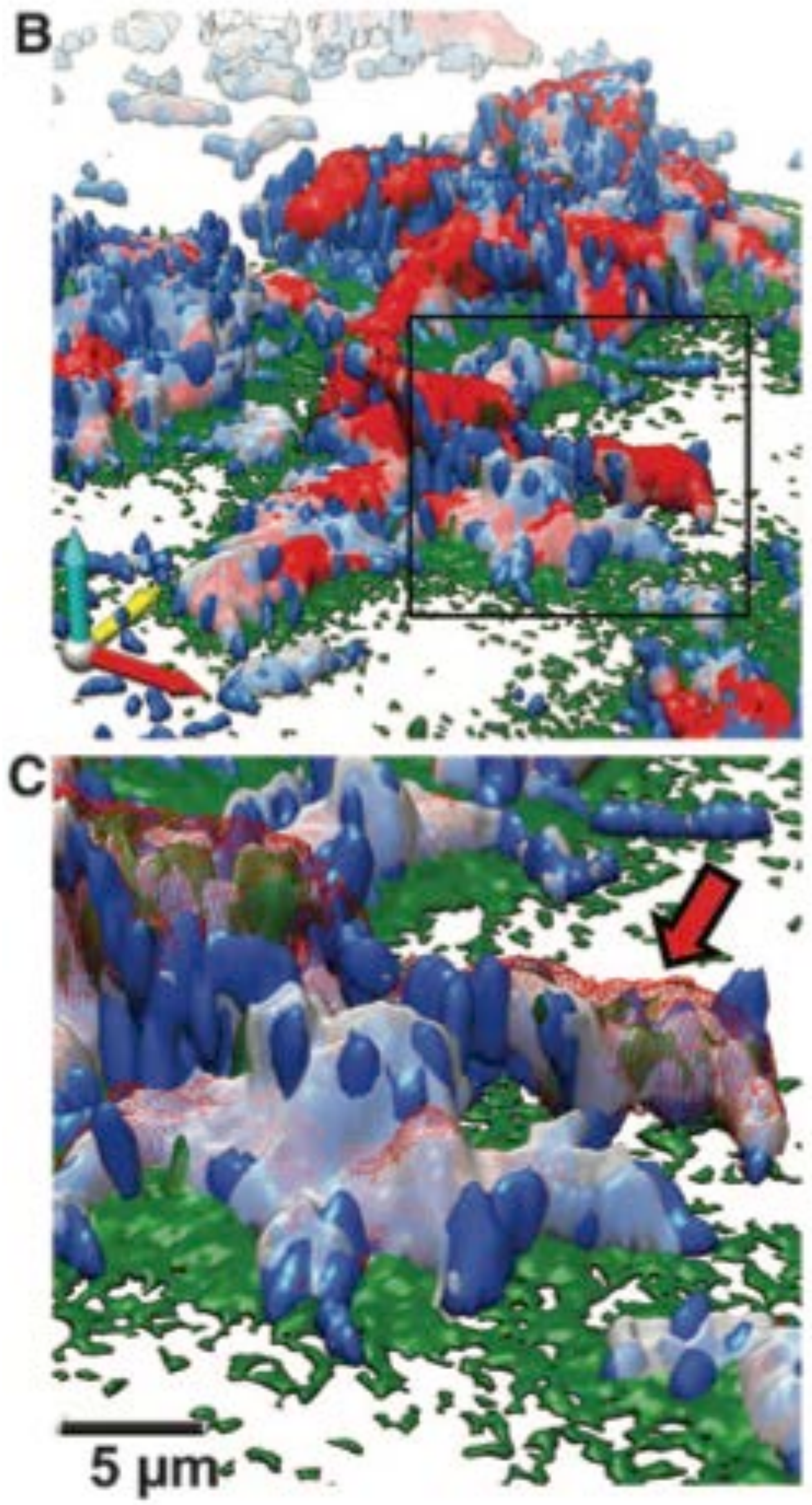
Coordination of matrix production

A precise spatial pattern

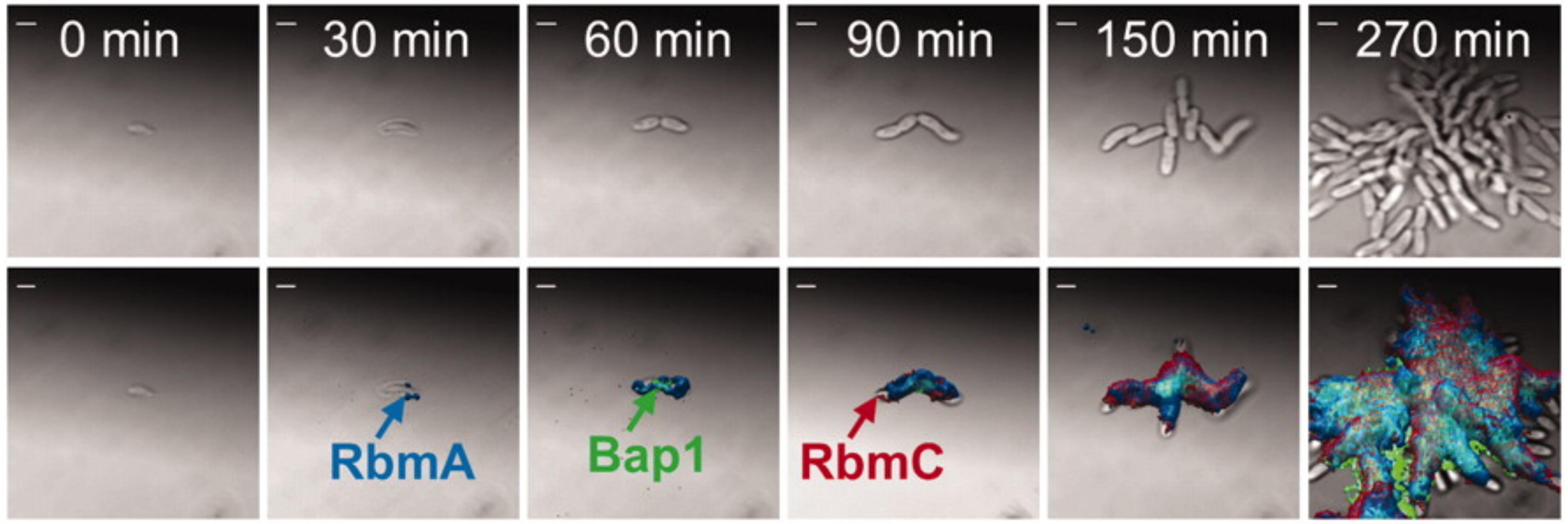
Vibrio produces 4 main matrix components

- proteins: RbmA, Bap1, RbmC
- polysaccharide: vps

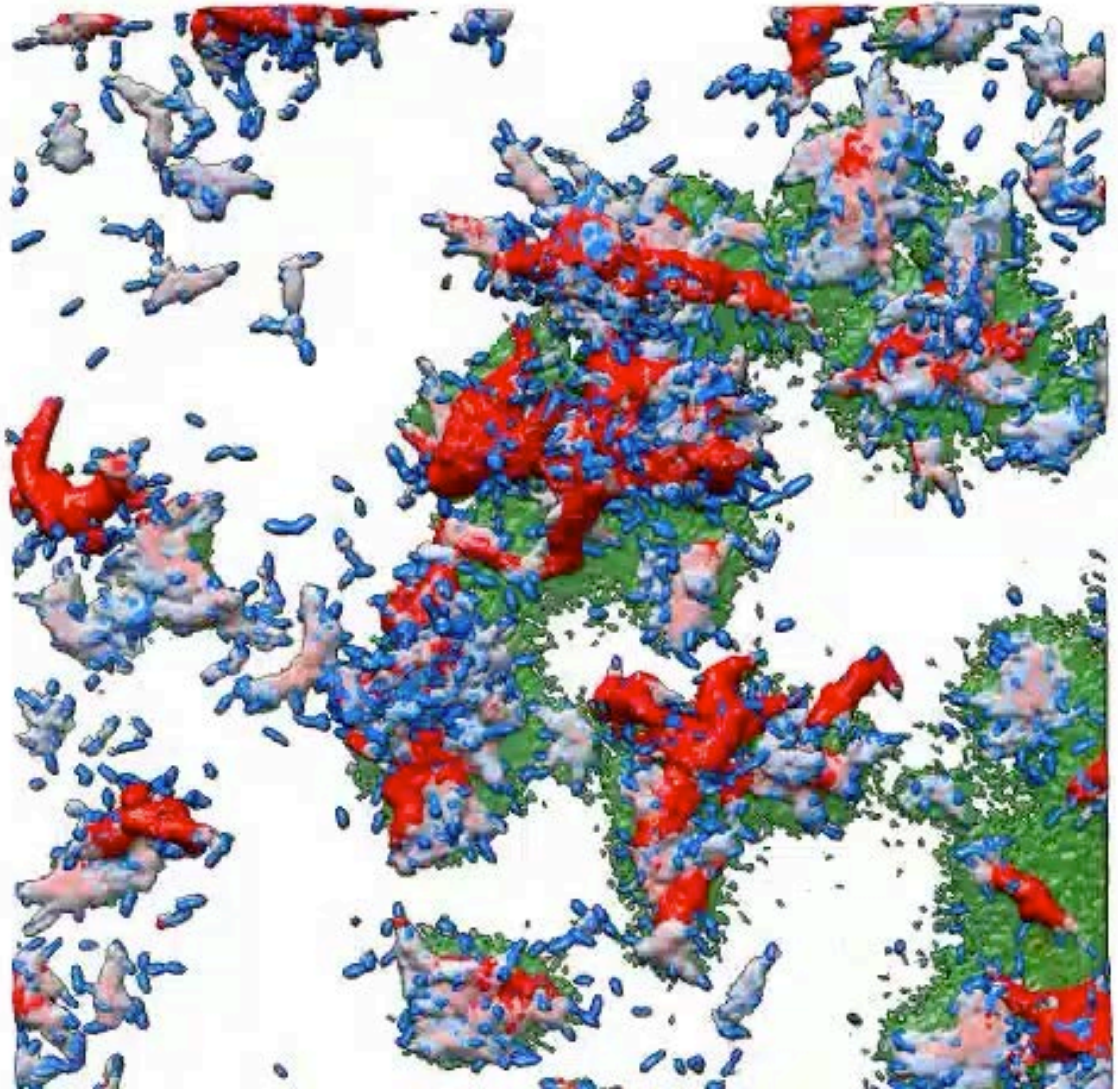
Berk, Science 2012



fluorescently-labeled antibodies can be used to specifically localize the diverse sticky EPS molecules

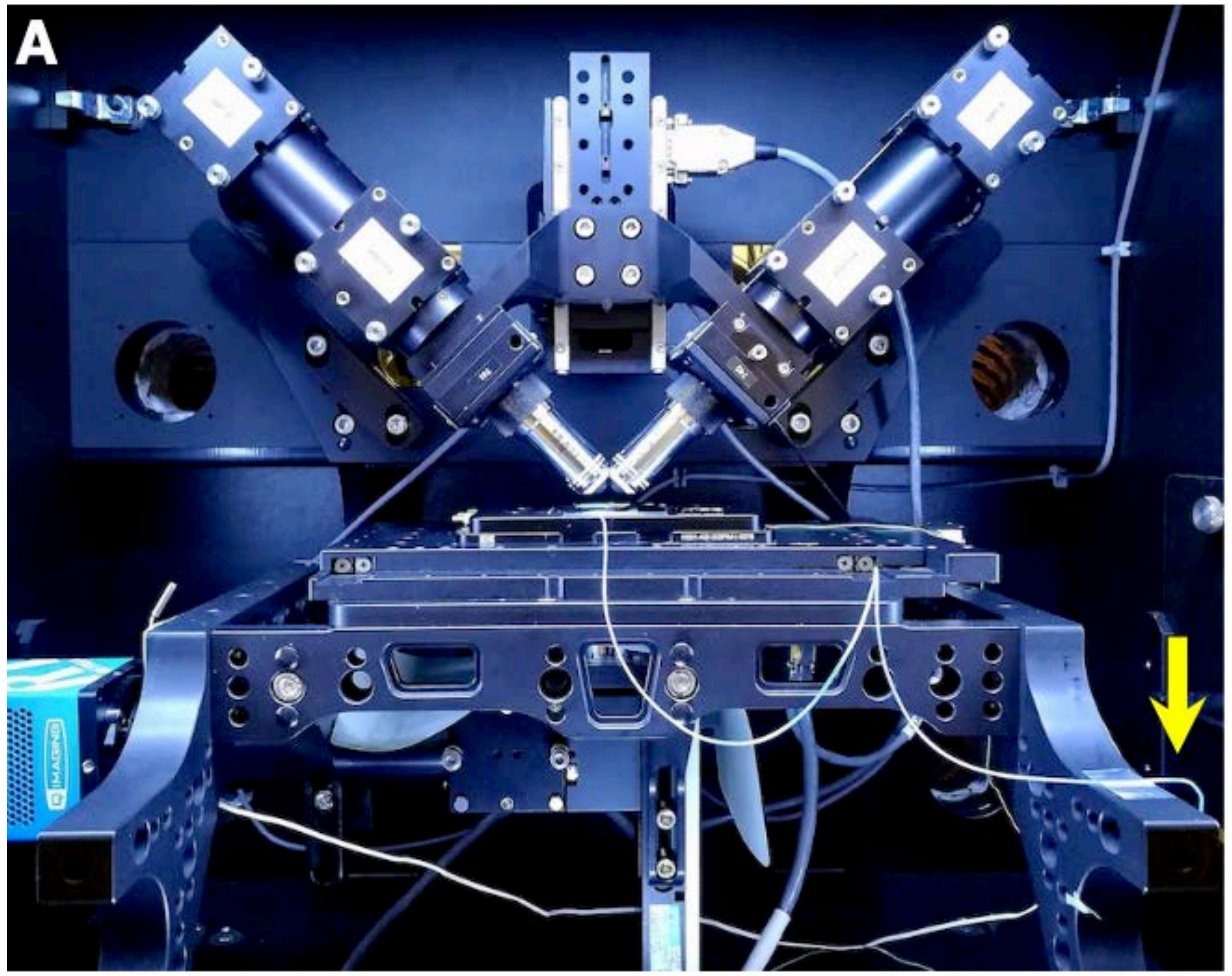


the three different secreted EPS are localized in space and time

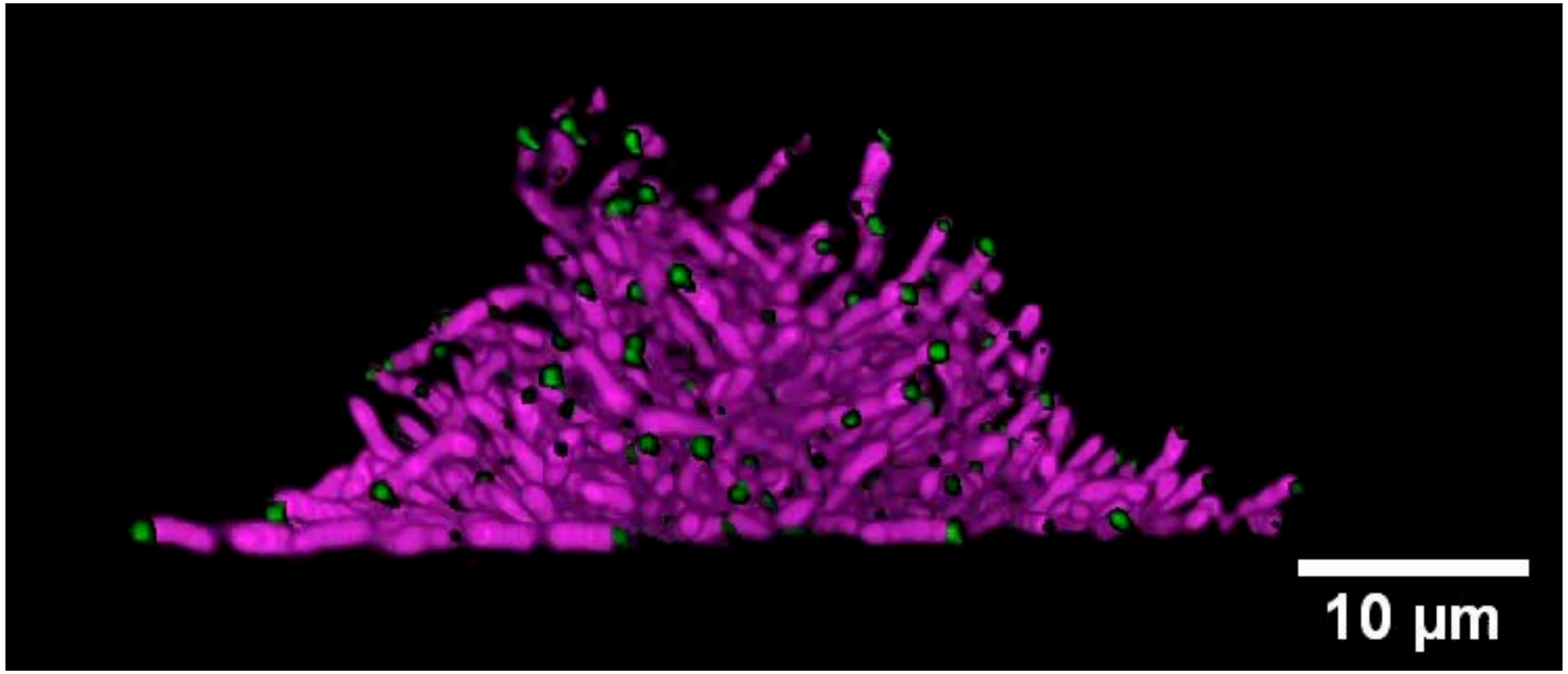


RbmA (gray), RbmC (red), Bap1 (green) and cells (blue)

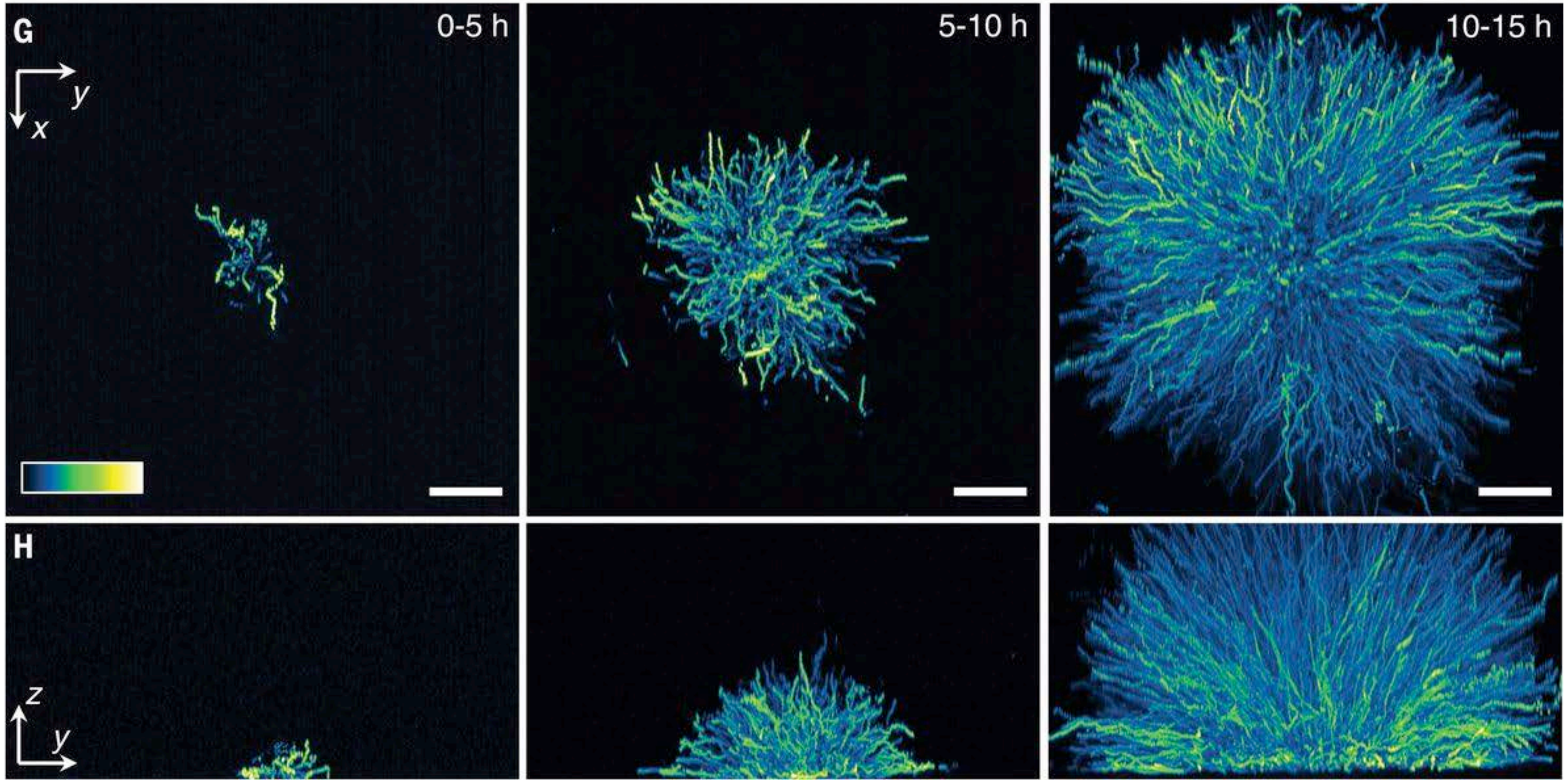
Imaging live biofilms in 3D



di-SPIM microscope (dual-view light sheet)



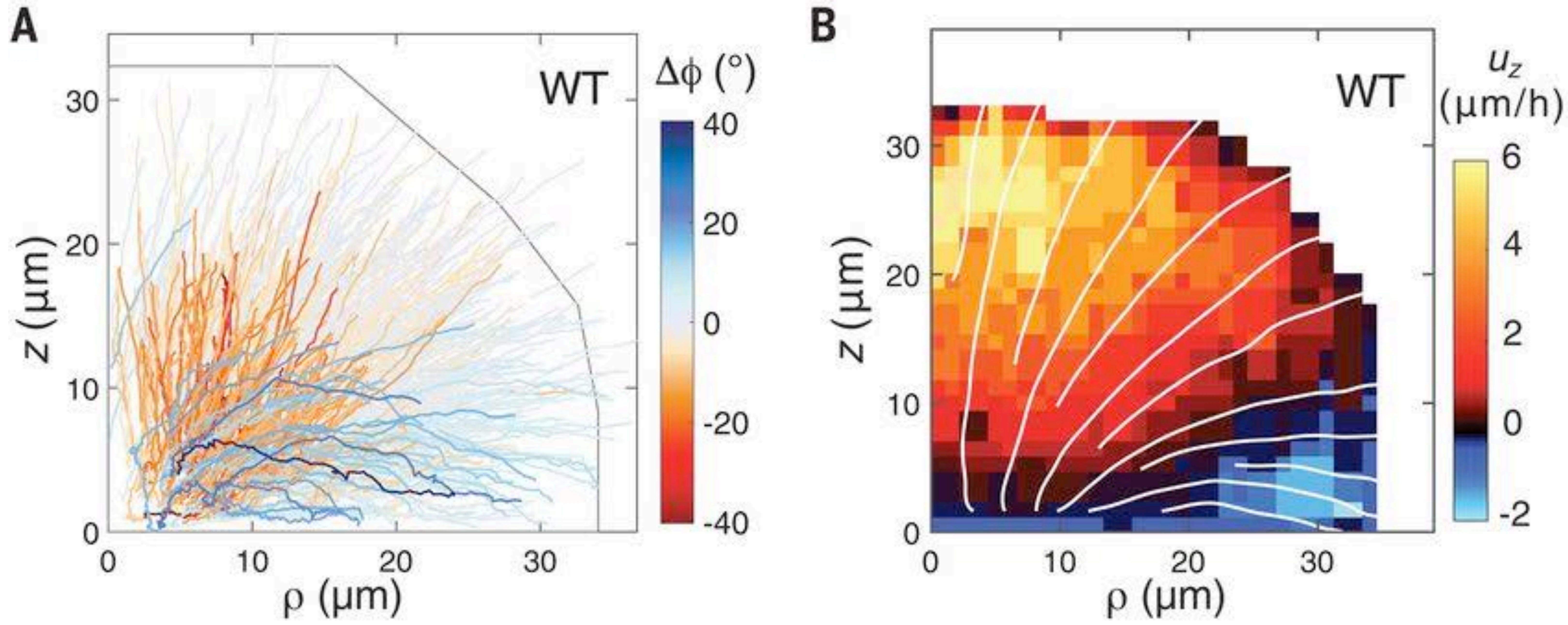
Single cell trajectores in growing biofilms



Single cells “flow” during morphogenesis

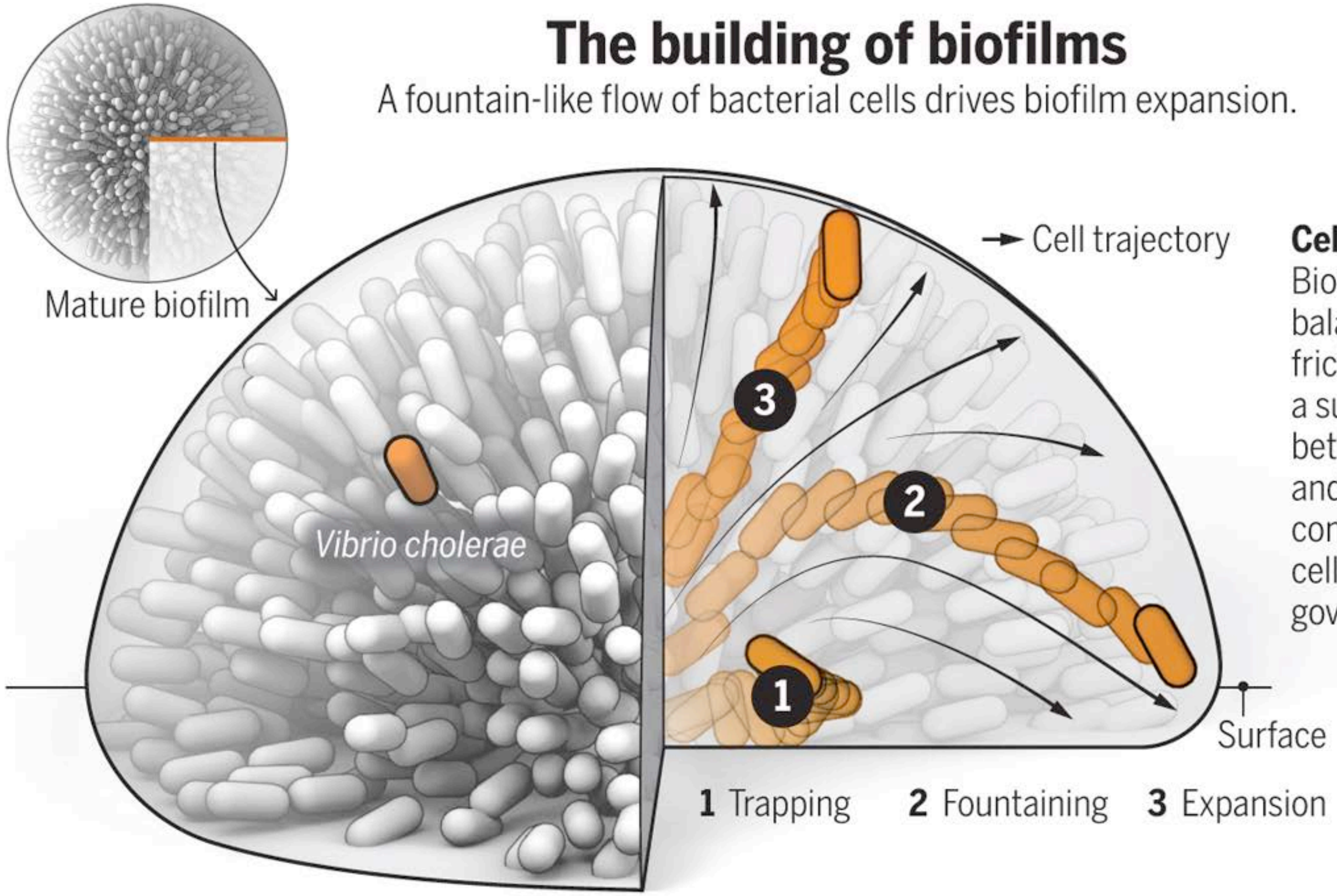
Fountain-like cellular flow

Trajectories **Cellular flow field**



The building of biofilms

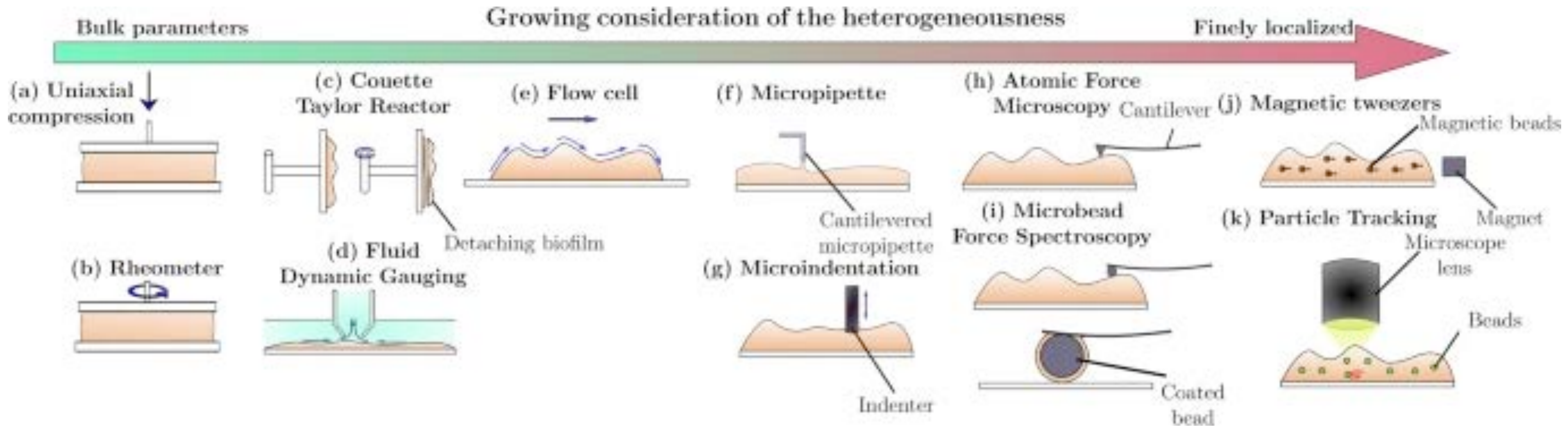
A fountain-like flow of bacterial cells drives biofilm expansion.

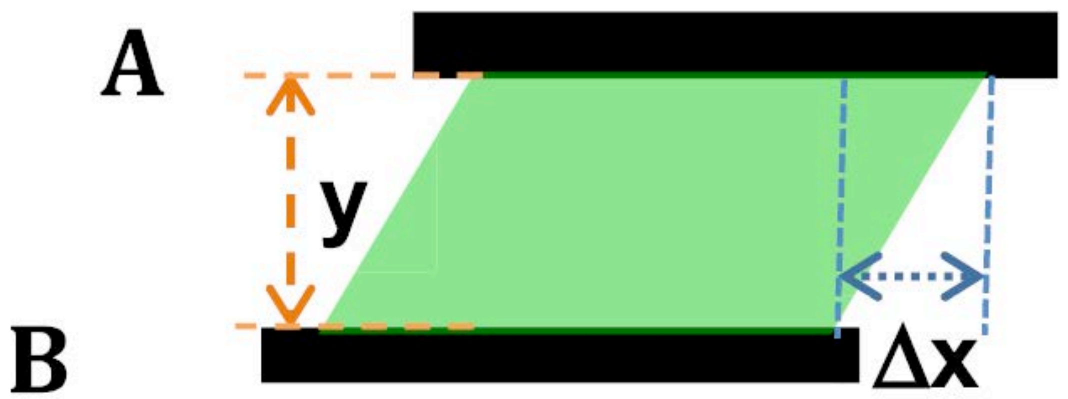


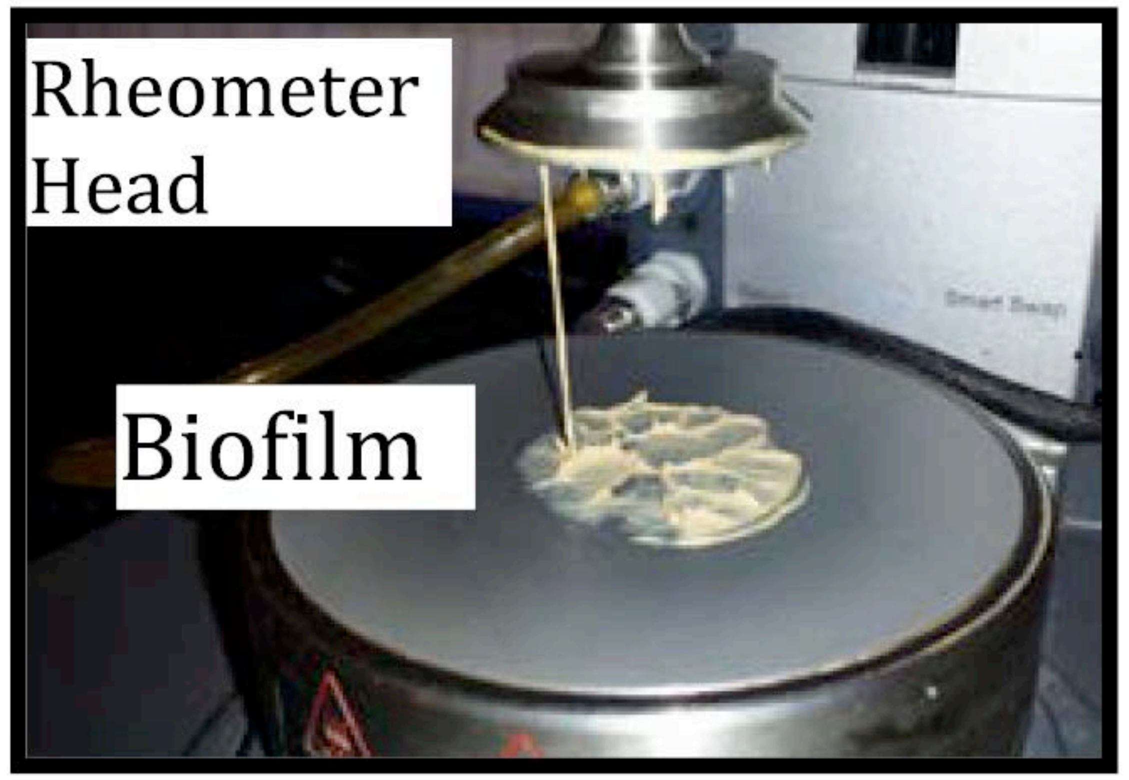
Cellular choreography
Biofilm expansion balances growth with friction between cells and a surface. Interactions between bacterial cells and cell matrix components mediate cellular movement and govern cell position fates.

Biofilm mechanics

Characterization

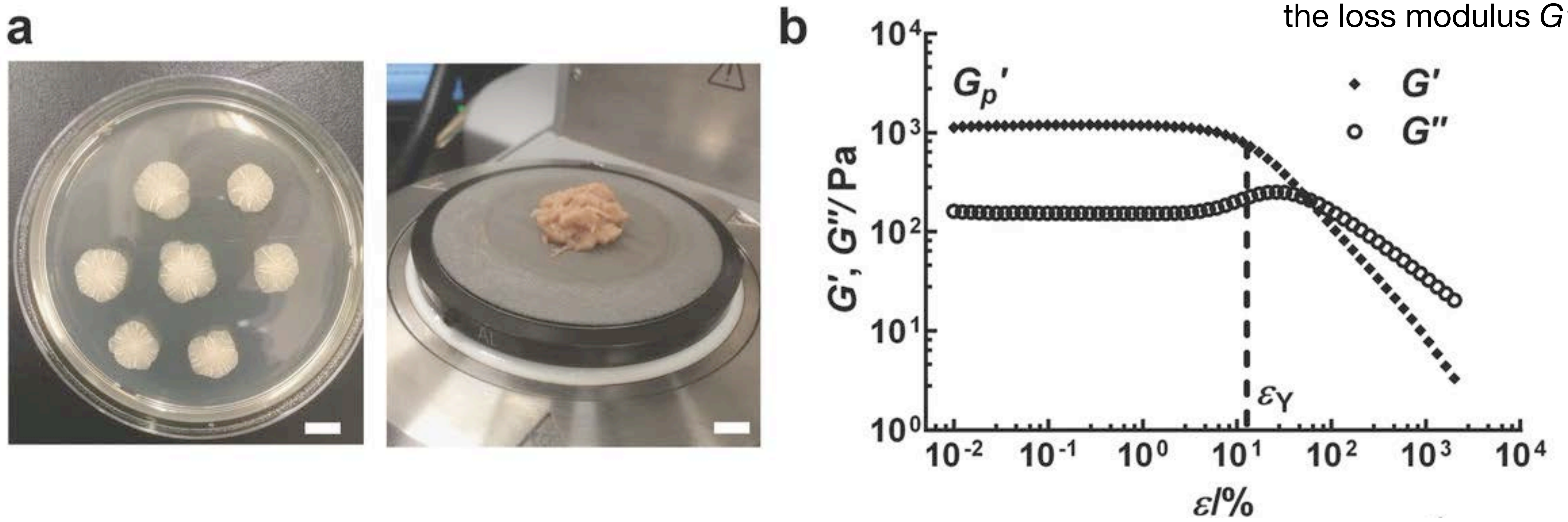






Bulk rheology of biofilms

storage modulus G' (representative of the elastic, solid-like properties) and the loss modulus G'' (representative of the viscous, fluid-like properties)



Above a critical strain, referred to as the yield strain ε_Y, the biofilm starts to yield with a dramatic decrease in G'

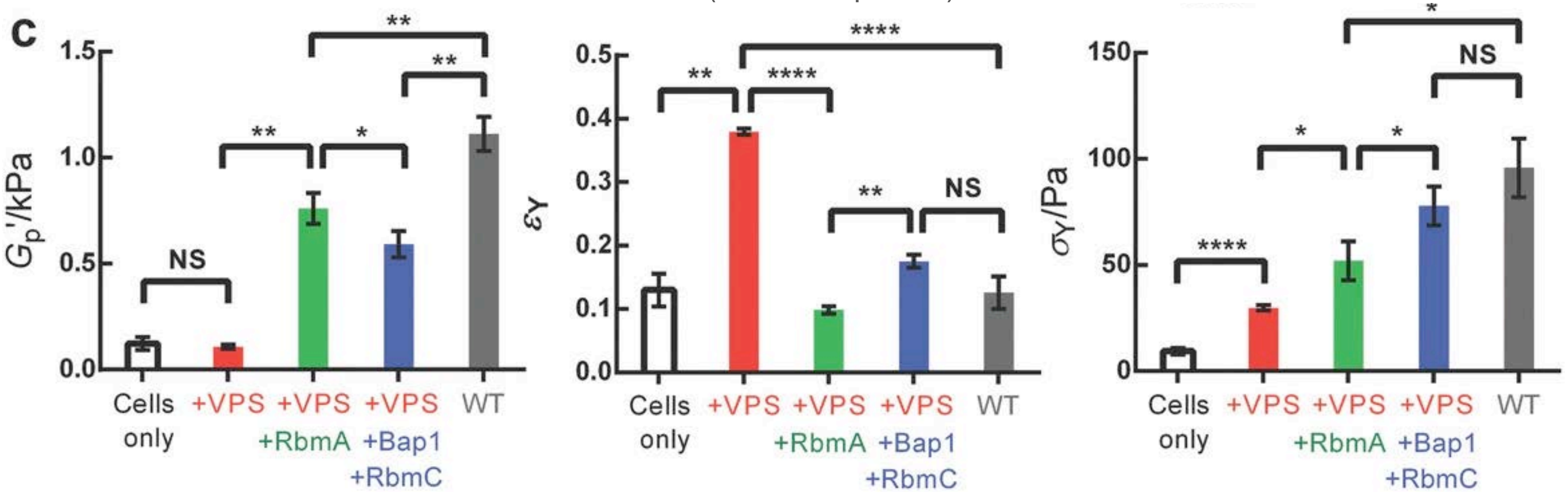
Mechanical properties of matrix components

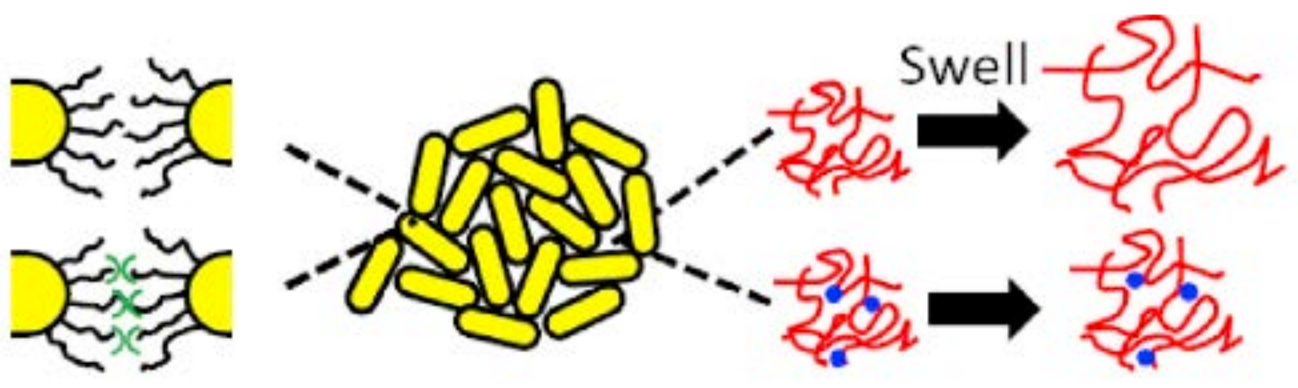
Matrix components have distinct functions

Storage modulus

Yield strain
(elastic to plastic)

Yield stress
(elastic to plastic)



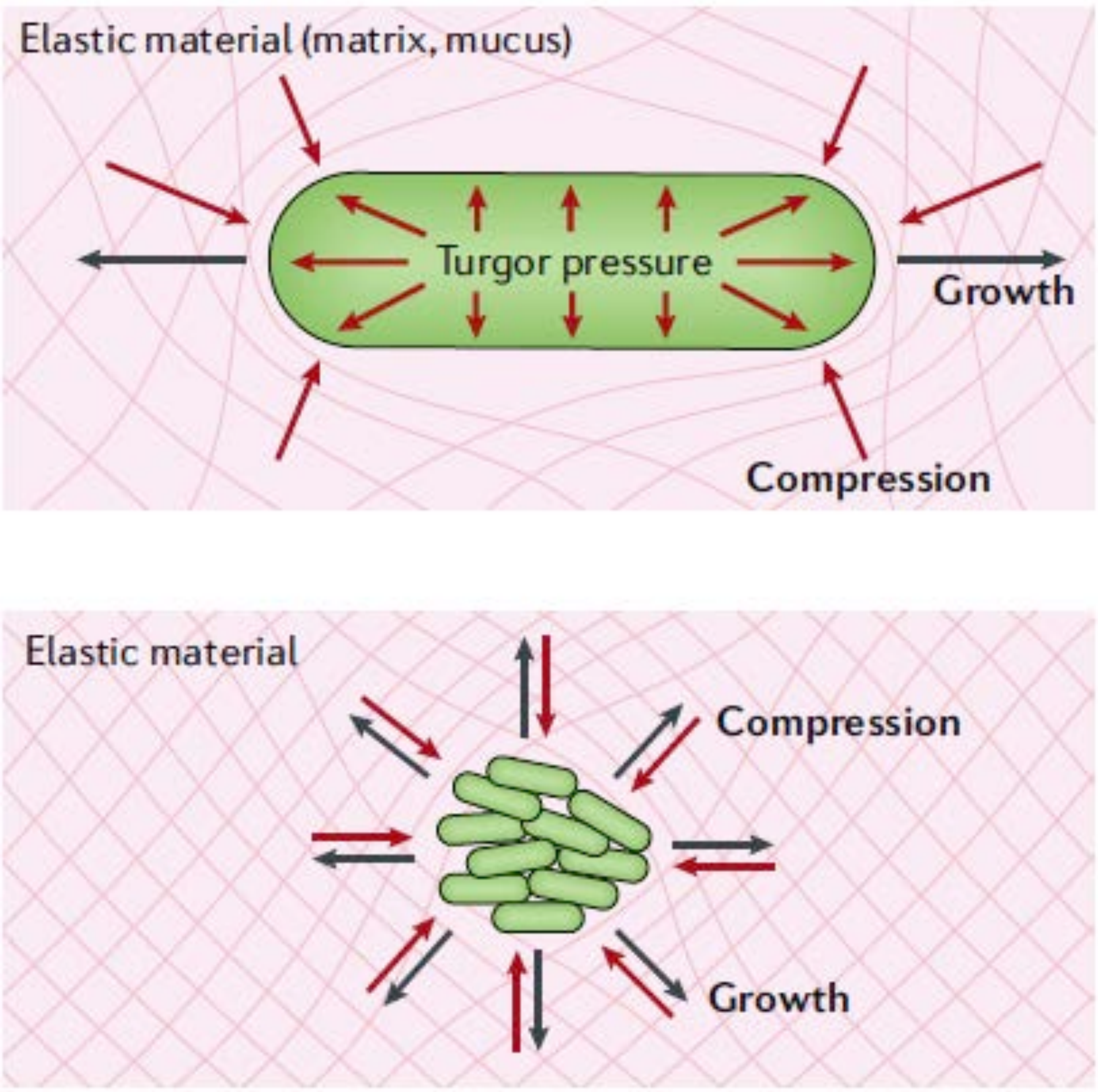


RbmA → cell-cell cohesion

Vps → hydrogel matrix

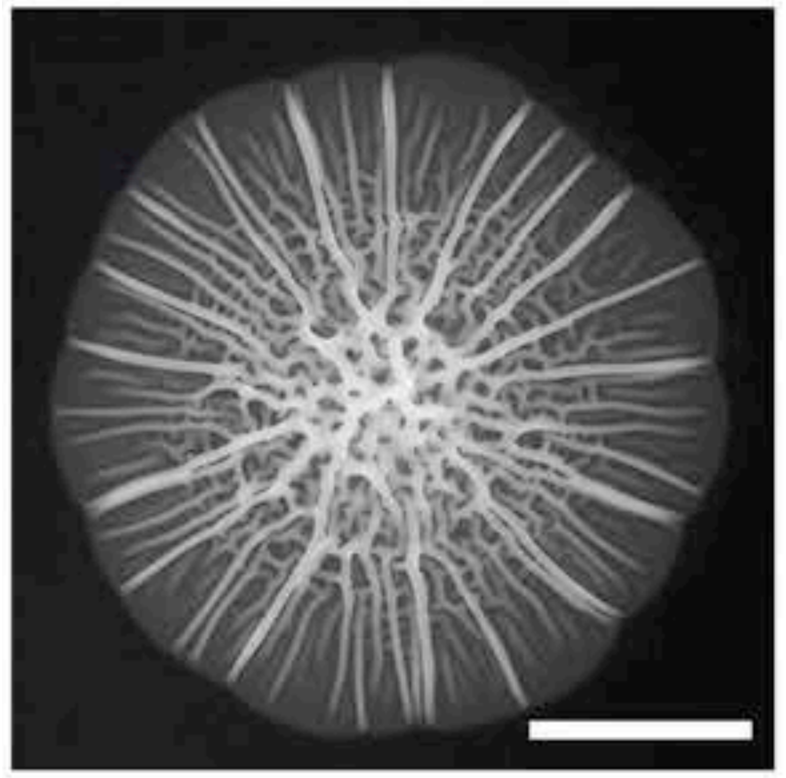
RbmC → robustness to swelling

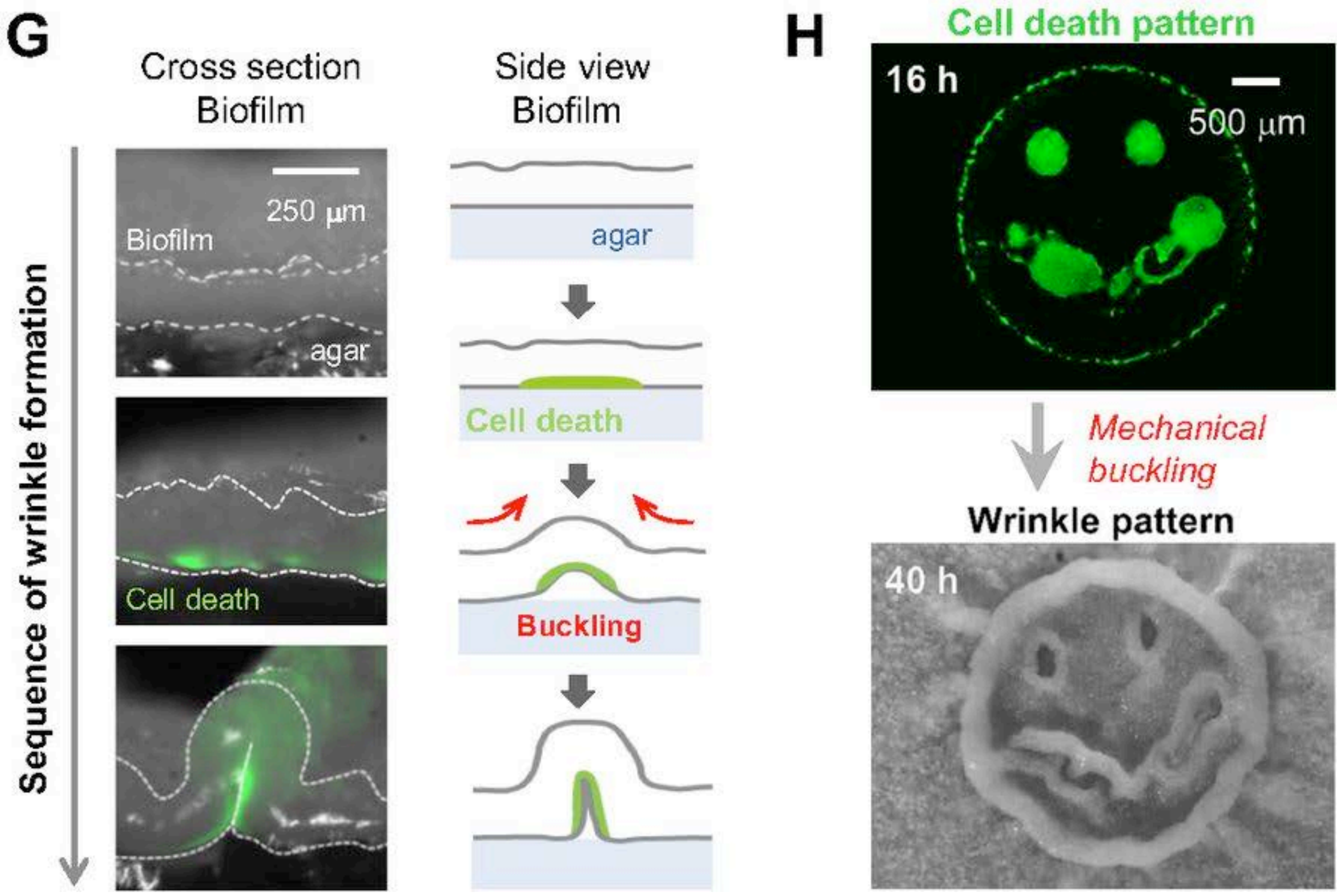
Growing in an elastic material



Biofilm-dwelling cells need to grow against the matrix
-> generates internal stress

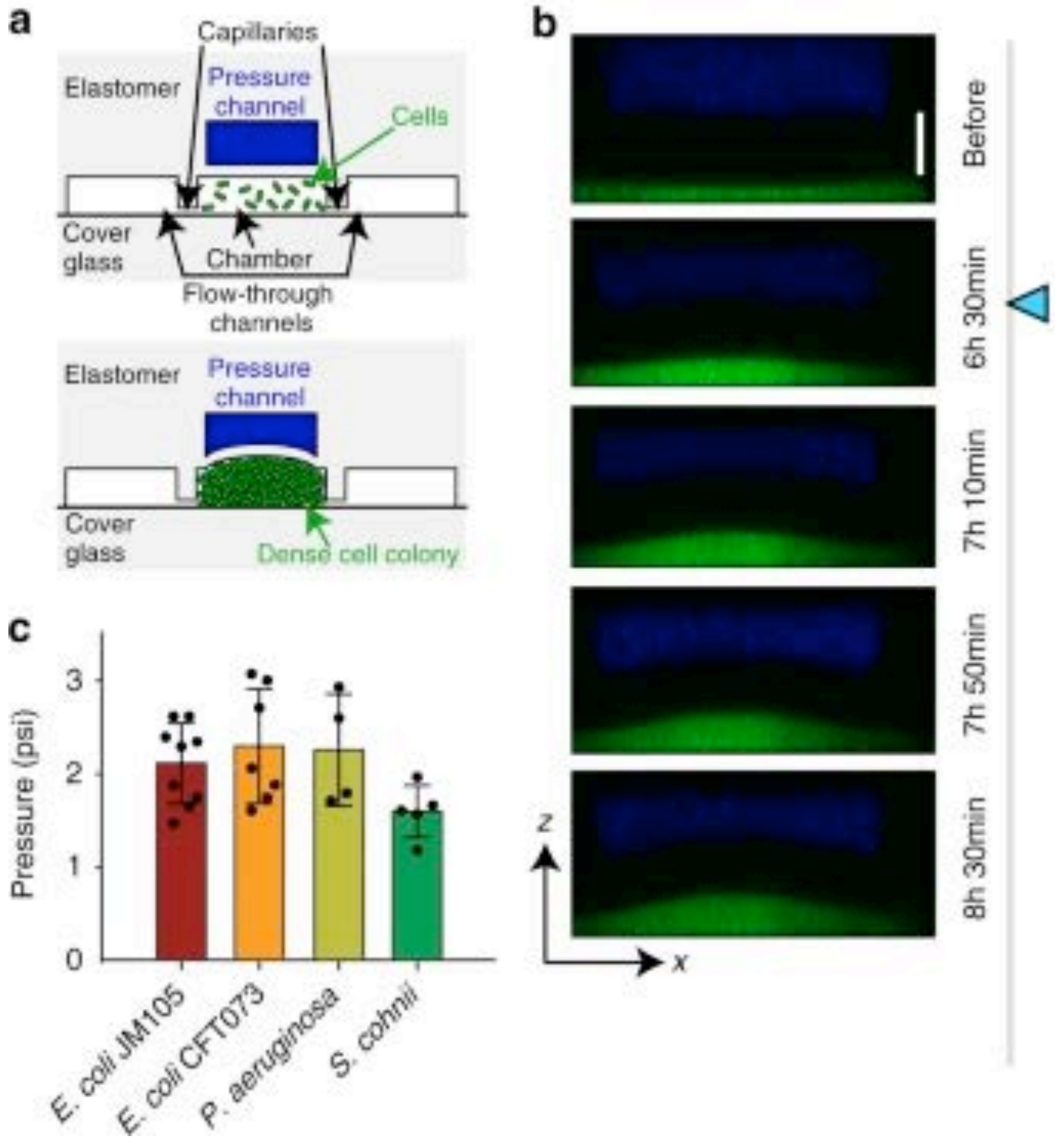
Buckling



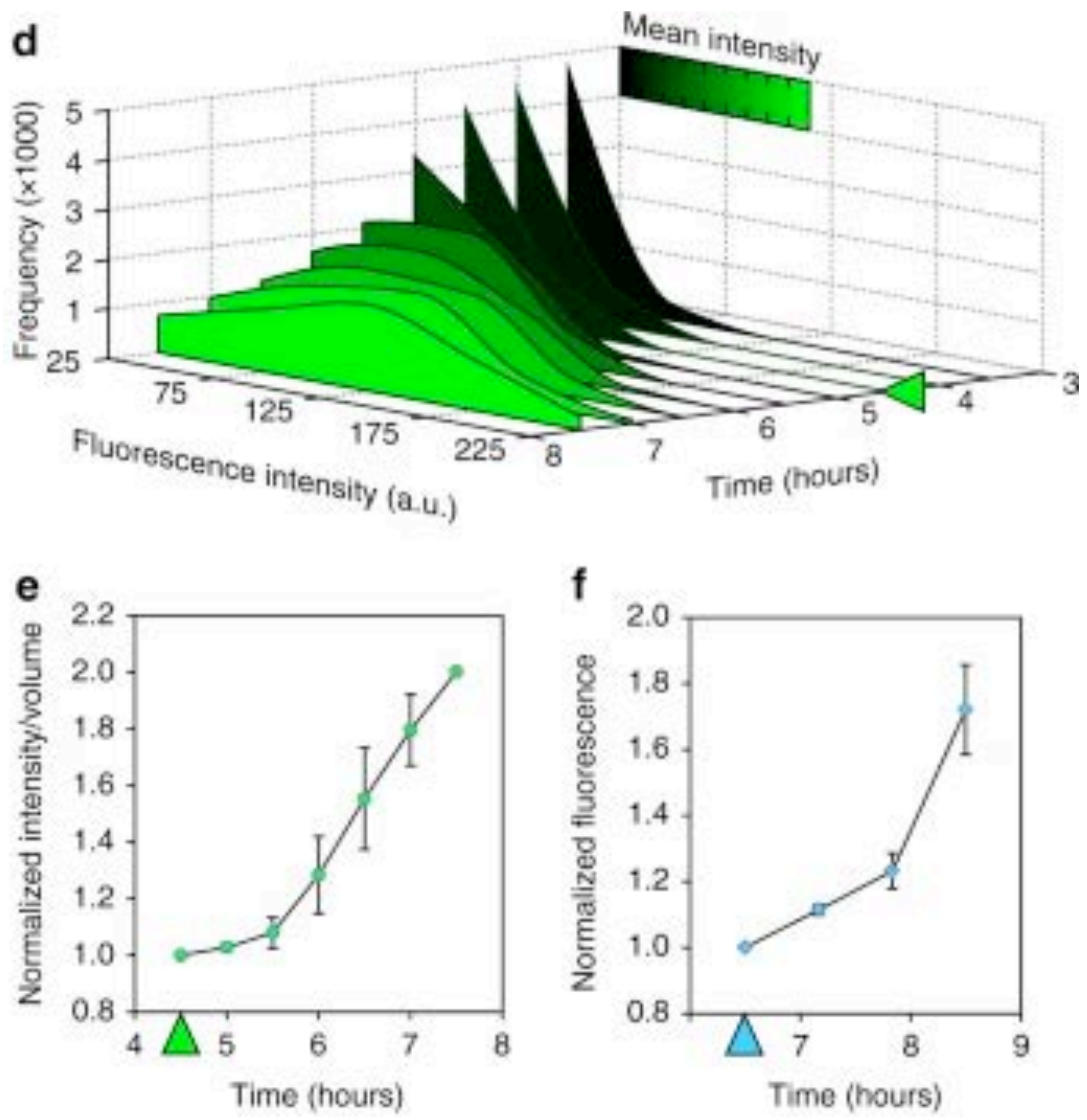


Pressure sensing in biofilms

Pressure measurement in microchamber

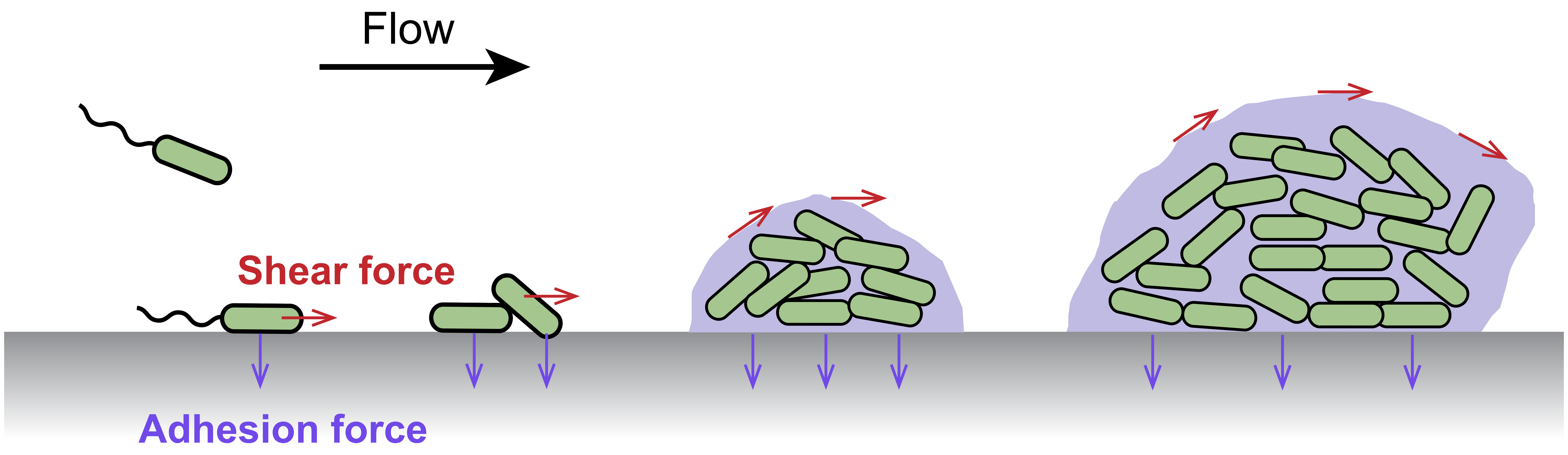


Gene expression measurement during self-induced compression



Confined growth regulates expression of biofilm-associated genes

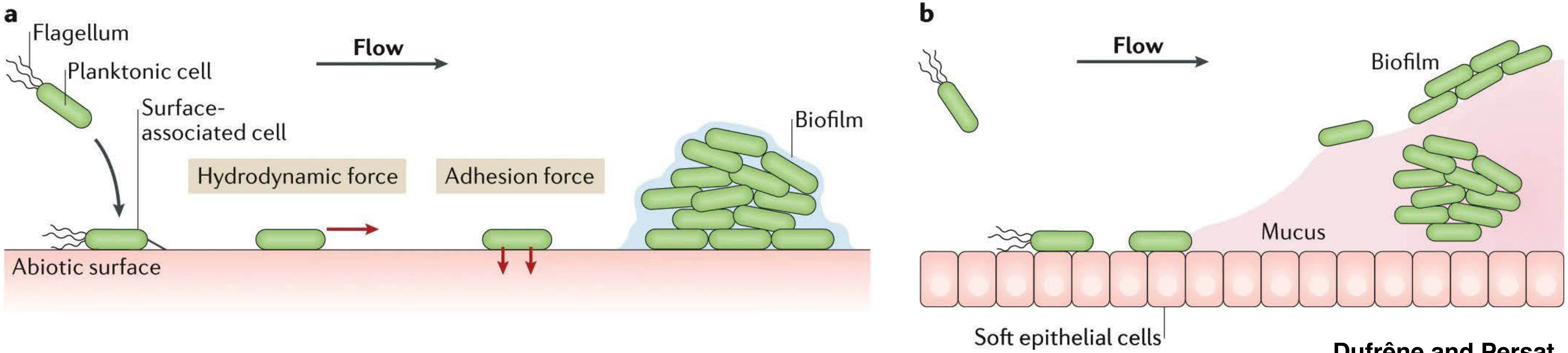
Forces on biofilms



How do mechanics contribute to biofilm morphogenesis?

Dufrene, *Nat Rev Micro*, 2020

Biofilms in the real world

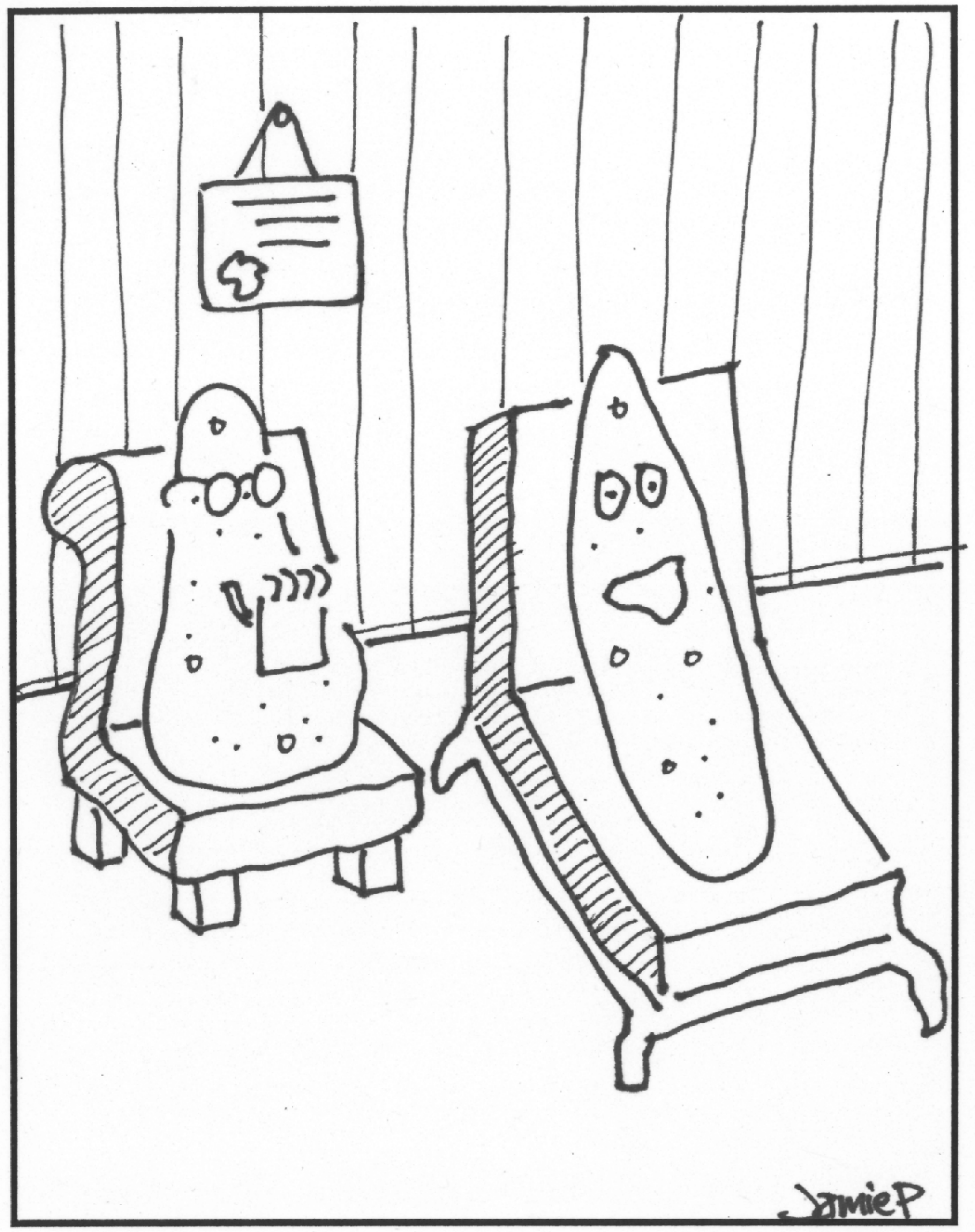


Dufrêne and Persat,

***Nat. Rev. Microbiology* 2020**

Do surface mechanics and flow regulate biofilm formation?

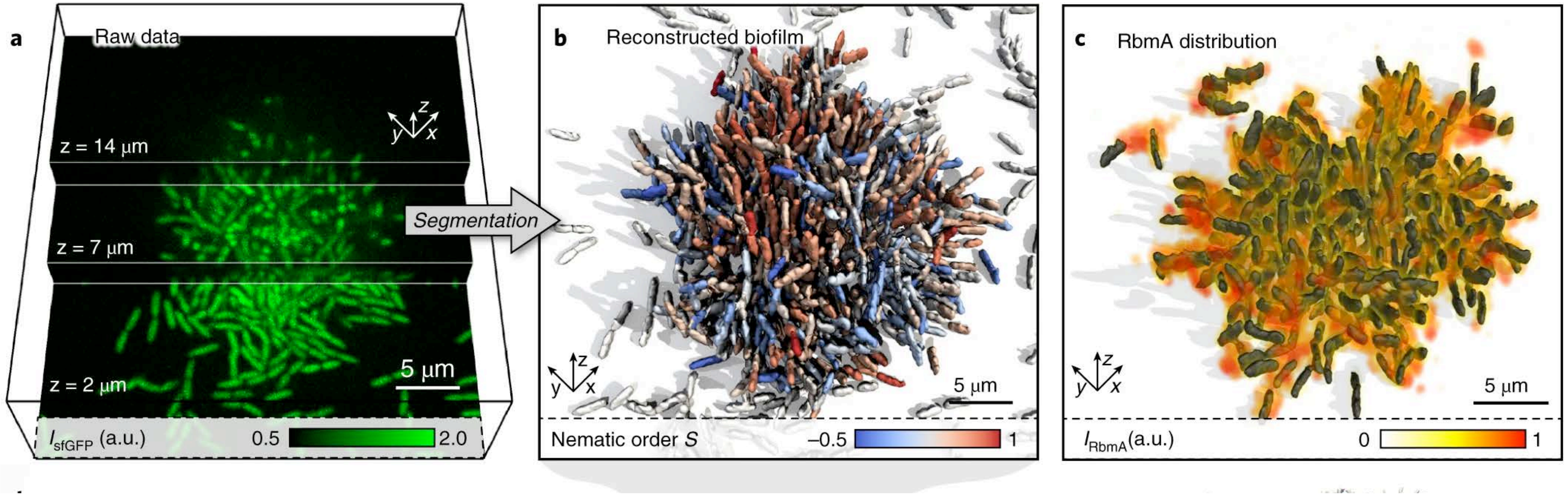
Biofilms in flow



I just can't go with the flow anymore.
I've been thinking about joining a biofilm.

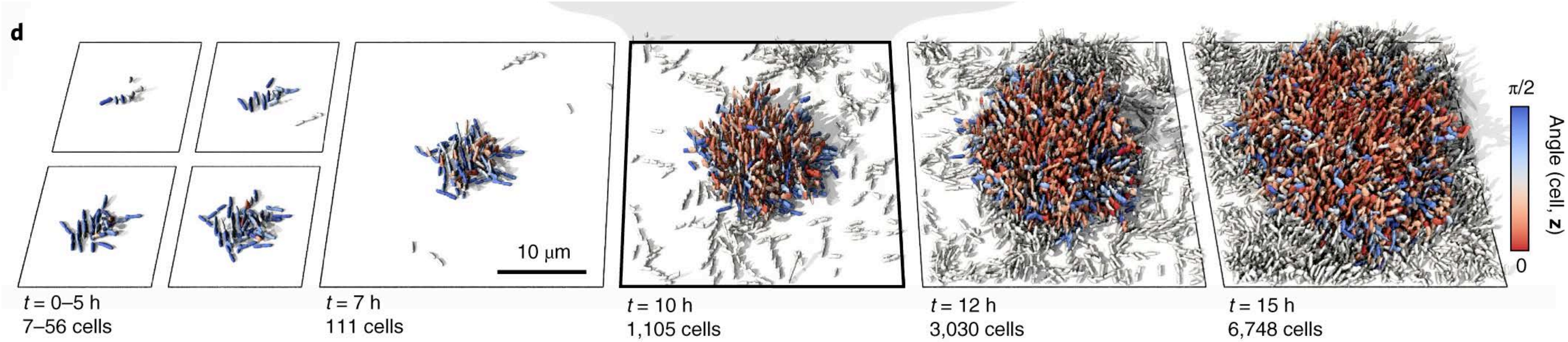
Vibrio cholerae biofilms

flow vs cell-cell cohesion



High resolution microscopy for single cell reconstruction

Biofilm formation in flow

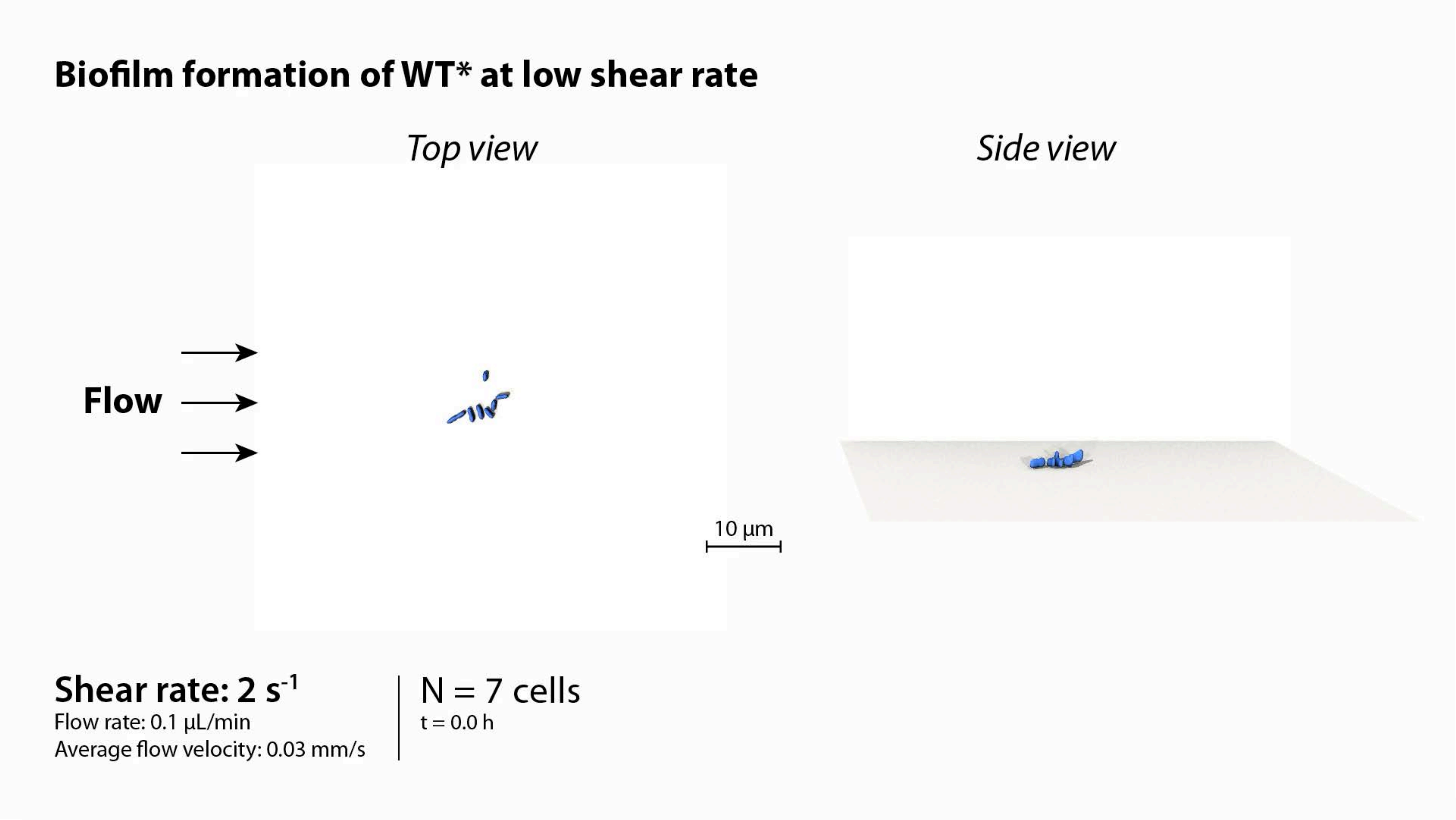


Single cell segmentation and tracking enables quantification of cellular order (physics analogy with liquid crystals)

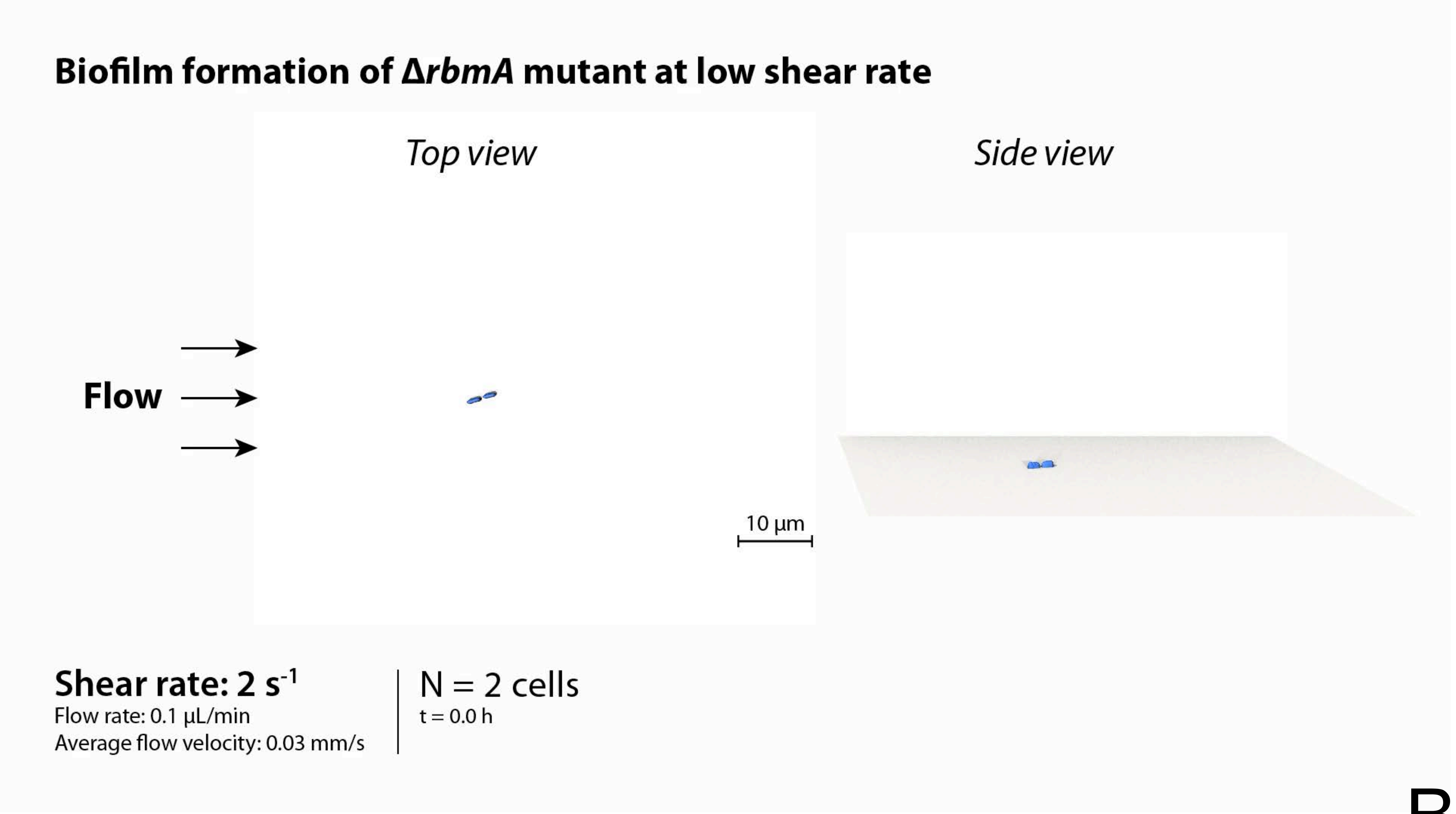
Nematic order parameter:

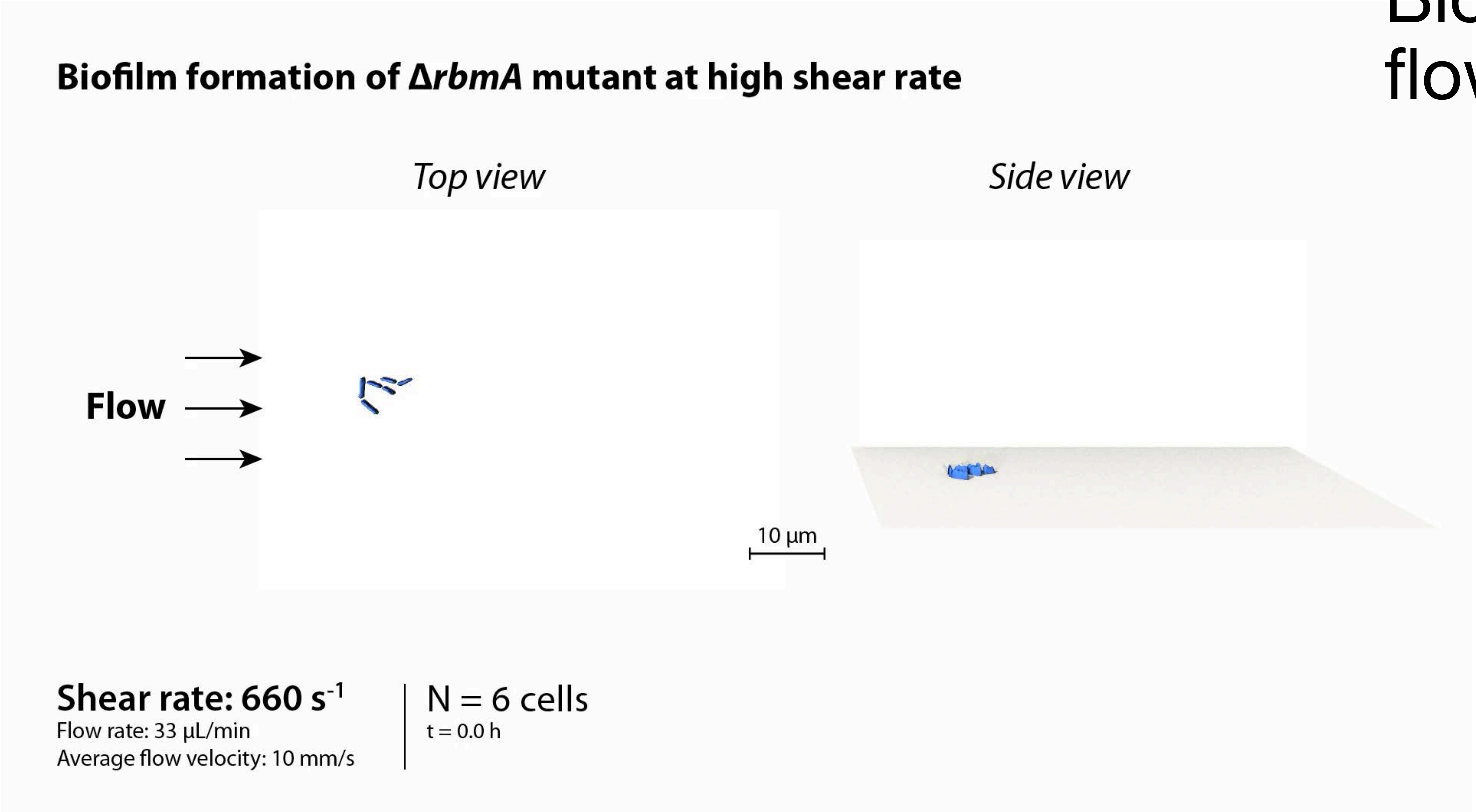
$$S = \langle 3/2(\hat{n}_i \cdot \hat{n}_j)^2 - 1/2 \rangle$$

$\boldsymbol{n}_i$ and $\boldsymbol{n}_j$ refer to the orientation vectors of cells i and j, respectively



Flow reorients bacteria during biofilm formation

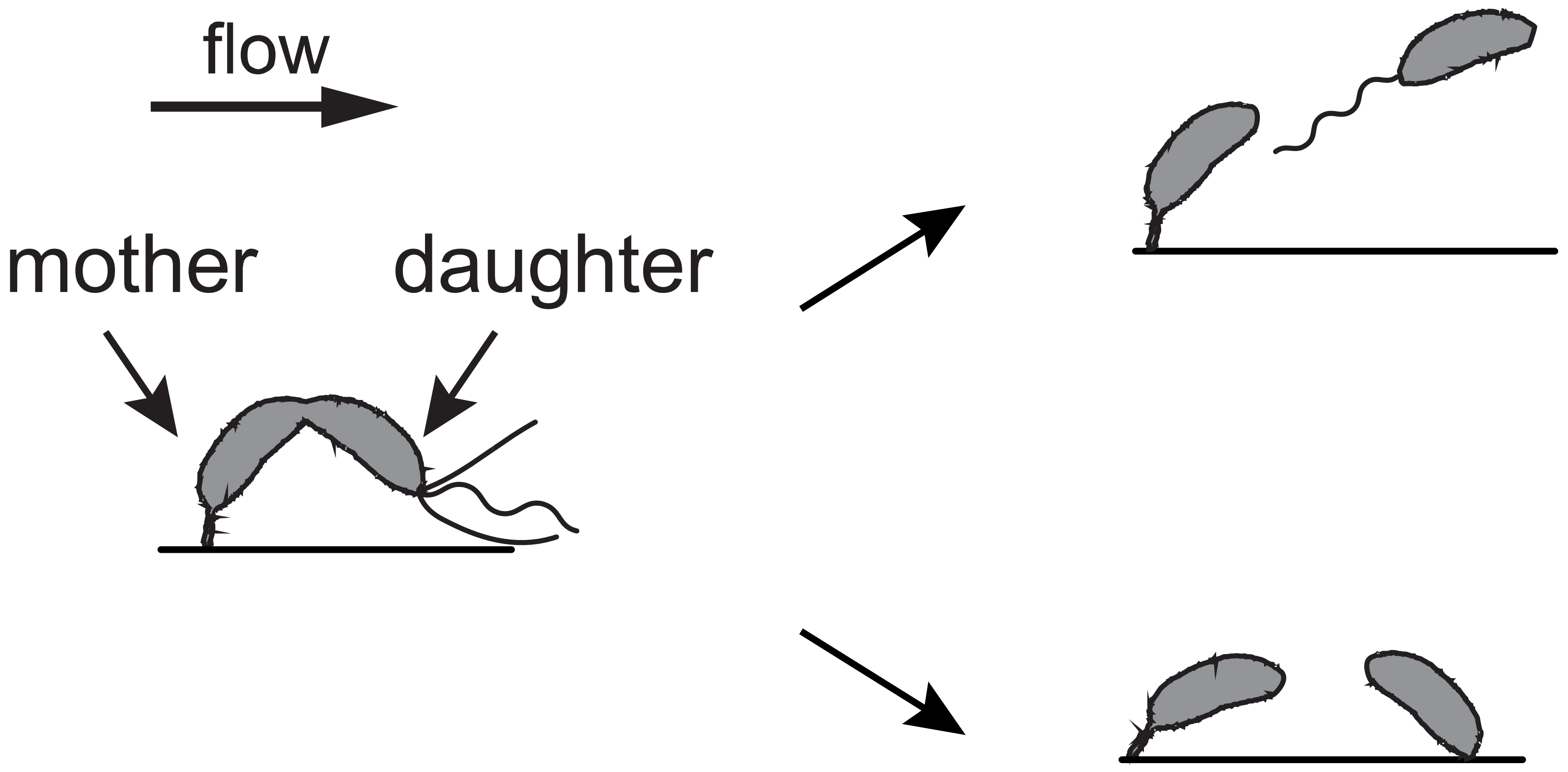




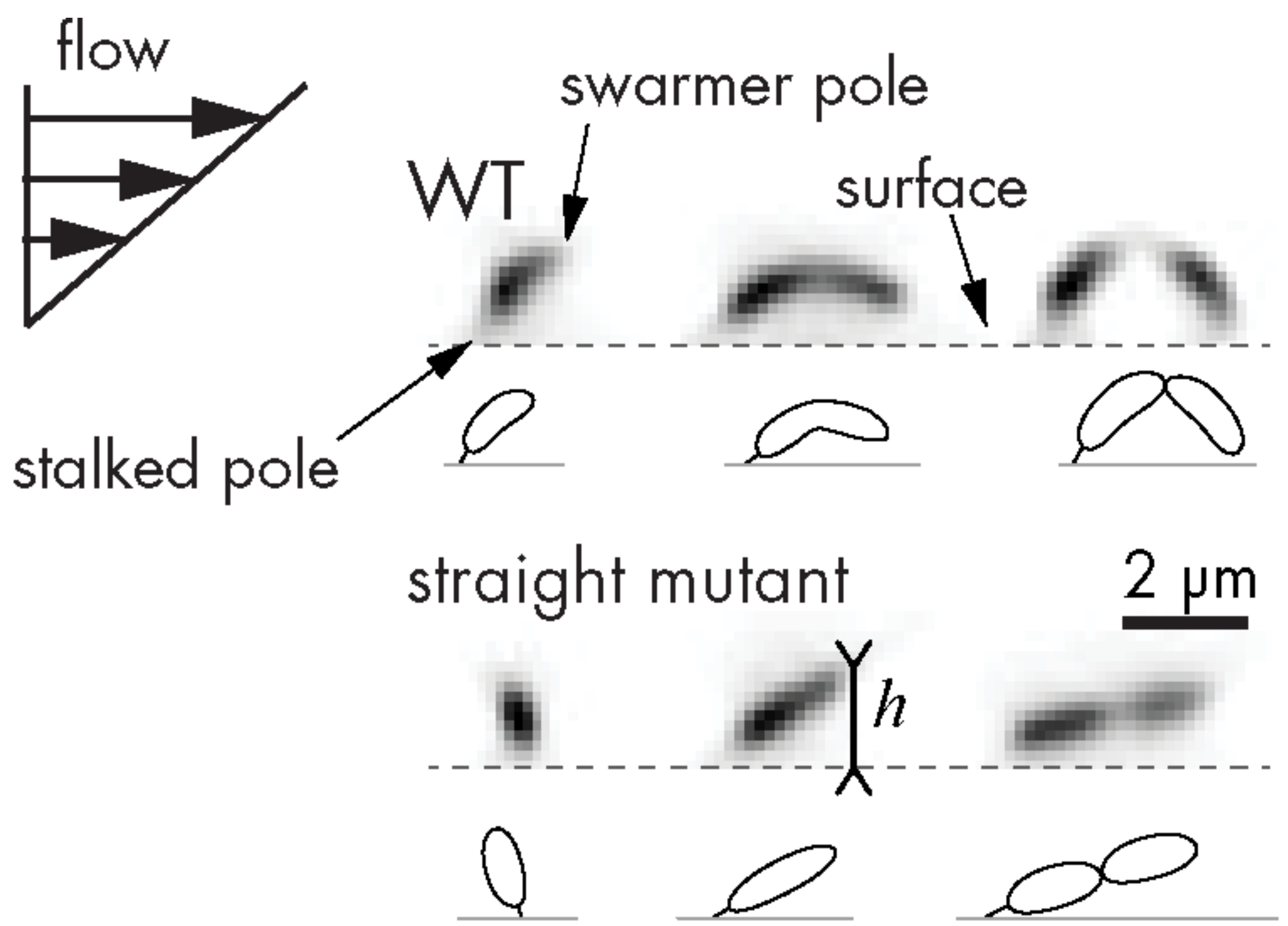
Biofilm matrix mitigates flow effects

Caulobacter crescentus biofilms

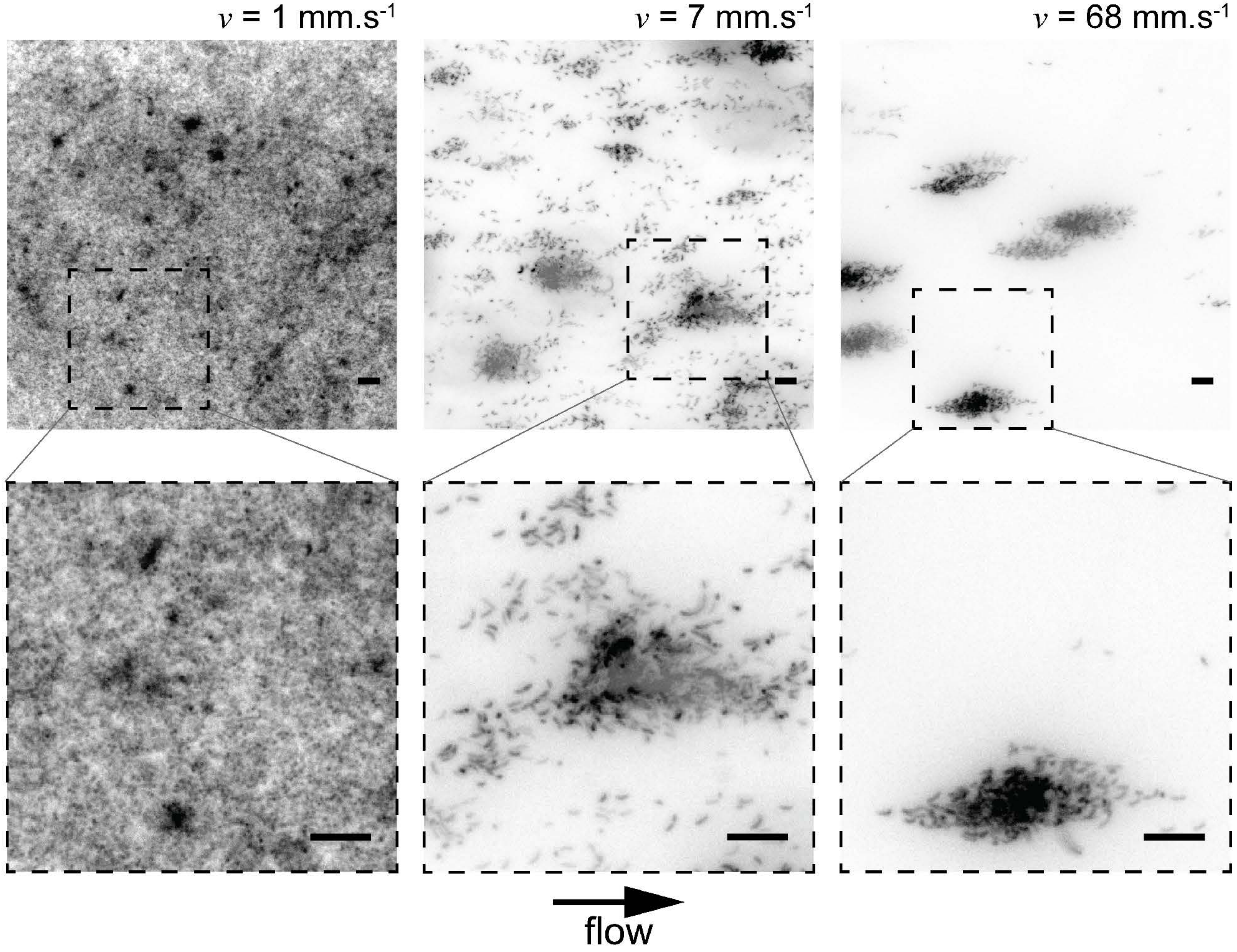
C. crescentus division in flow



C. crescentus curved shape promotes biofilm formation in flow

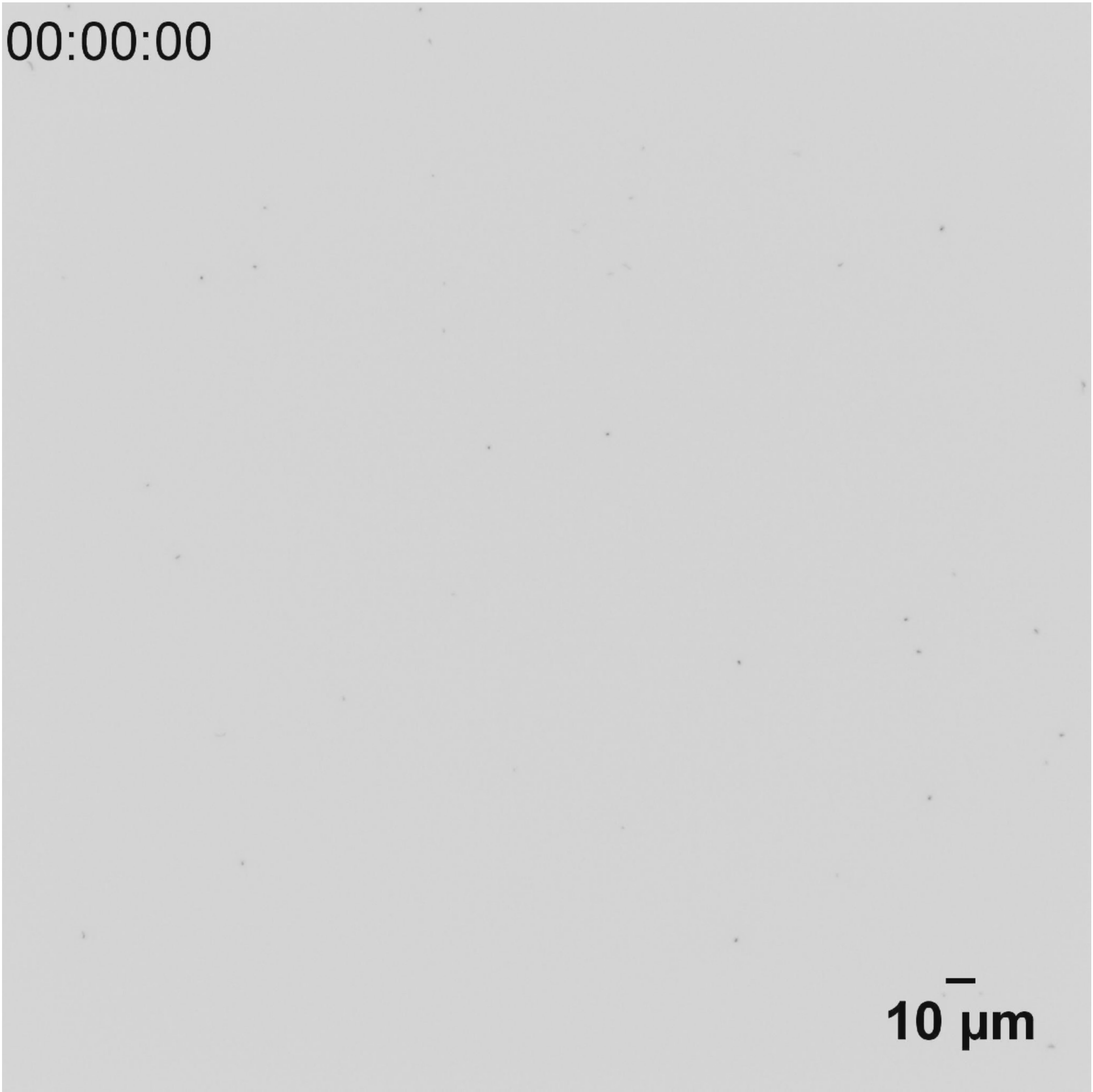


Flow shapes colonization patterns

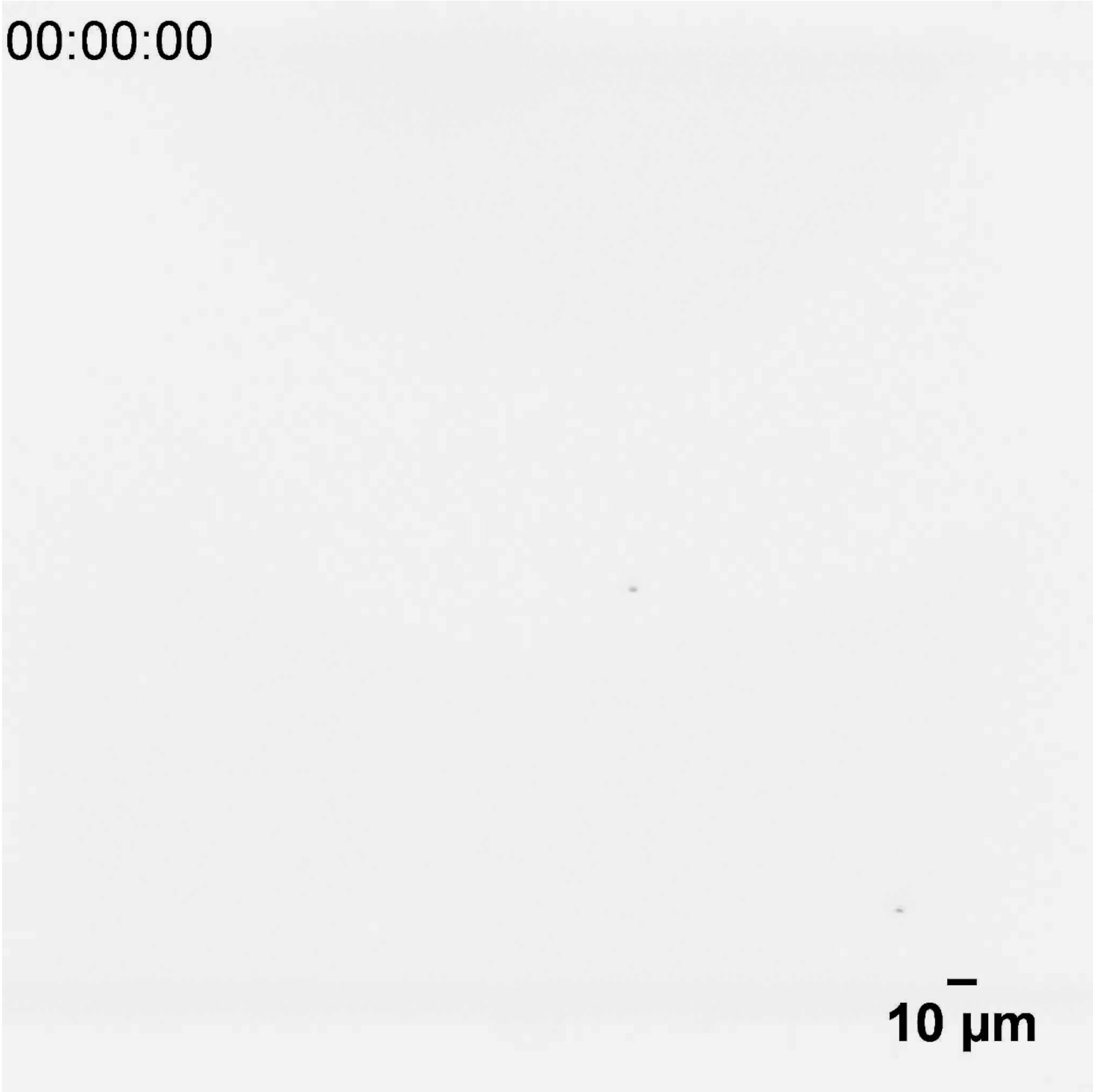


Rossy et al., Nat. Com. 2019

Colonization dynamics in flow



2.5 $mm.s^{-1}$

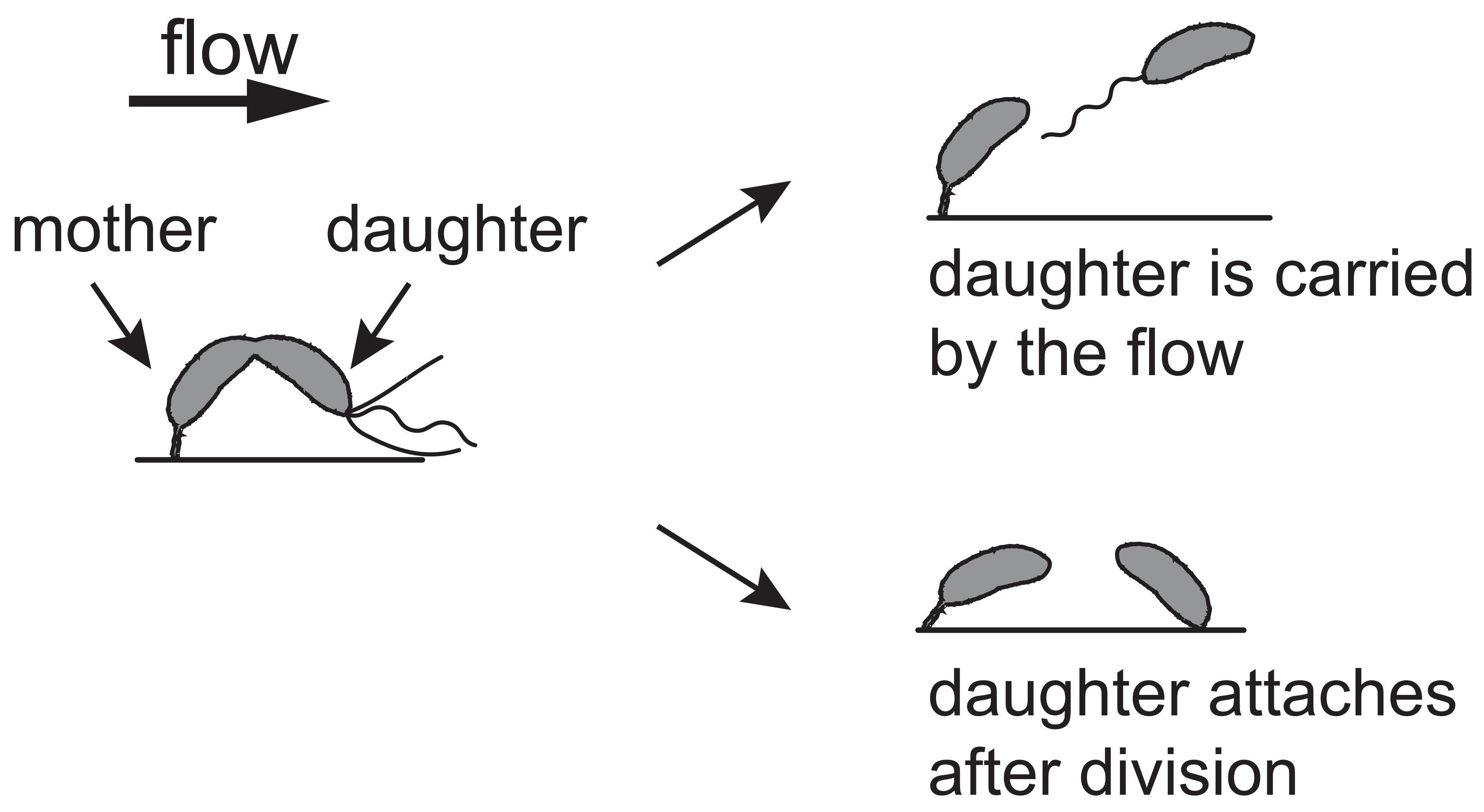


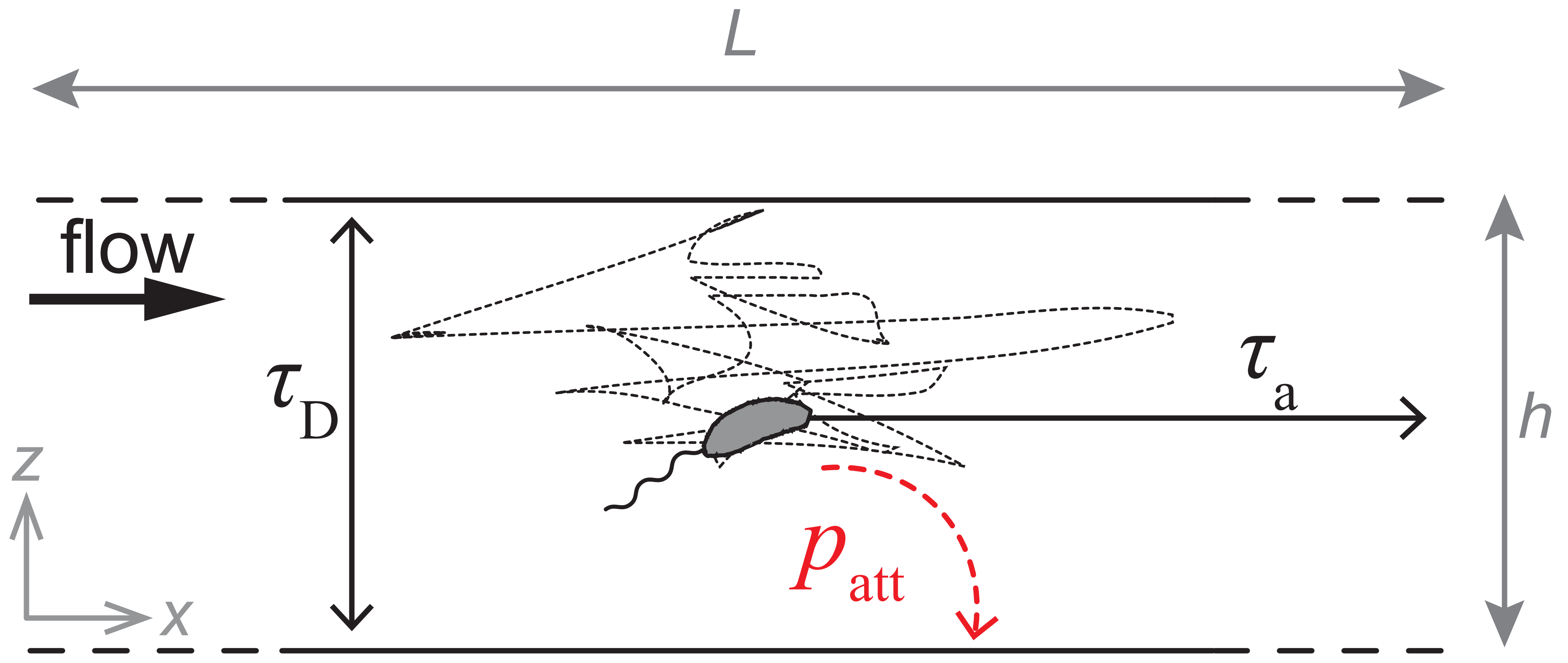
27 $mm.s^{-1}$

Weak flow allows for reattachment of planktonic cells

Rossy et al., Nat. Com. 2019

Cellular advective-diffusion model



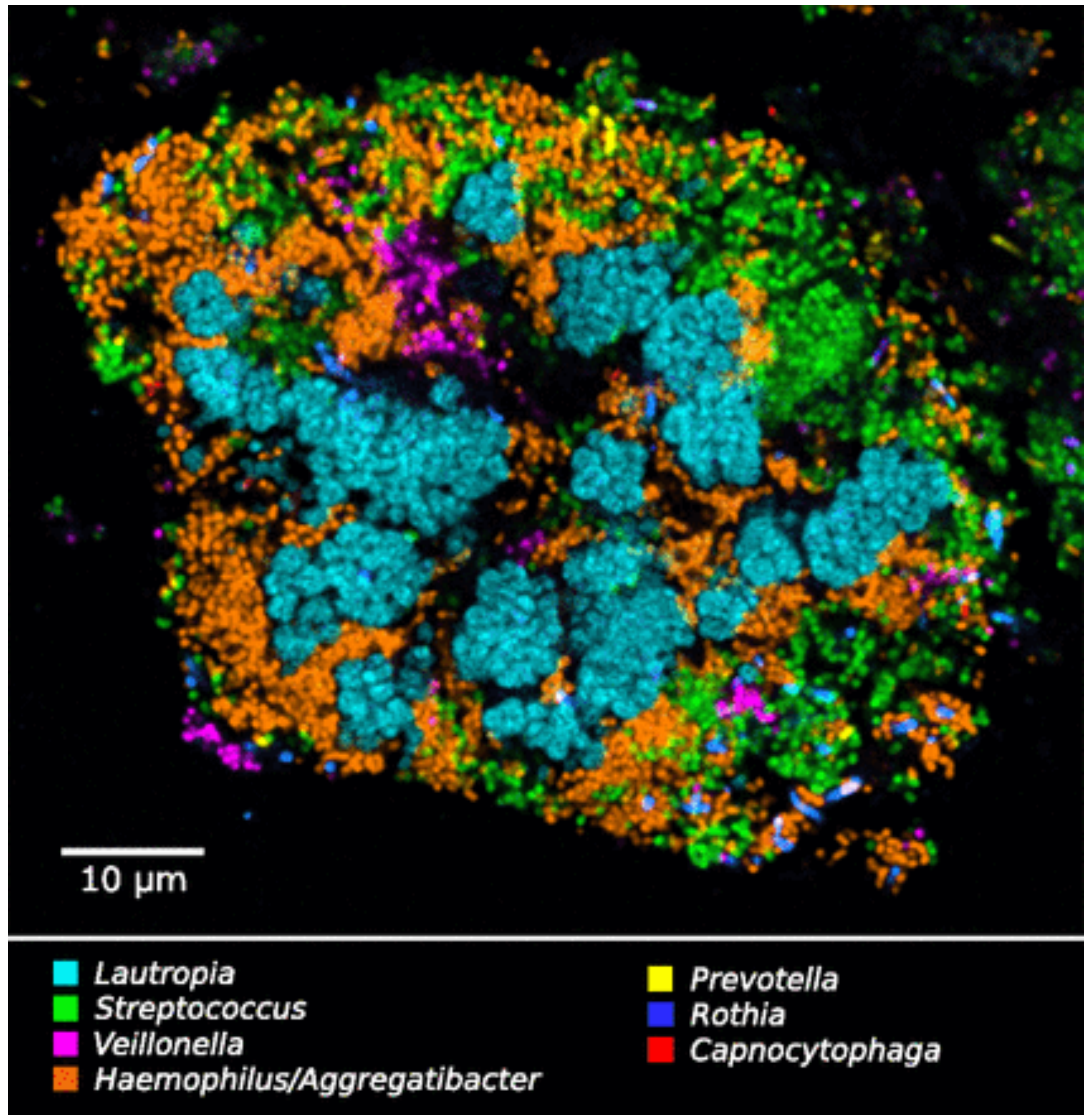


Weak flow: swimming and reattachment

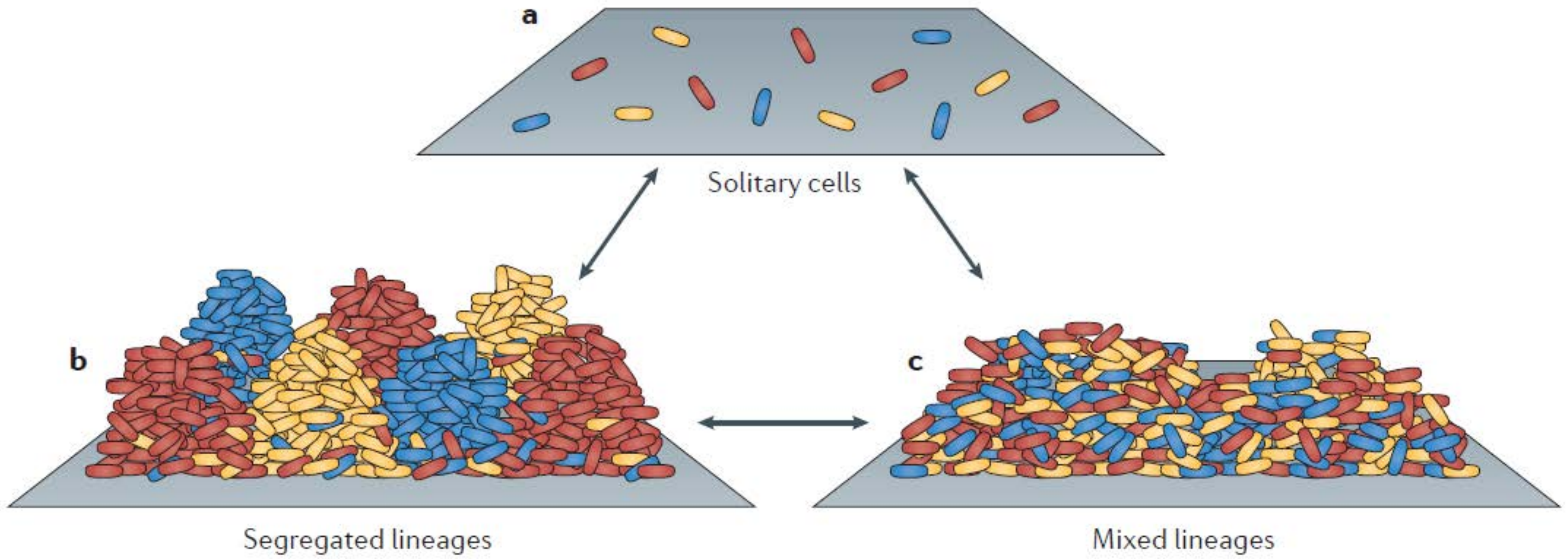
Strong flow: advective transport away from mother

Rossy et al., Nat. Com. 2019

Spatial organization of biofilms



Welch et al., PNAS 2016



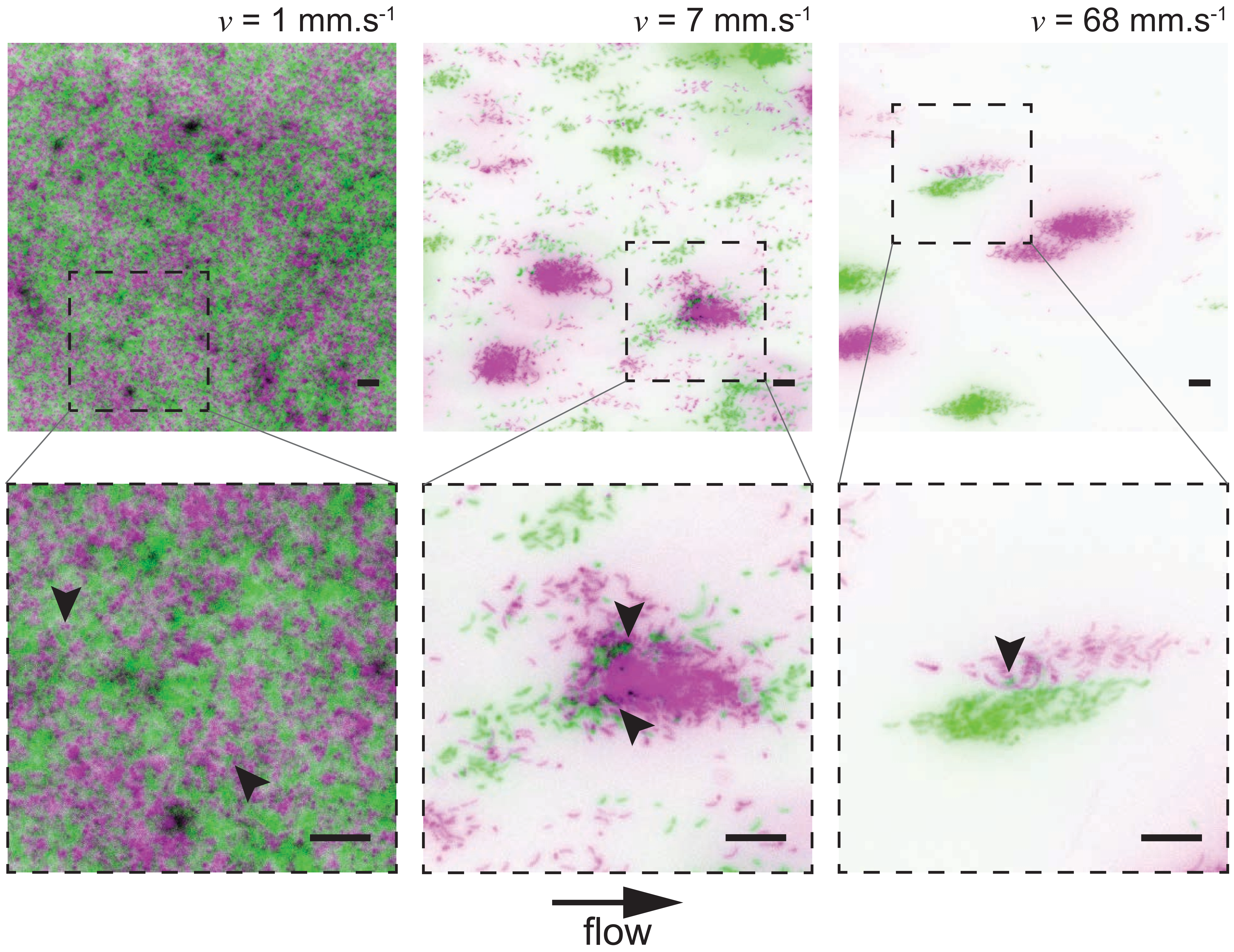
Nadell et al., Nat Rev. Micro. 2016

Does cellular advective diffusion influence biofilm spatial organization?

Clonal mixing in flow

Biofilms in flow, two clones:

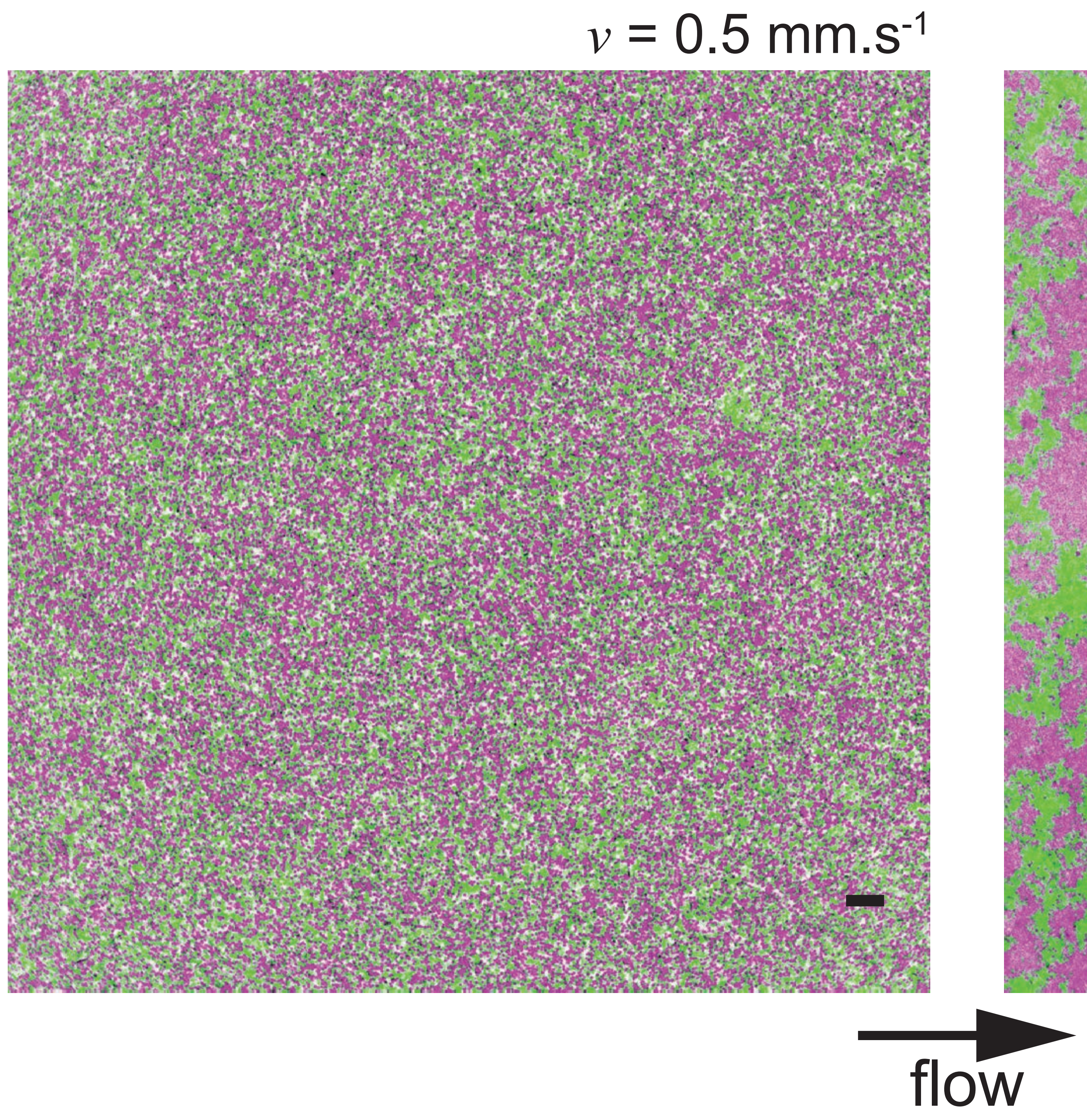
WT *C. crescentus* mKate +
WT *C. crescentus* mVenus



Flow segregates colonal lineages

Rossy et al., Nat. Com. 2019

Biofilms in flow

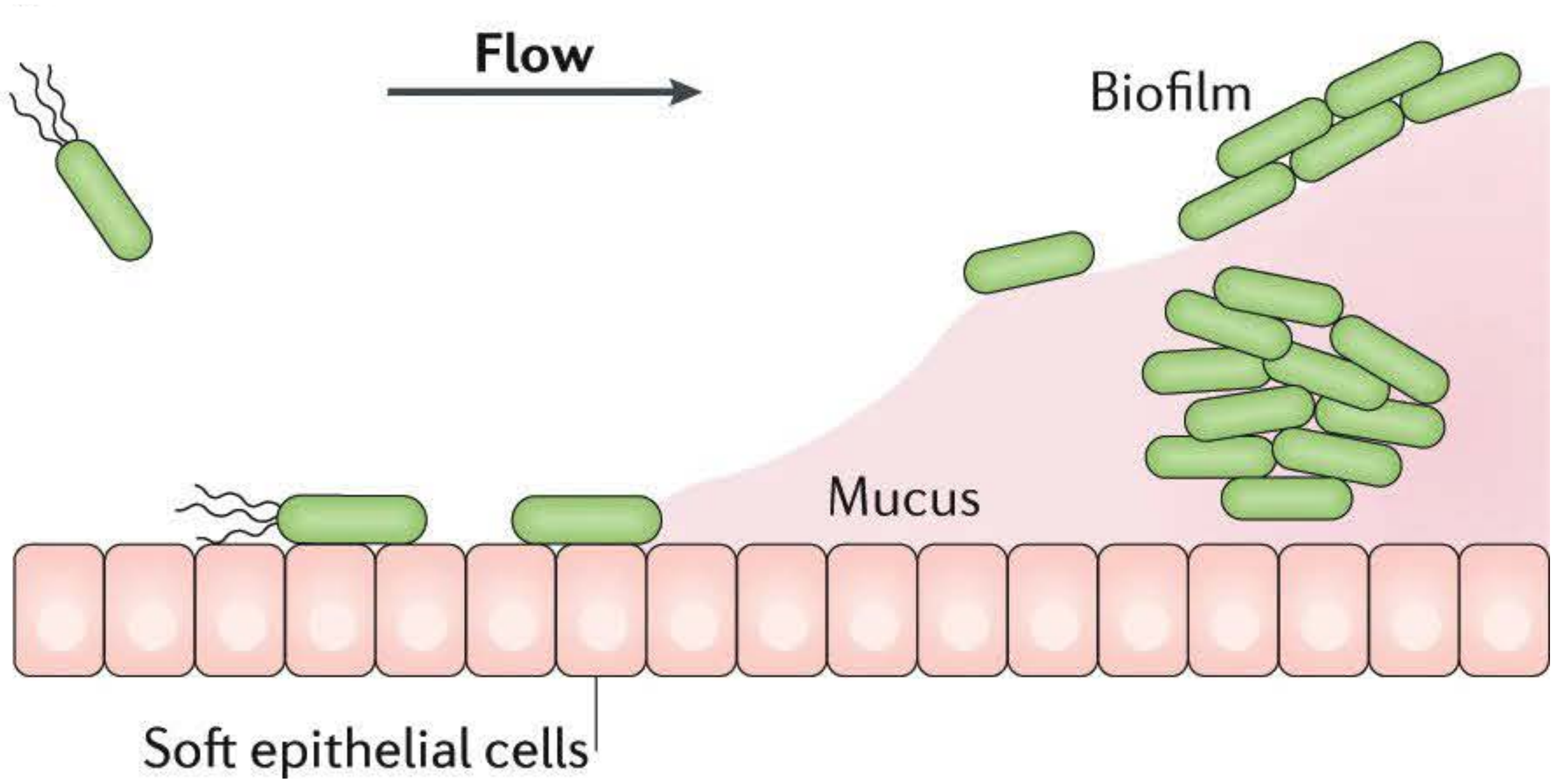


- **Flow shapes biofilm architecture and surface colonization dynamics**
- **Flow regulates biofilm spatial organization**

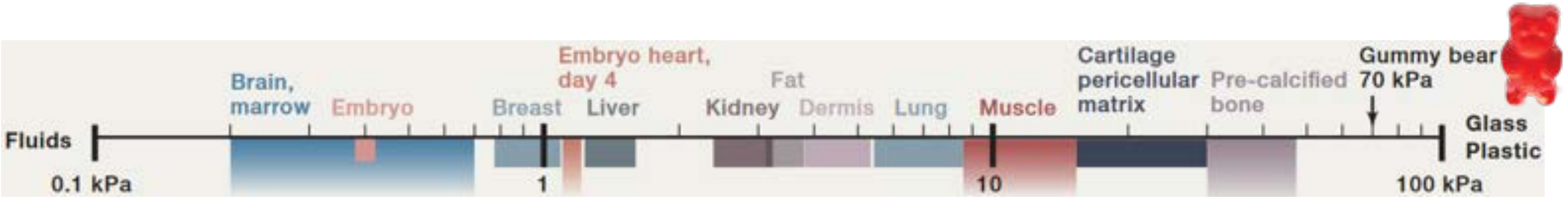
▸ **Impact on social interactions**

Rossy et al., Nat. Com. 2019

Biofilms on soft surfaces

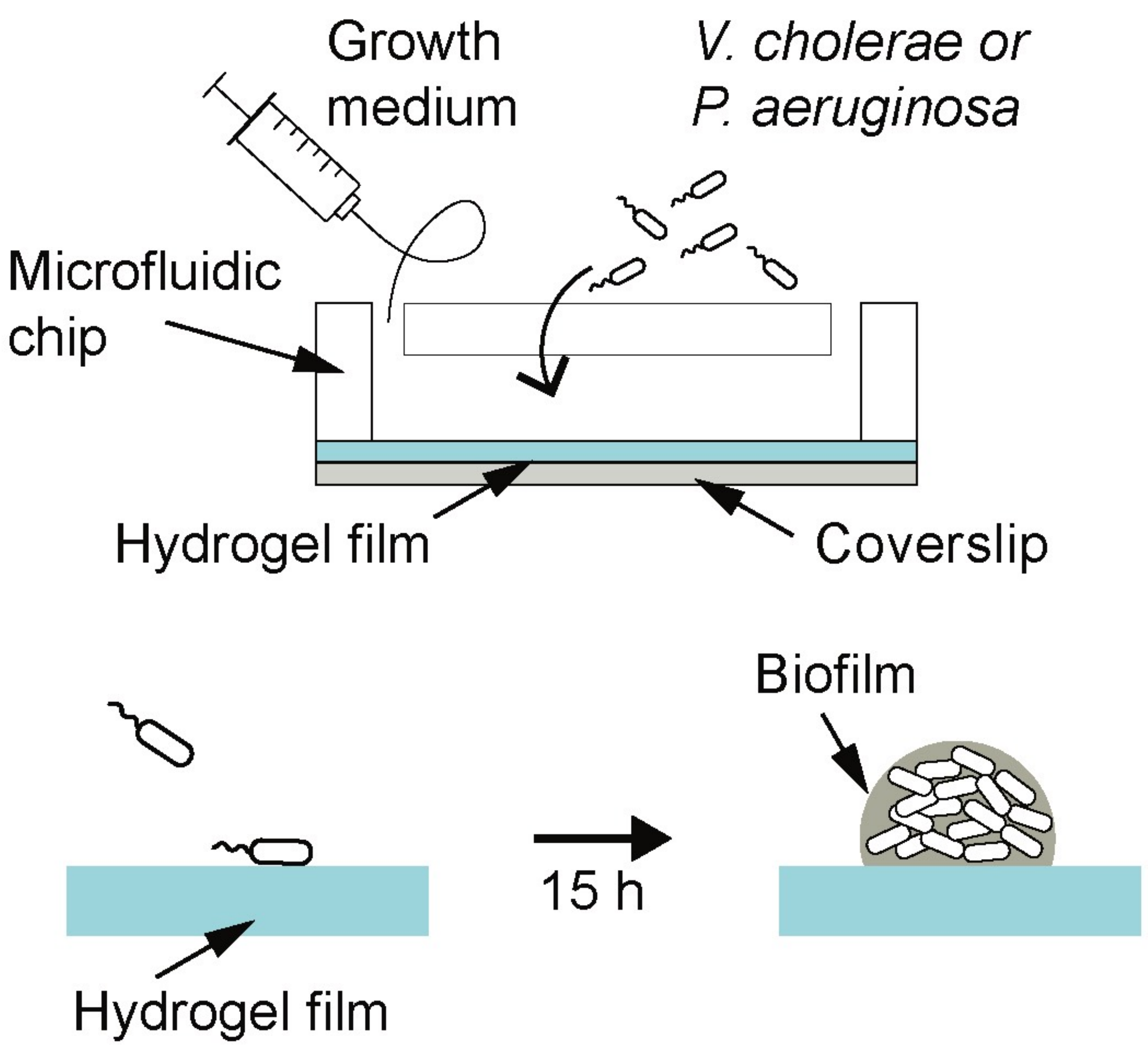


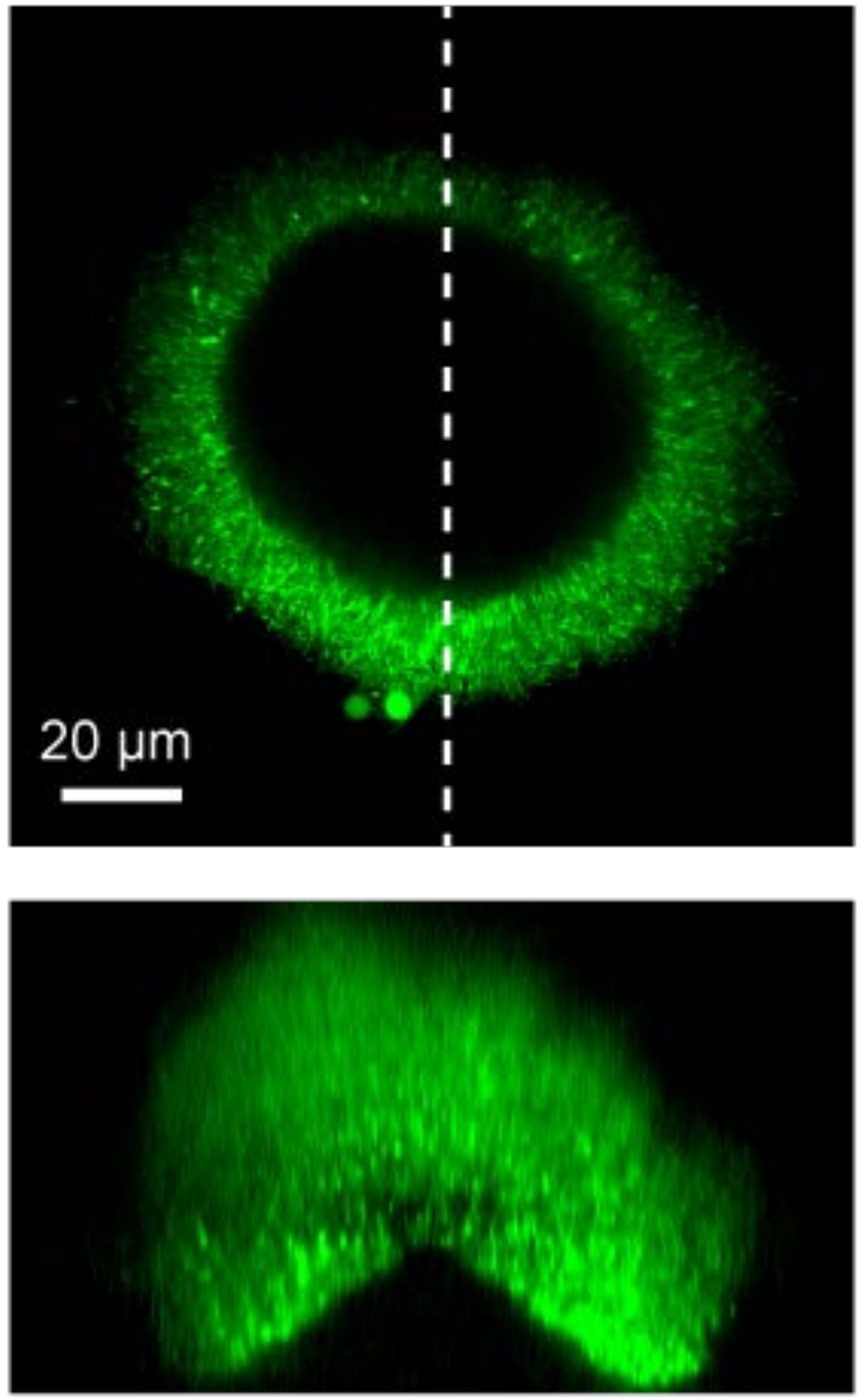
in vivo biofilms form on soft materials



Does surface stiffness regulate biofilm biogenesis?

Biofilms on synthetic hydrogels

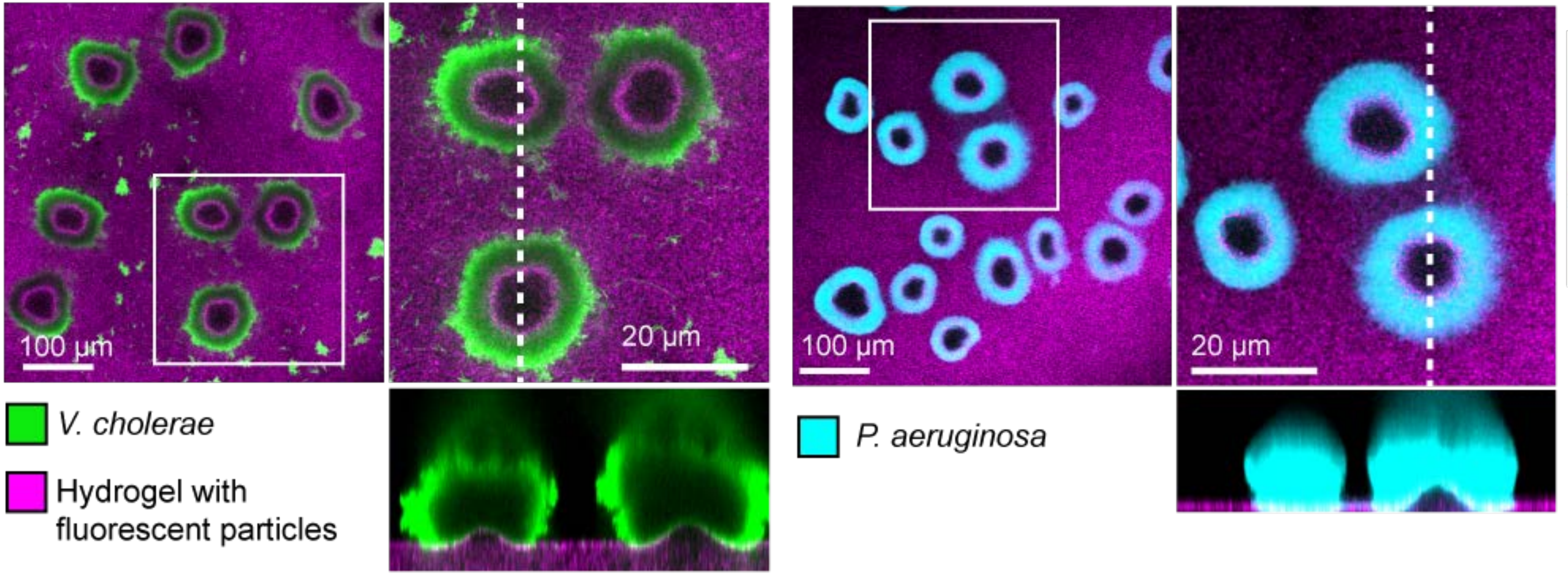




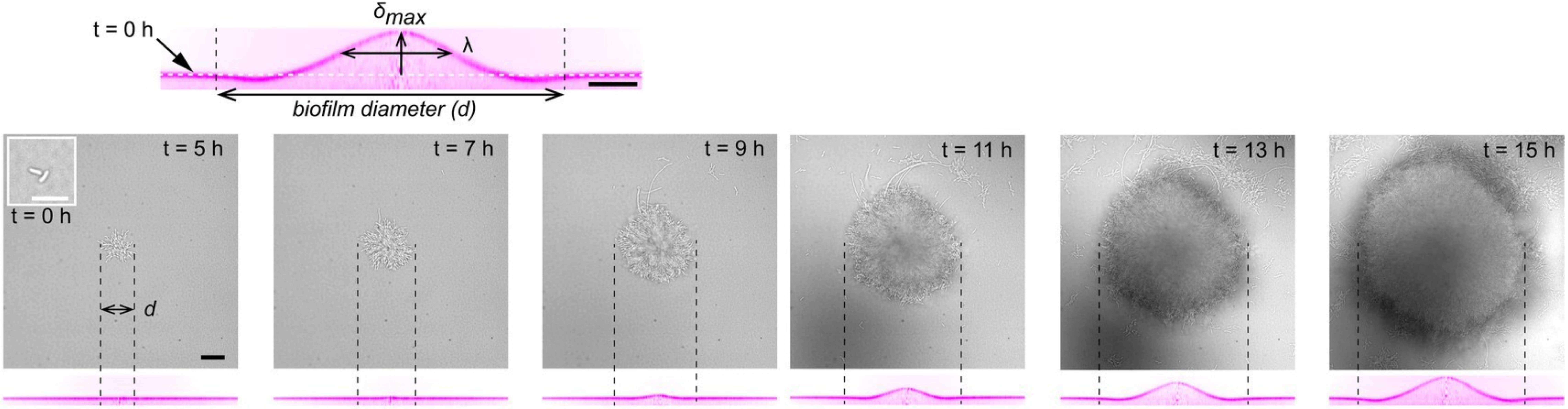
Vibrio cholerae

“rugose” strain

Biofilms deform soft substrates

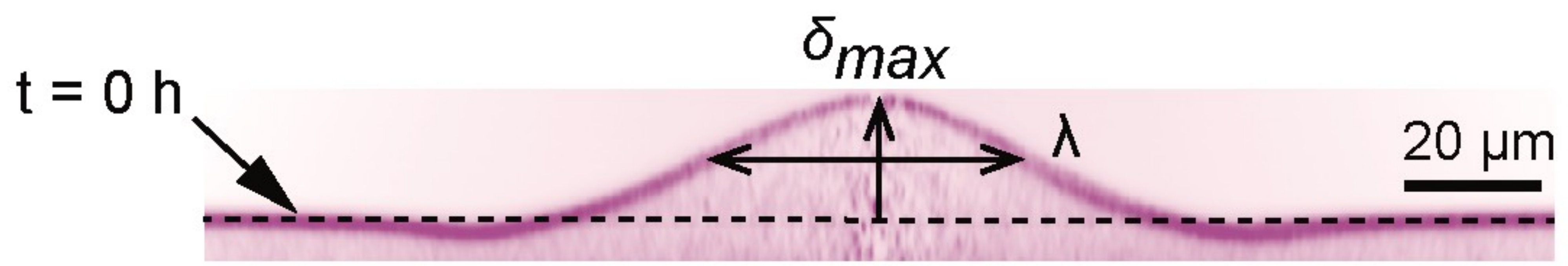


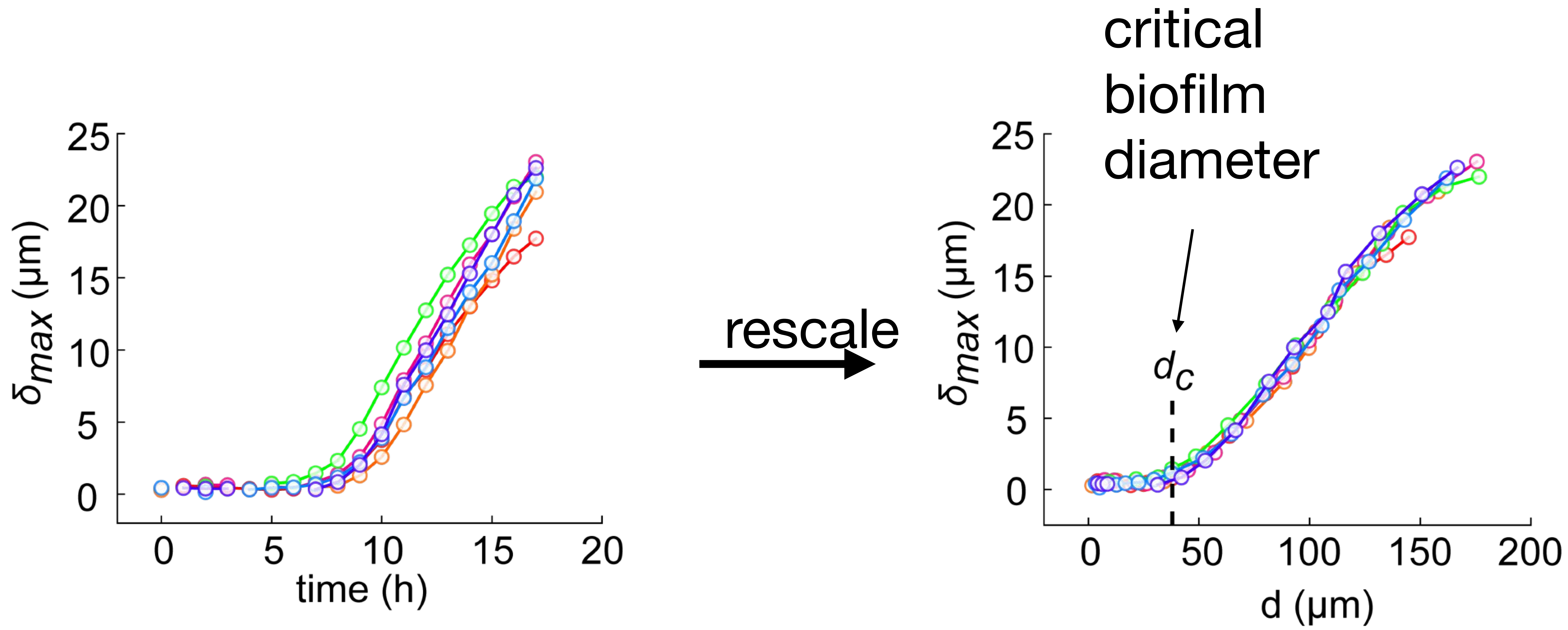
Deformations follow growth



Cont et al., eLife 2020

Deformation dynamics

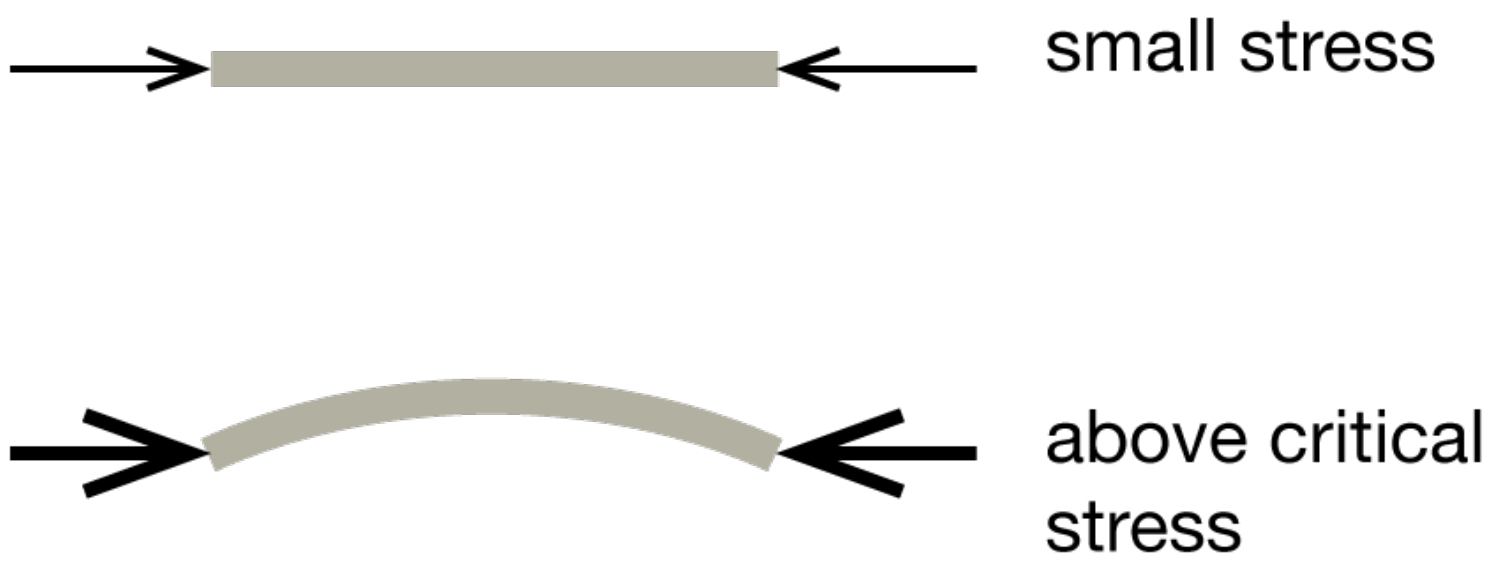




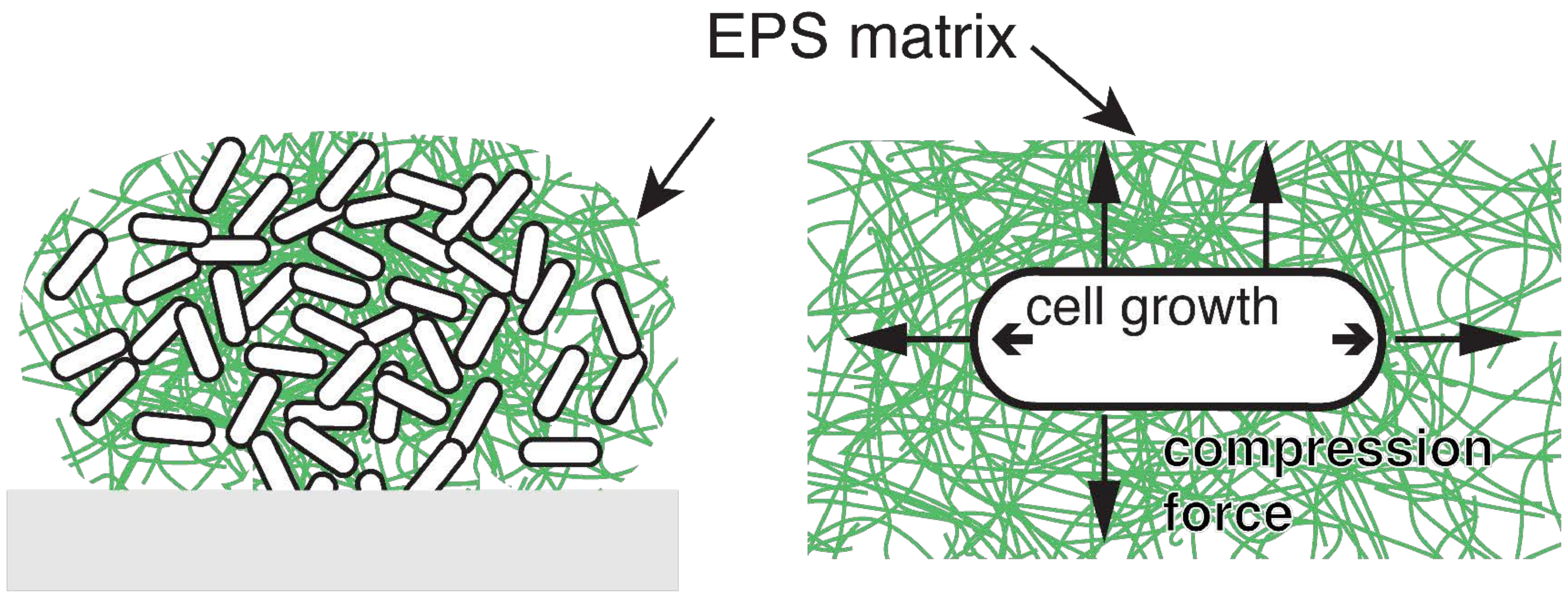
Threshold diameter suggests a buckling instability mechanism for deformation

Cont et al., eLife 2020

Buckling



Wang and Zhao, Sci rep 2015

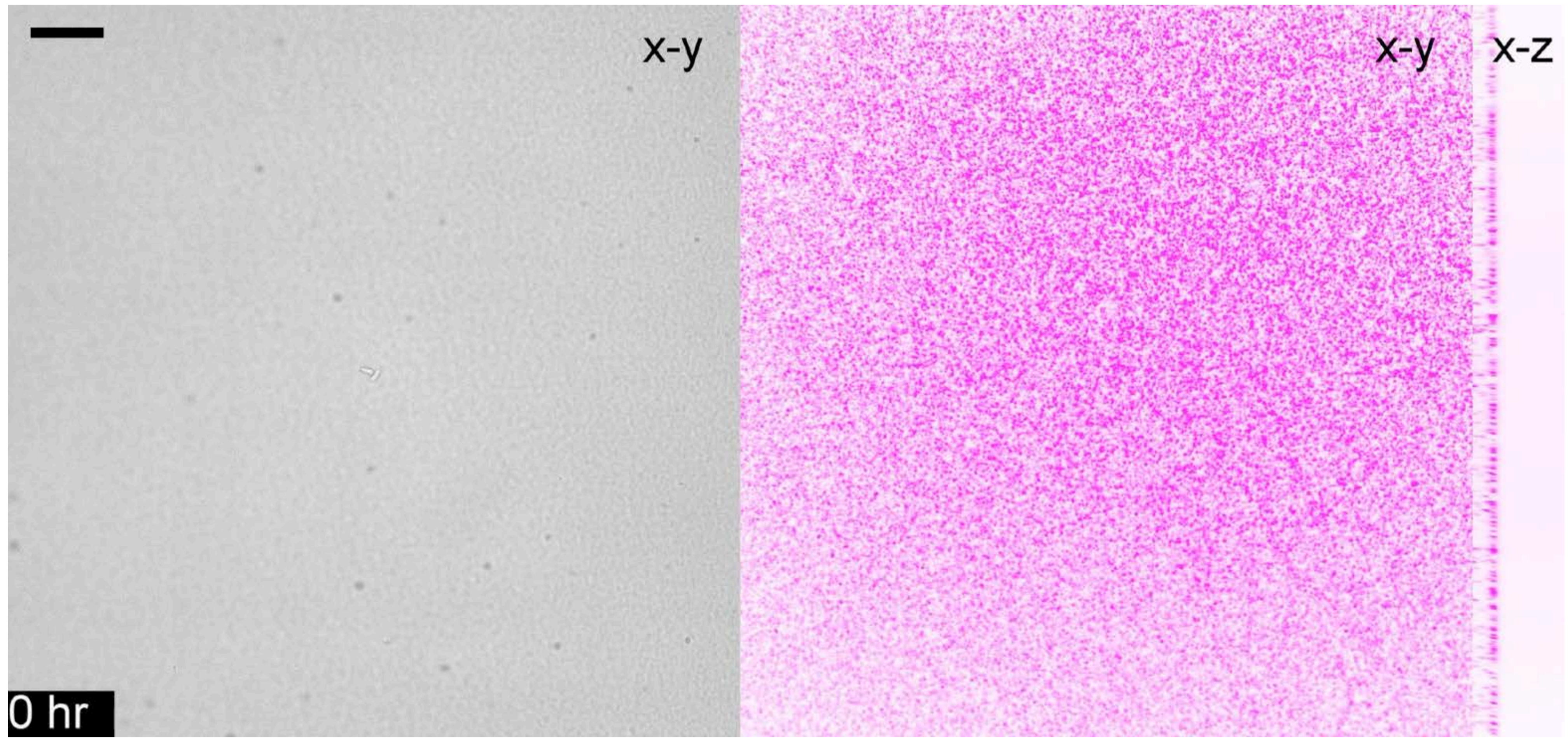


How is the force generated?

Growth + cell-cell cohesion

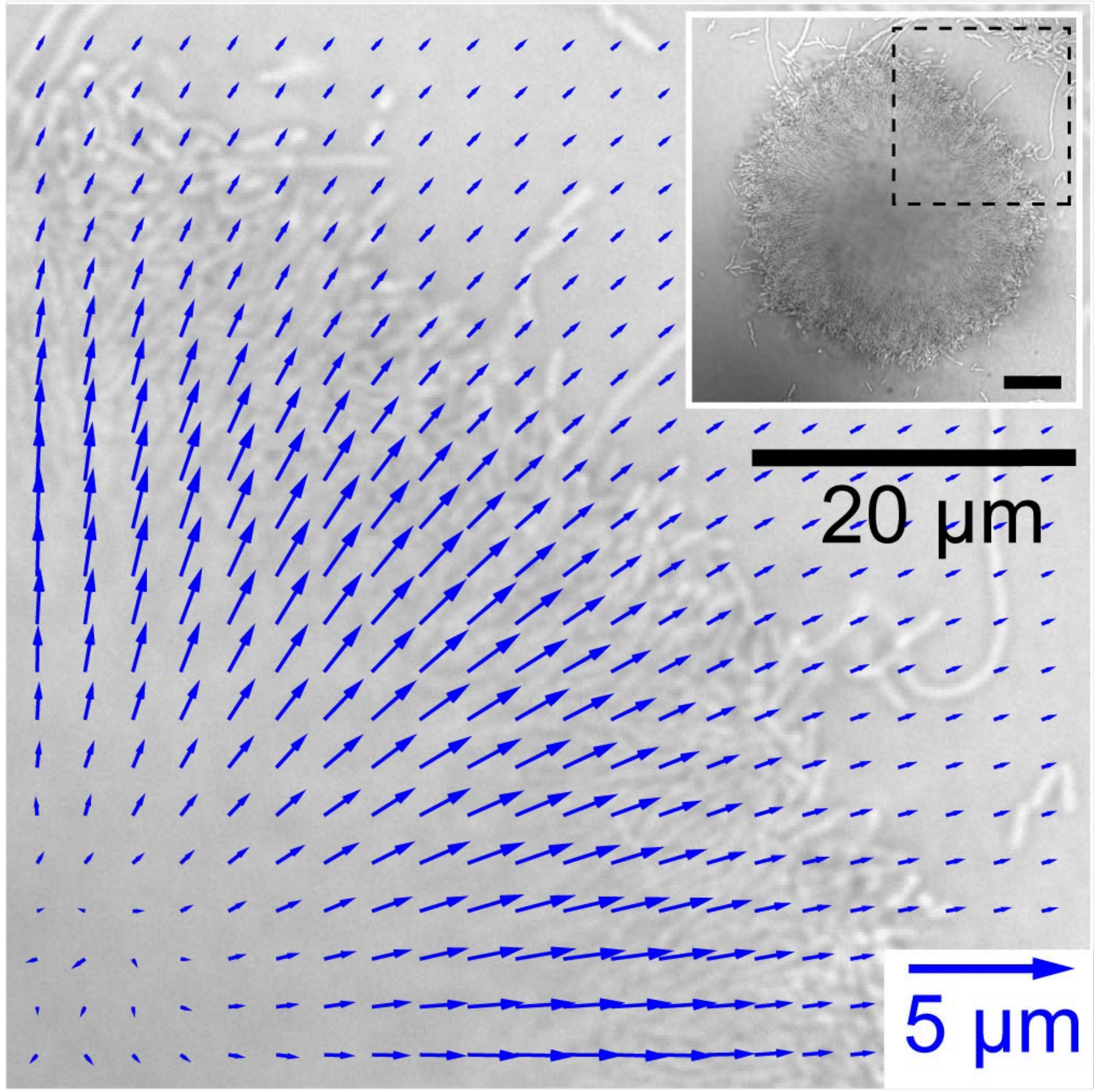
→ internal stress

Biofilms generate a force on their substrate



1. 3D timelapse imaging of tracers in hydrogel
2. image registration
3. digitial volume correlation (DVC)
4. traction force microscopy

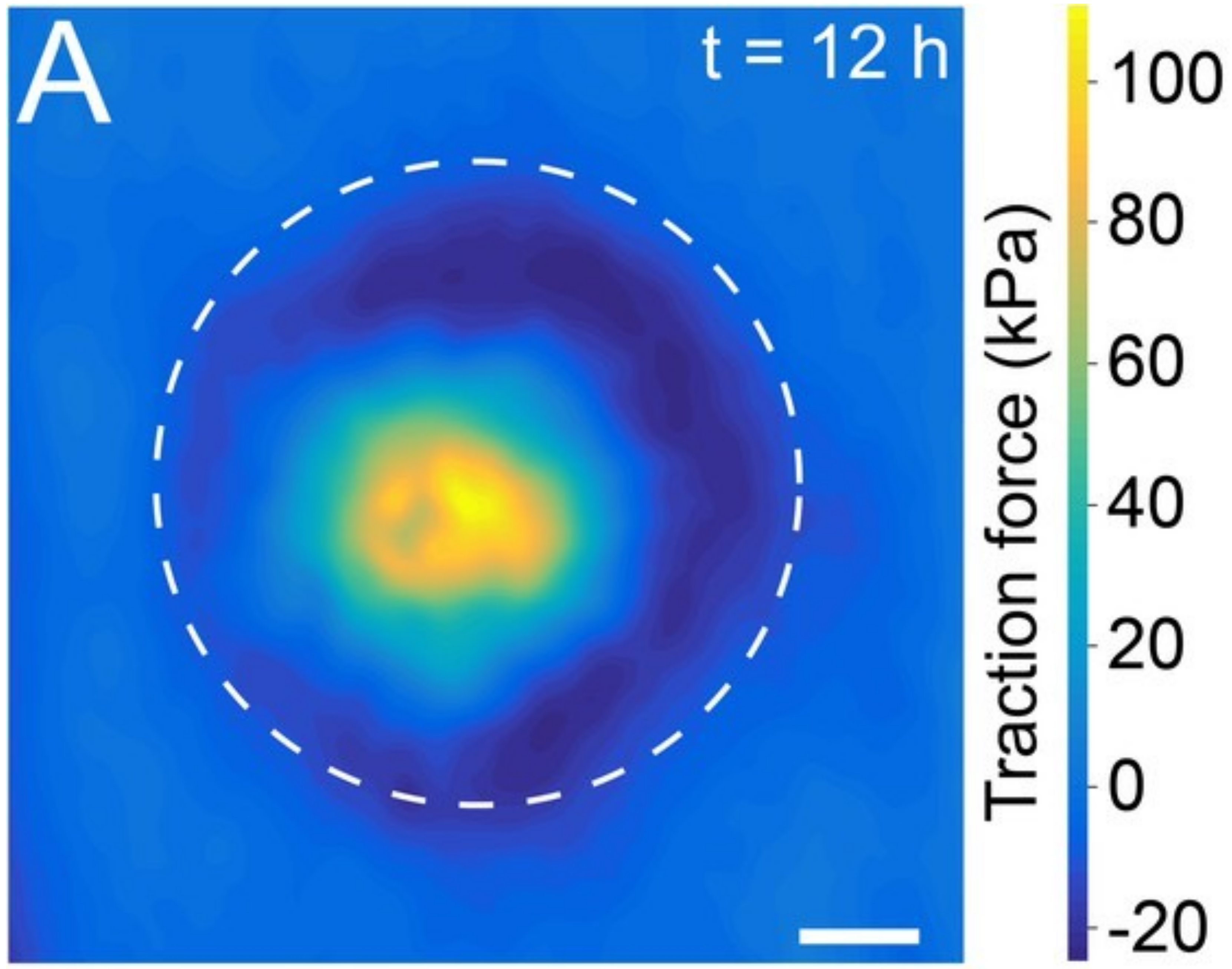
Bar-Kochba, Exp. Mech., 2014
github.com/FranckLab



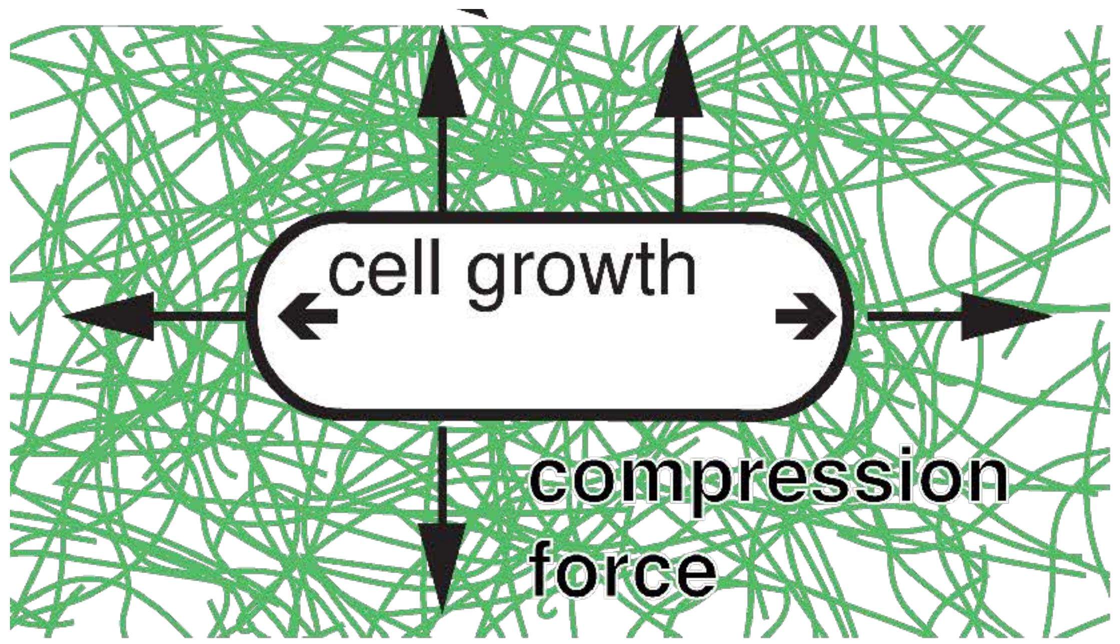
Growing biofilms “push” on the substrate

→ internal stress builds up in the biofilm, force is oriented radially

Biofilms generate large mechanical stress



Large internal stress transmitted to substrate

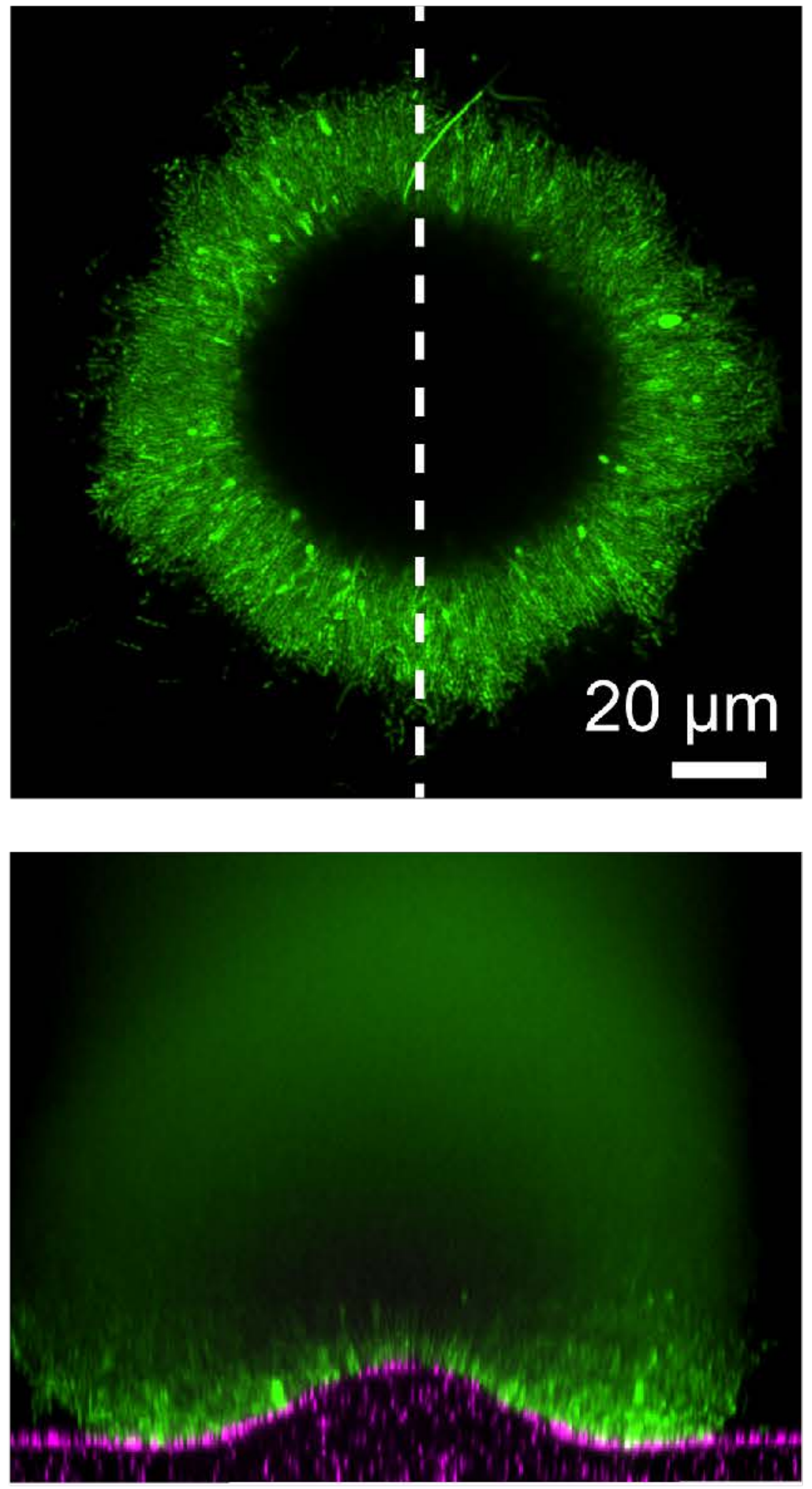


~ MPa turgor pressure drives cell growth within the matrix

Epithelial cell-cell junctions come apart under few kPa
→ What happens in tissue?

The role of matrix components

Vc WT*



Cell-cell cohesion promotes buildup of internal mechanical stress

→ **biofilm buckling**

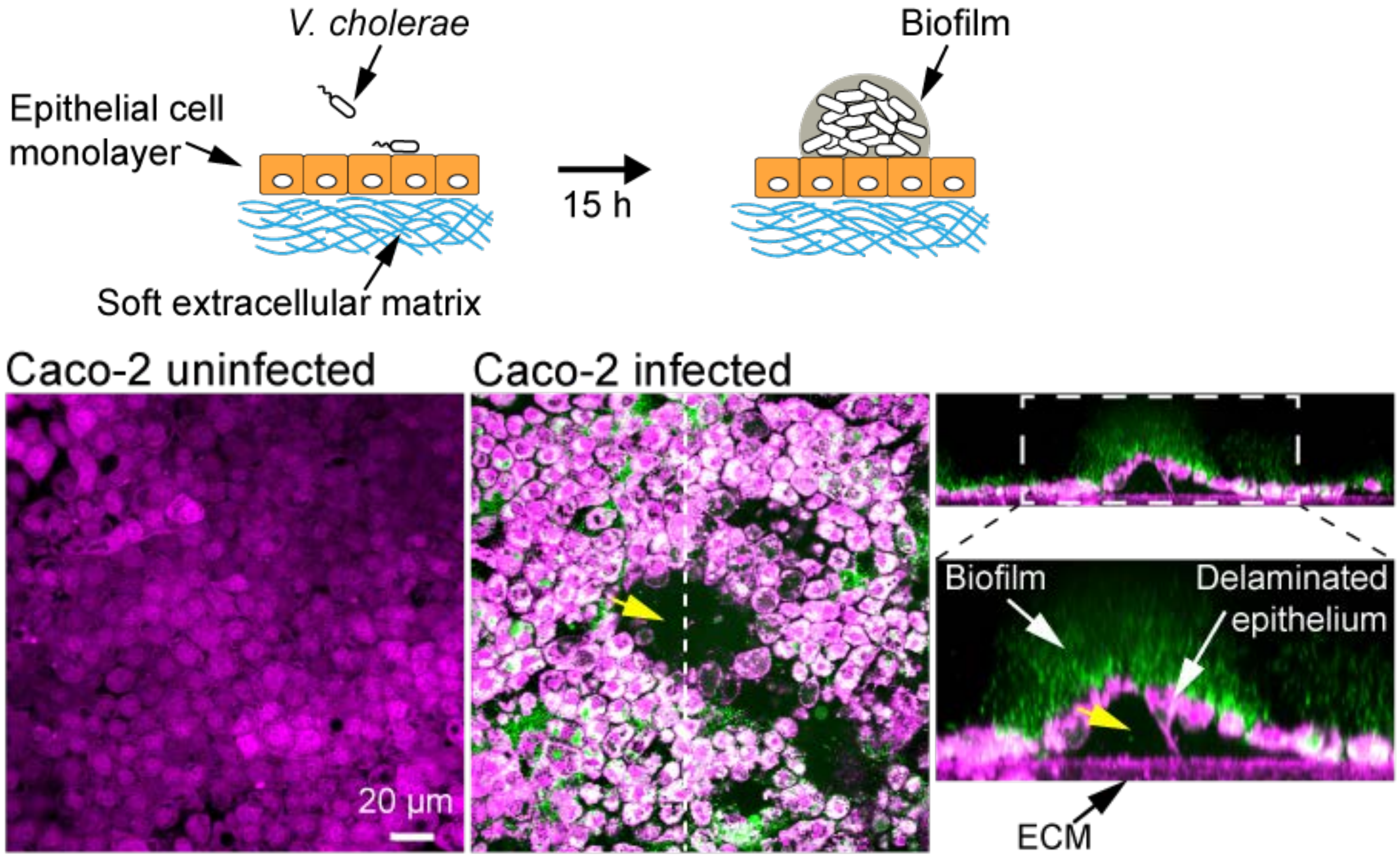
Biofilm-substrate adhesion helps transmit buckling-induced stress to substrate

→ **substrate deformation**

Cont et al., eLife 2020

Biofilms on epithelia

a mechanical mode of infection



Cont et al., eLife 2020

Mechanobiology in biofilms
summary

- Bacteria greatly benefit from forming multicellular structures
- The EPS matrix provides mechanical cohesion of the biofilm
- Single cells coordinate matrix patterning
- Each matrix component has a specific biomechanical function
- External stressor such as hydrodynamic forces influence biofilm architecture
- Mechanical stress within the biofilm has a strong impact on organization