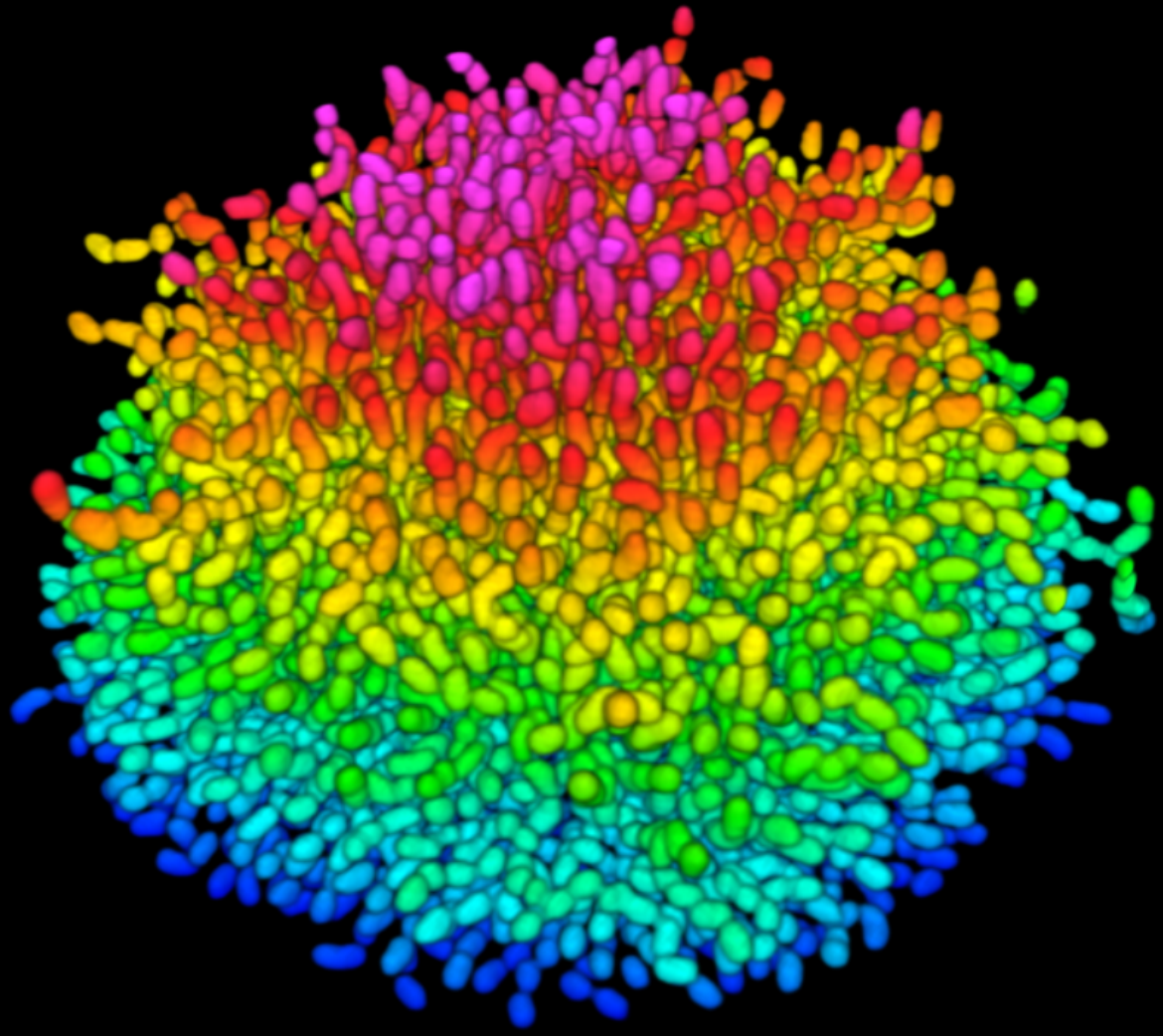
Building a biofilm the *Vibrio cholerae* way



Ned Wingreen – Princeton University

NOTICE.

PREVENTIVES OF

CHOLERA!

Published by order of the Sanatory Committee, under the sanction of the Medical Counsel.

BE TEMPERATE IN EATING & DRINKING!

Avoid Raw Vegetables and Unripe Fruit !.

Abstain from COLD WATER, when heated, and above all from *Ardent Spirits*, and if habit have rendered them indispensable, take much less than usual.

SLEEP AND CLOTHE WARM !

☞DO NOT SLEEP OR SIT IN A DRAUGHT OF AIR,

Avoid getting Wet !

Attend immediately to all disorders of the Bowels.

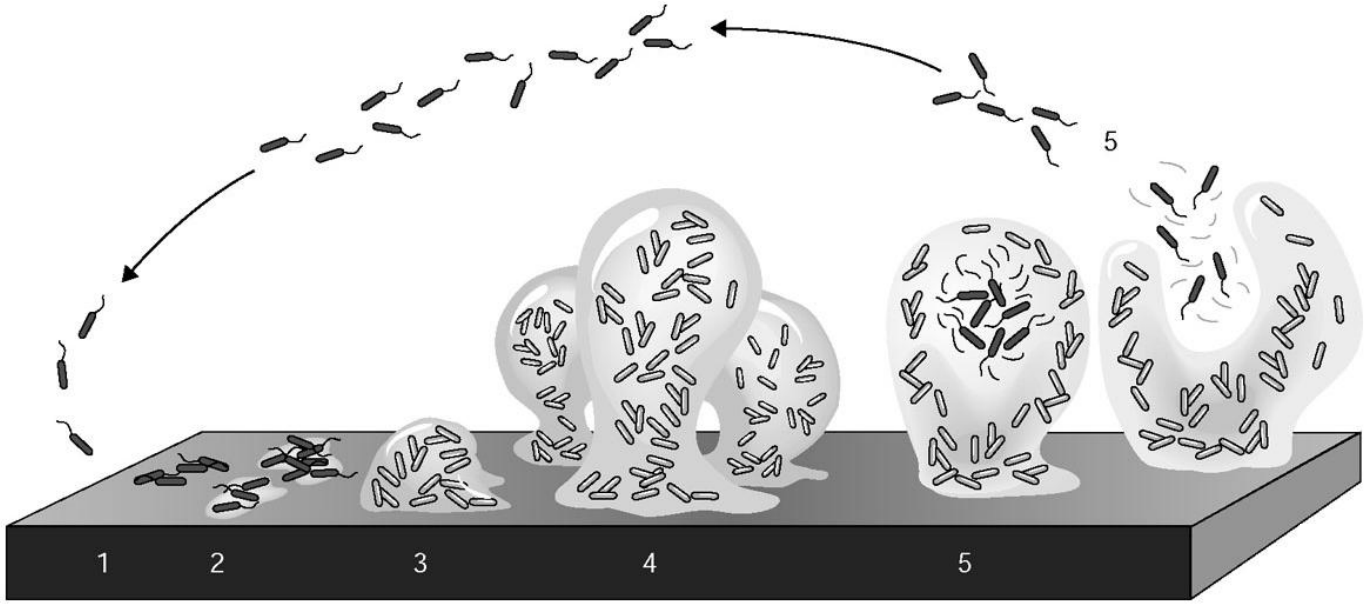
TAKE NO MEDICINE WITHOUT ADVICE.

Medicine and Medical Advice can be had by the poor, at all hours of the day and night, by applying at the Station House in each Ward.

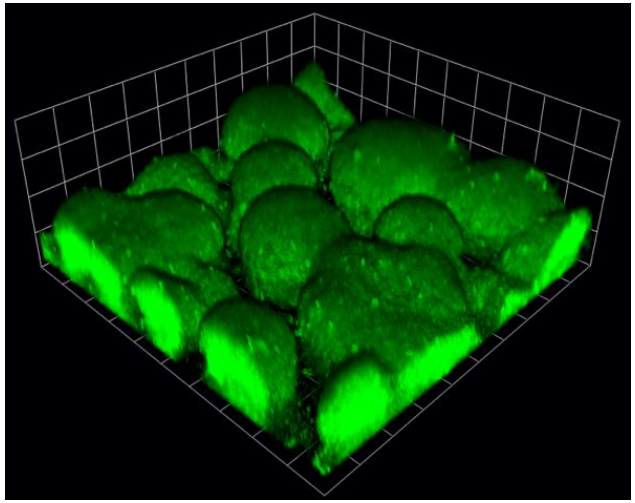
CALEB S. WOODHULL, *Mayor.*

JAMES KELLY, *Chairman of Sanatory Committee.*

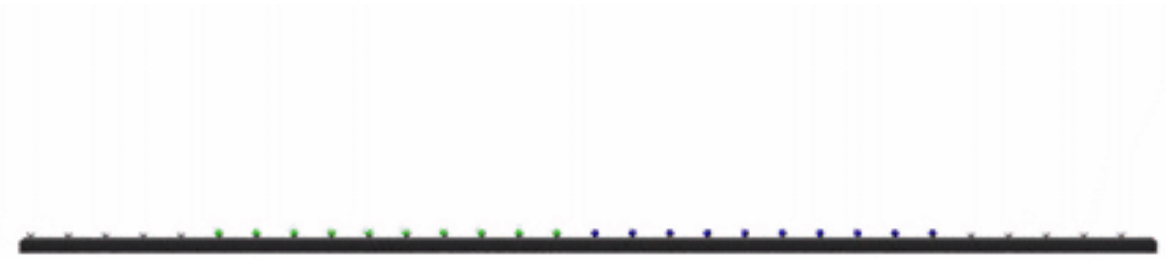
How to form a 3D biofilm?



Adapted from Stoodley (2001)

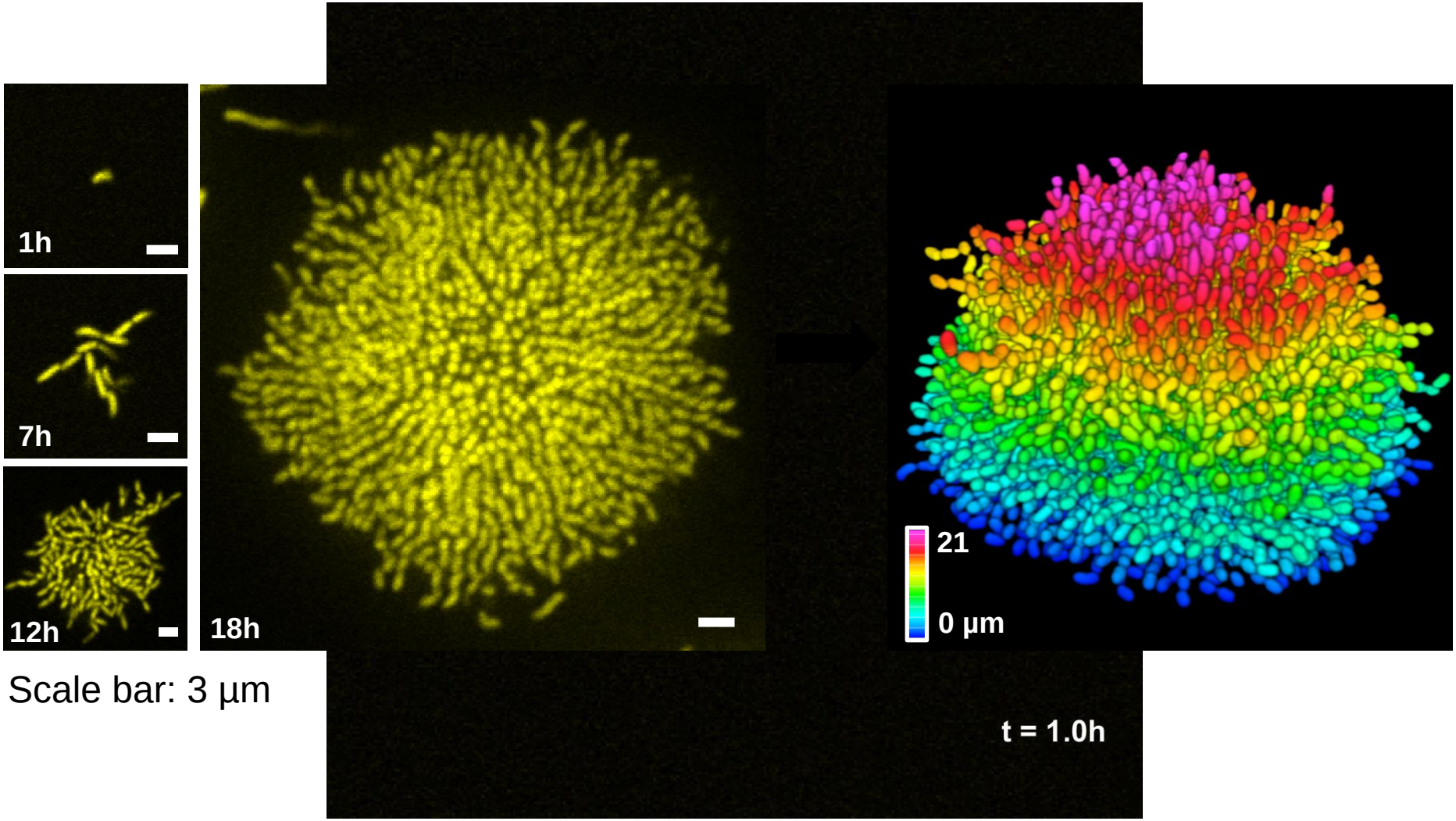


Harwood *et al*. (2005)



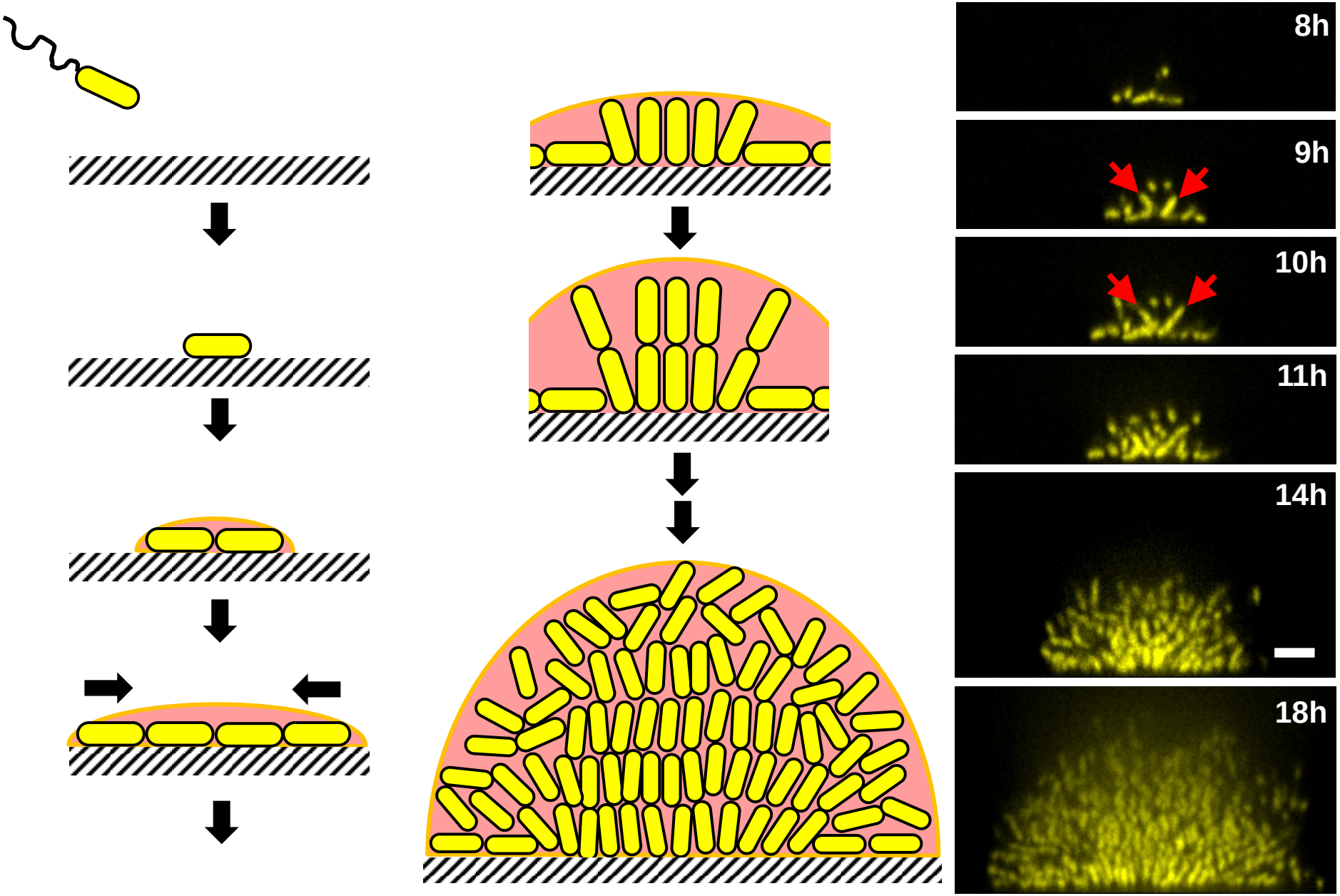
www.idynomics.org

Biofilm formation cell-by-cell

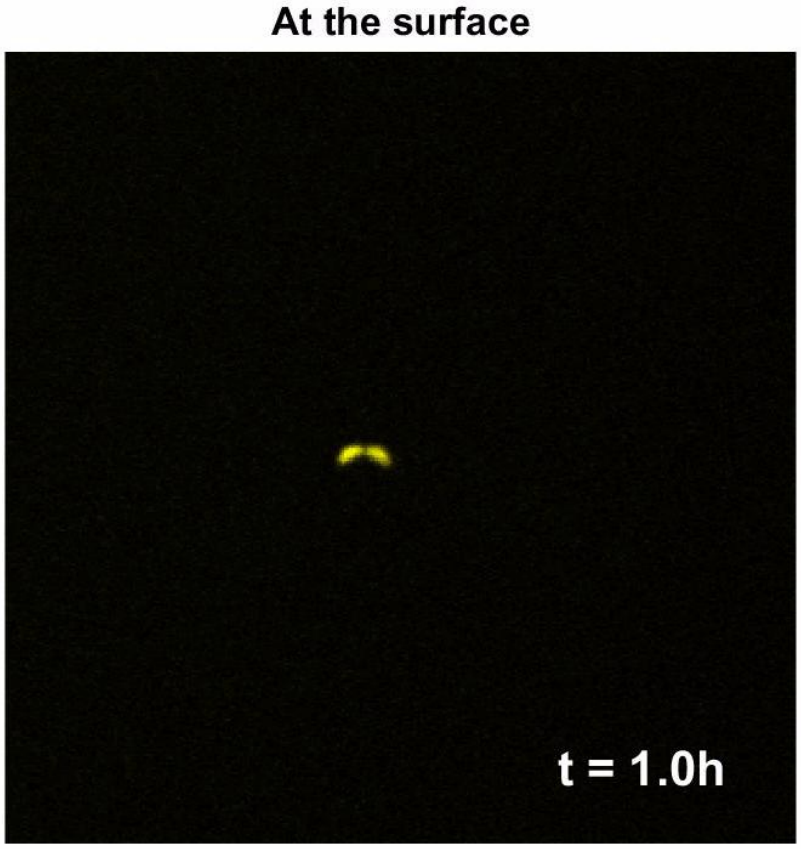


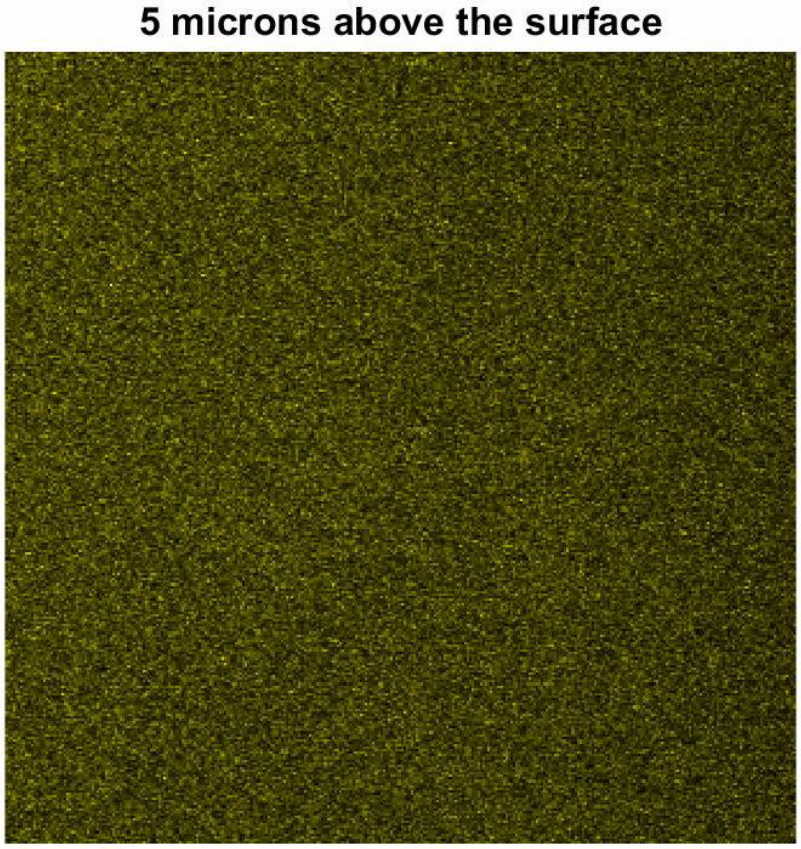
Scale bar: 3 µm

Schema for *V. cholerae* biofilm growth



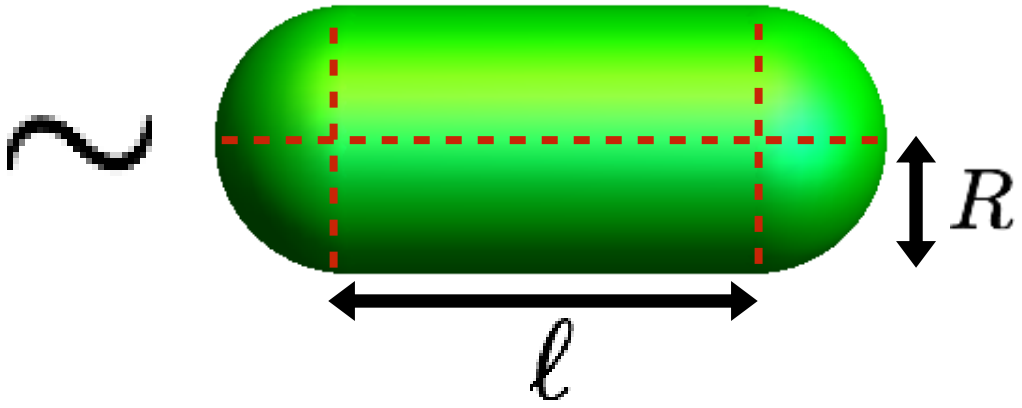
Role of cell-to-surface adhesion

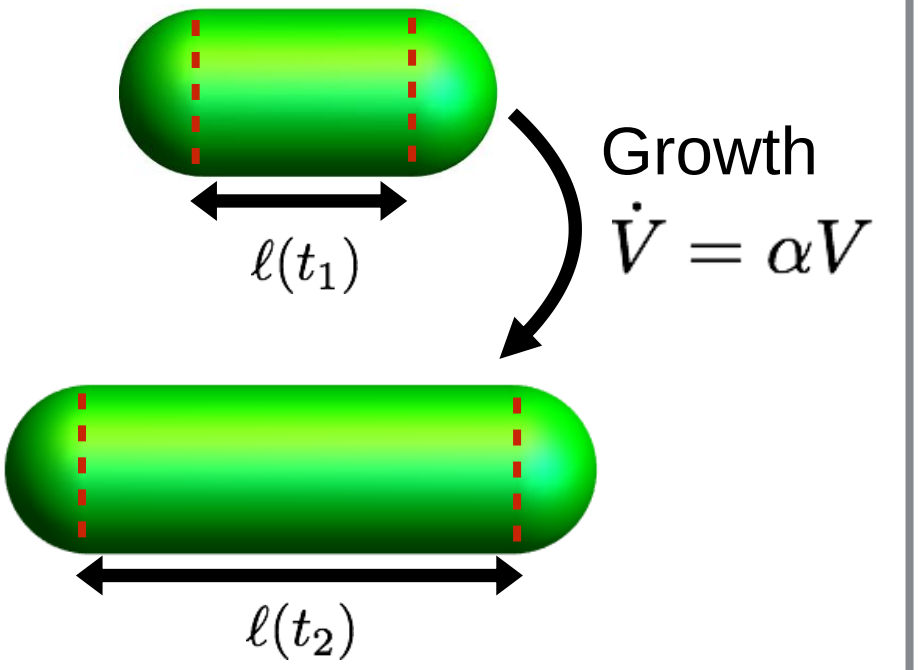


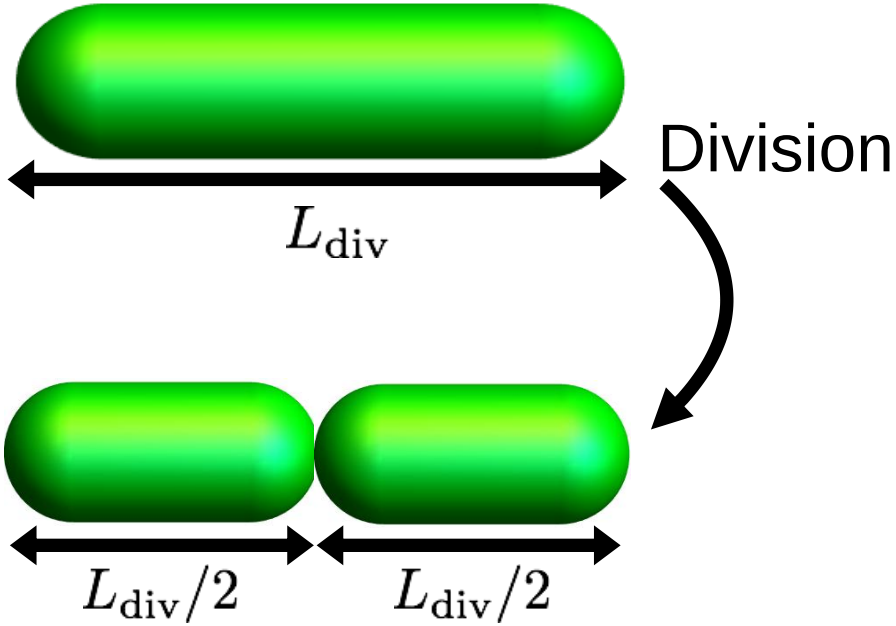


Δ*bap1* Δ*rbmC* mutant

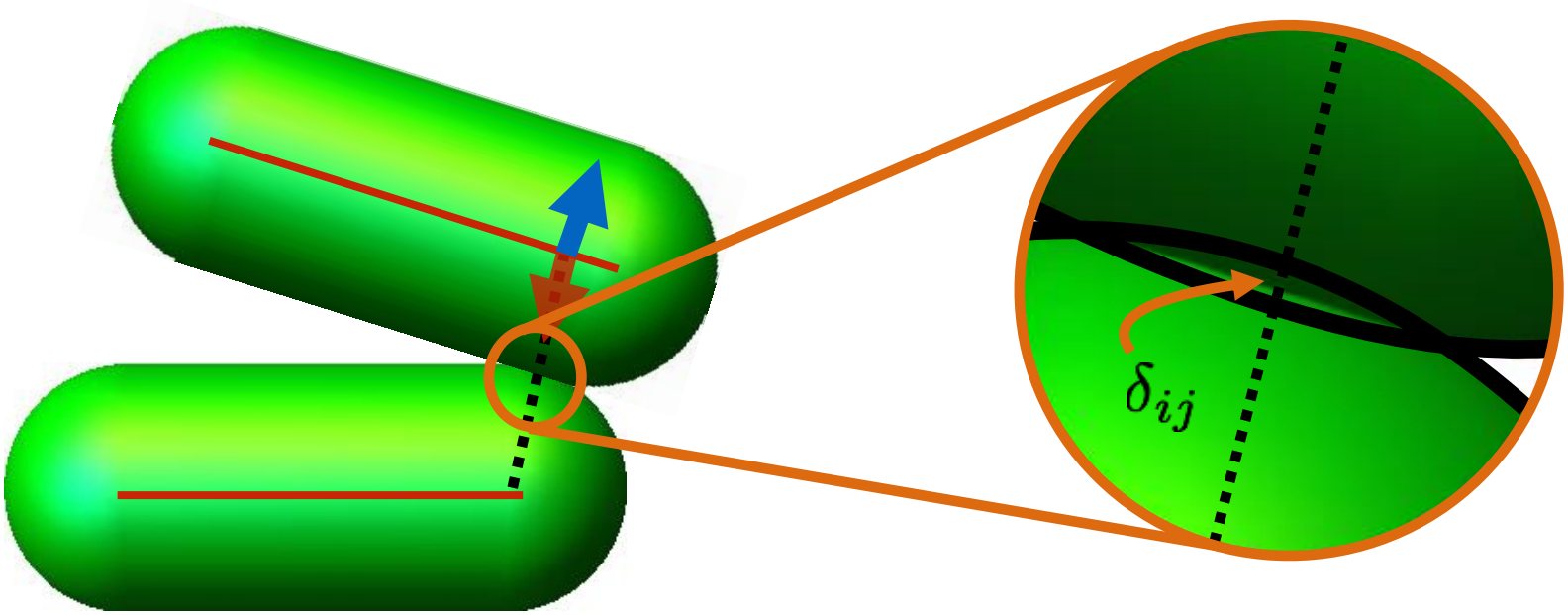
Agent based simulation







Cell-cell interaction

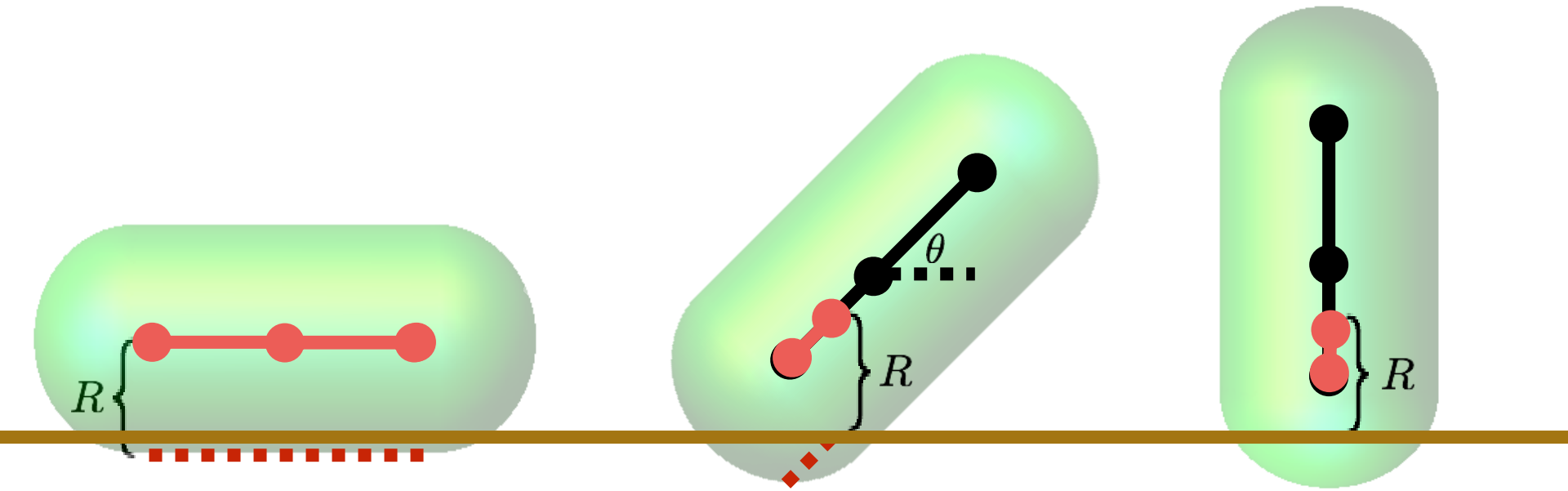


Repulsion due to elastic energy

Attraction due to adhesive energy

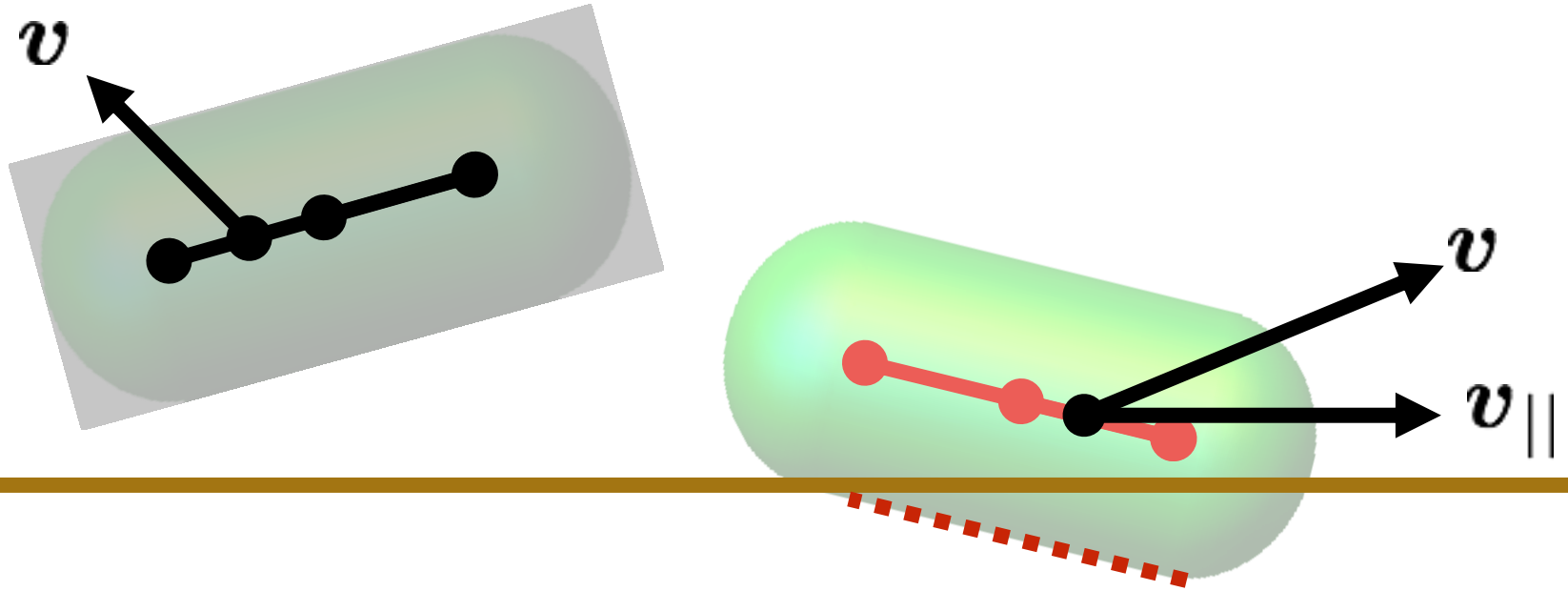
$$E_{\text{cell}-\text{cell}} = E_0 R^{1/2} \delta_{ij}^{5/2} - A_0 R^{-1} \delta_{ij}$$

Cell-surface interaction interpolates between cylindrical and spherical contacts



$$\delta_{\text{eff}}^{5/2} = \delta_{ij}^{2} \cos^{2}(\theta) + R^{1/2} \delta_{ij}^{3/2} \sin^{2}(\theta)$$

Viscous drag



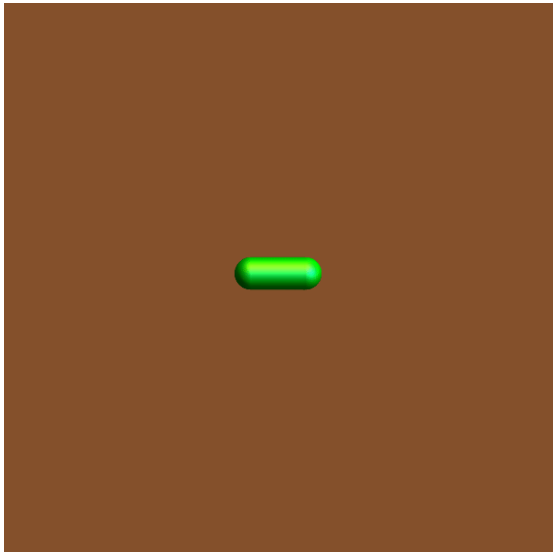
$$\boldsymbol{F}_{\text{drag}} = \nu_0 \boldsymbol{v} + \nu_1 A_{\text{eff}} \boldsymbol{v}_{||}$$

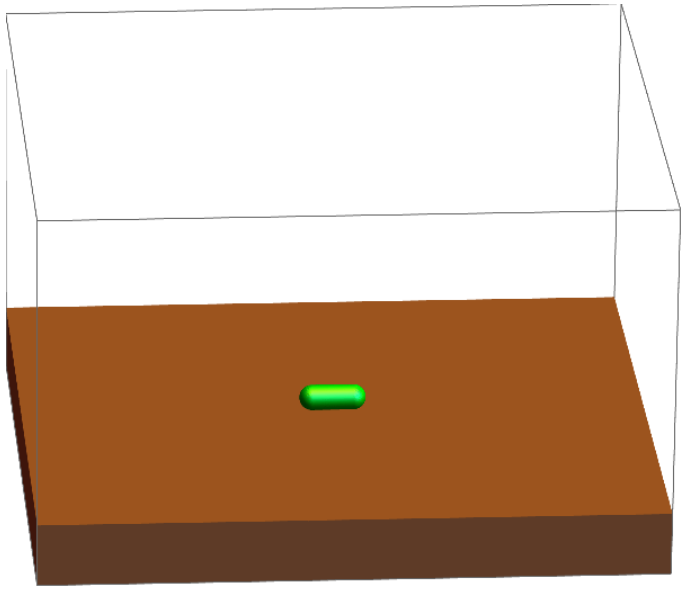
Ambient drag

Surface drag

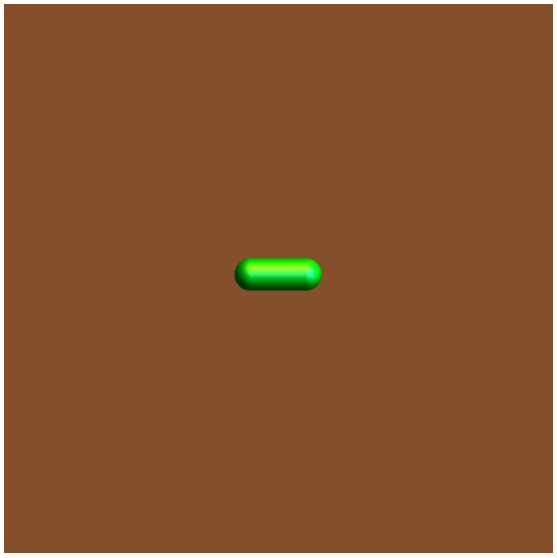
Surface interaction facilitates 3D growth

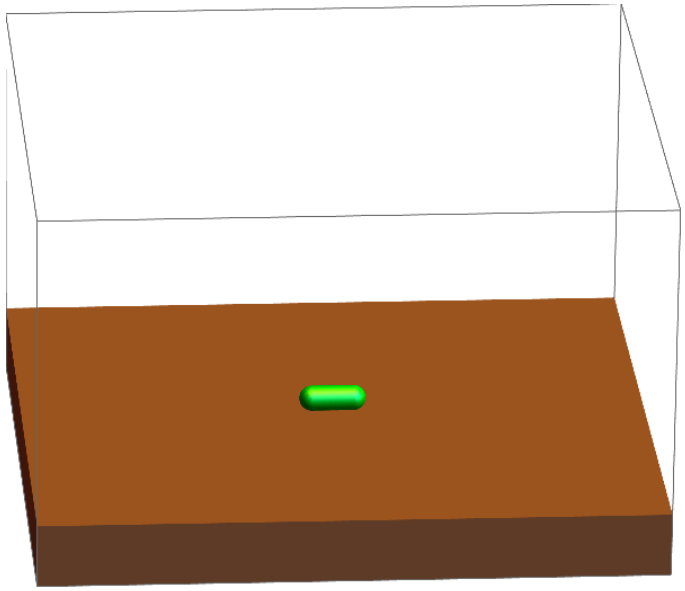
Ambient drag only



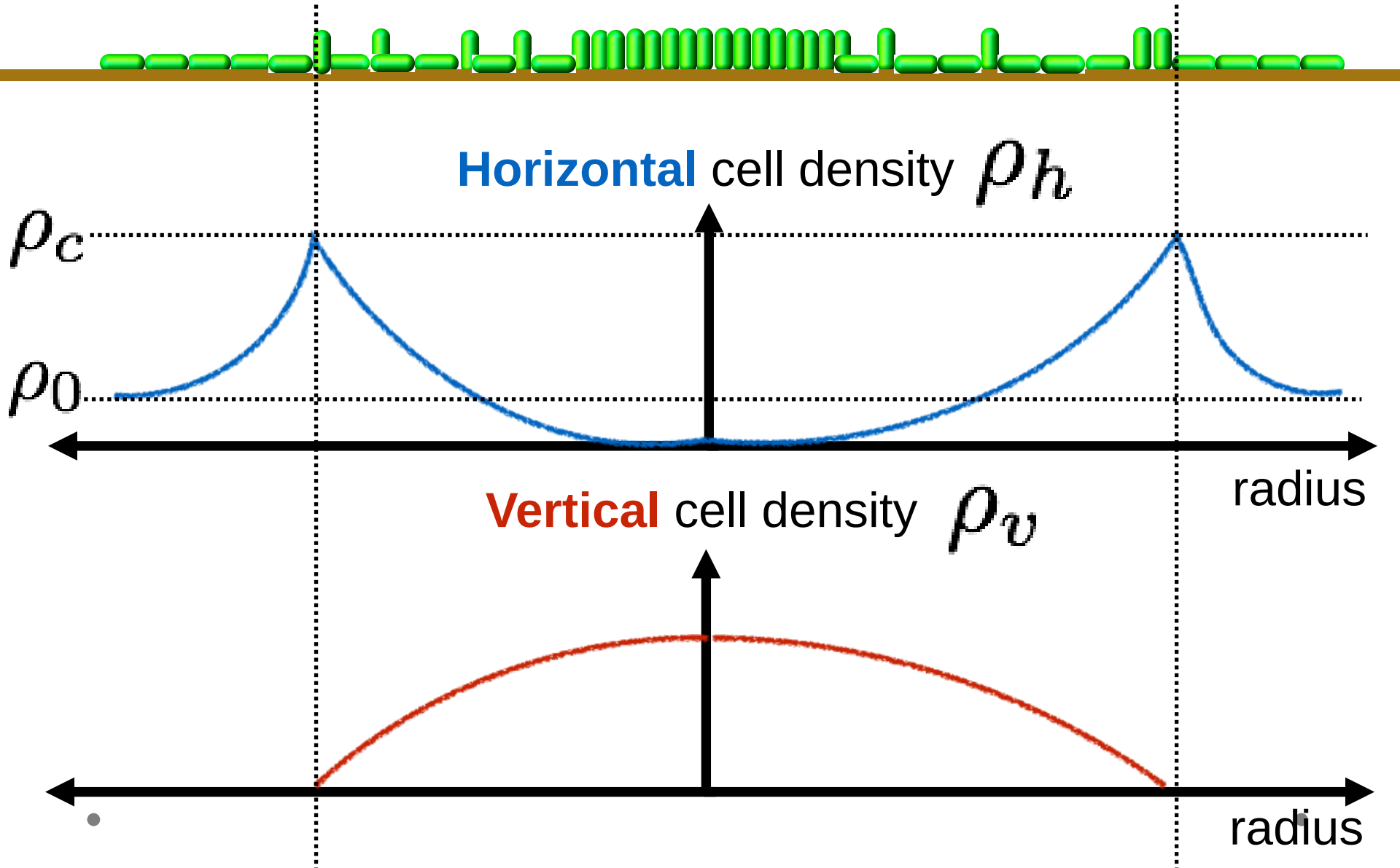


Ambient drag plus surface drag





Continuum model for “verticalization”



Continuity equations for horizontal and vertical cells

Horizontal cells

$$\dot{\rho_h} + \nabla \cdot (\rho_h \boldsymbol{v}) = \alpha \rho_h$$

Cell flux

growth rate

pop-up rate ("verticalization")

$$\dot{\rho_v} = \beta \Theta(p - p_c) \rho_h$$

Vertical cells

Cells push on their neighbors

Approximation: pressure ~ cell density (above packing density)

$$p = \begin{cases} \lambda(\rho_h + \xi\rho_v - \rho_0) & \text{if } \rho_h + \xi\rho_v > \rho_0 \\ 0 & \text{otherwise} \end{cases}$$

Force balance: $-\eta\rho_h \boldsymbol{v}_h = \nabla p$

Equations of motion

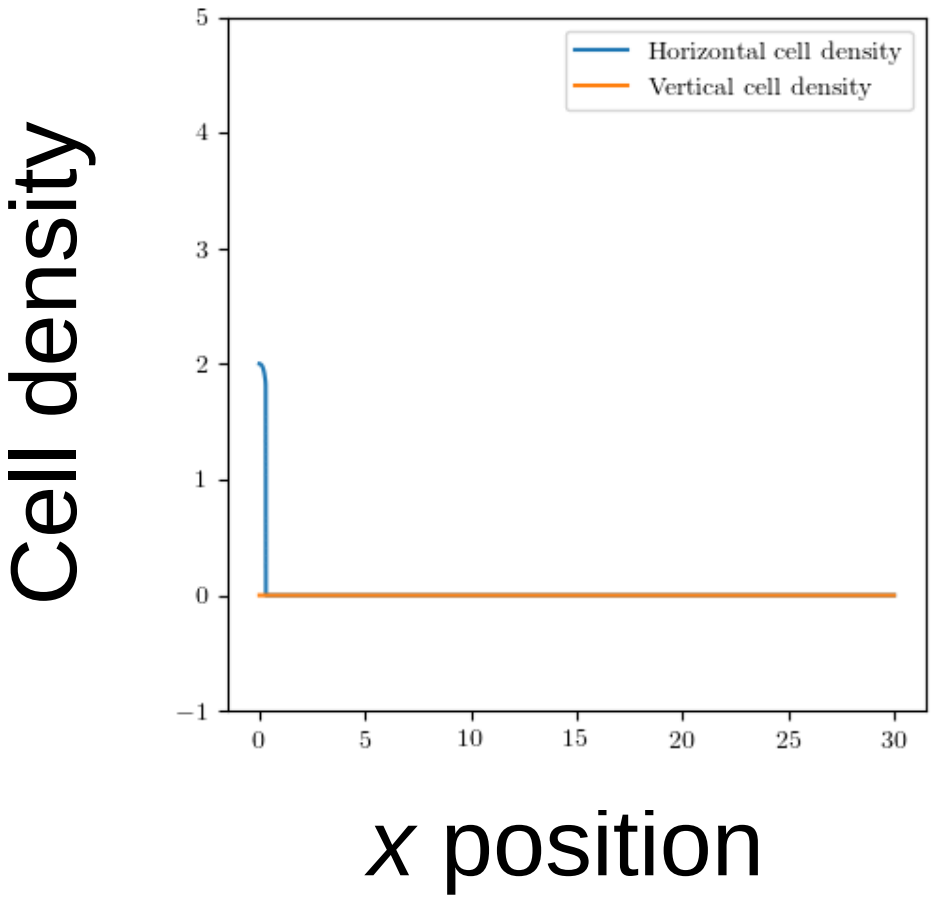
Horizontal cells

$$\dot{\rho_h} = \gamma \nabla^2 (\rho_h + \xi \rho_v) + [\alpha - \beta \Theta(p - p_c)]\, \rho_h$$

Vertical cells

$$\dot{\rho_v} = \beta \Theta(p - p_c) \rho_h$$

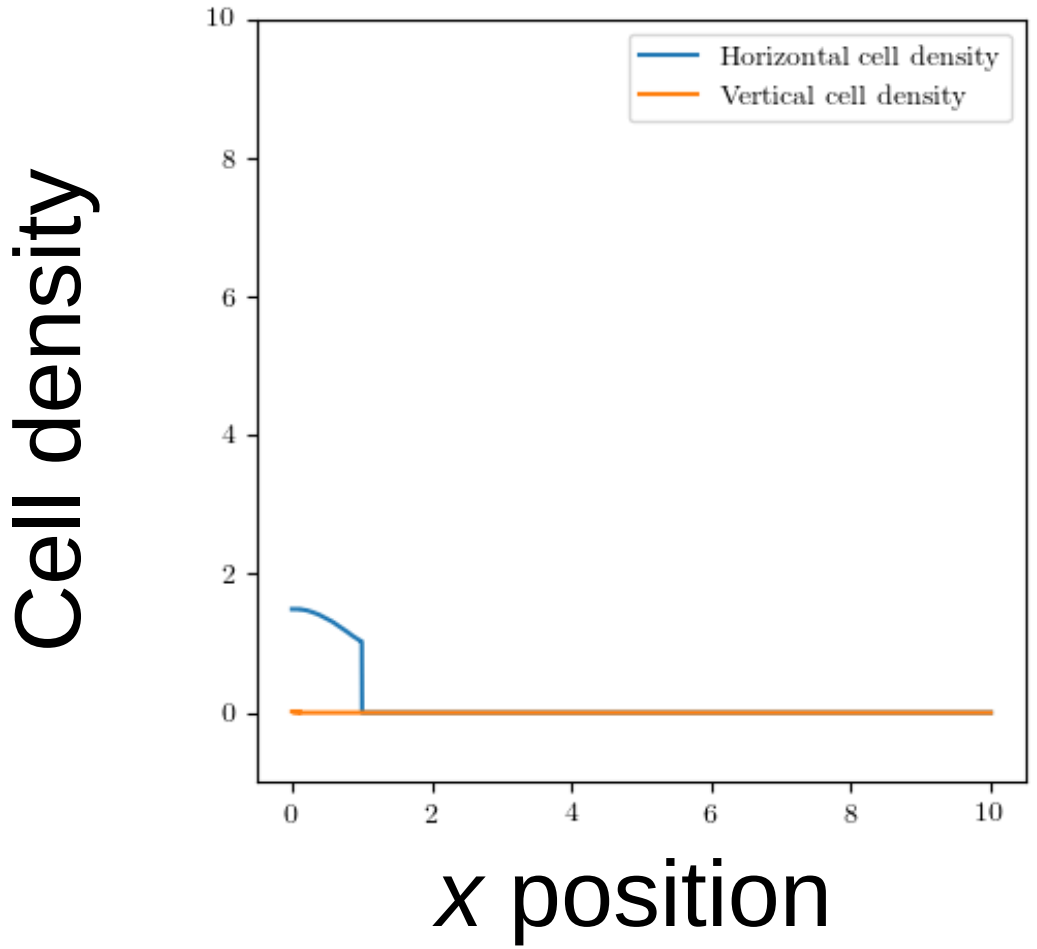
Numerical solution yields moving front



- Traveling "shark fin" of horizontal cells
- Front moves with constant speed

Can we predict the speed and shape?

If verticalization is fast → “isobaric” regime

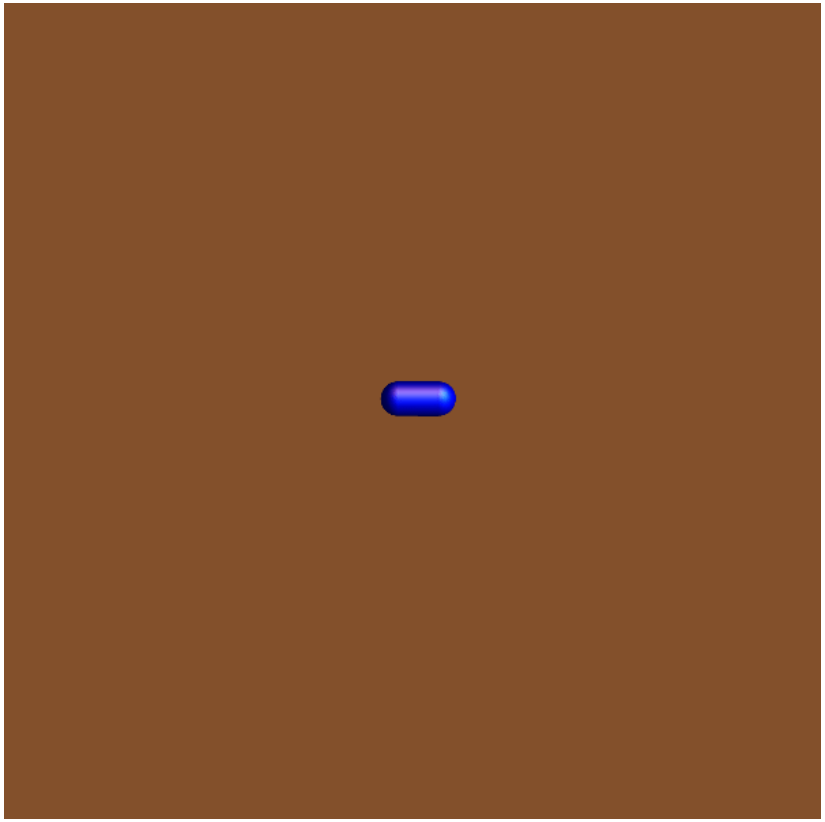


Isobaric regime if

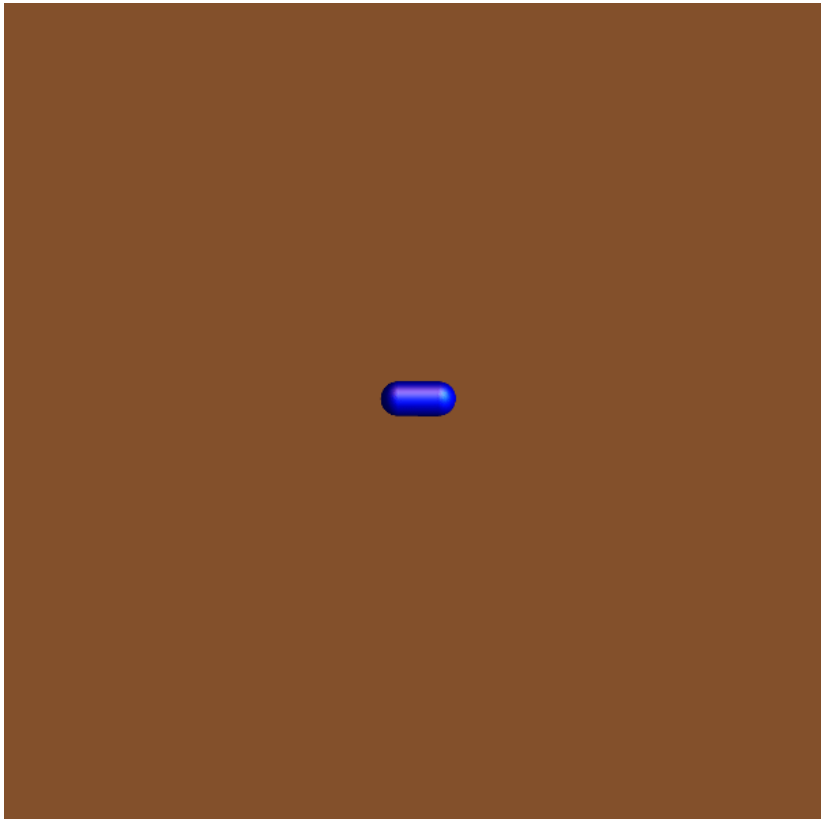
$$\alpha/\beta < 1 - \xi$$

α = growth rate
β = verticalization rate
ξ = ratio of vertical to horizontal footprint

Cell length influences colony shape

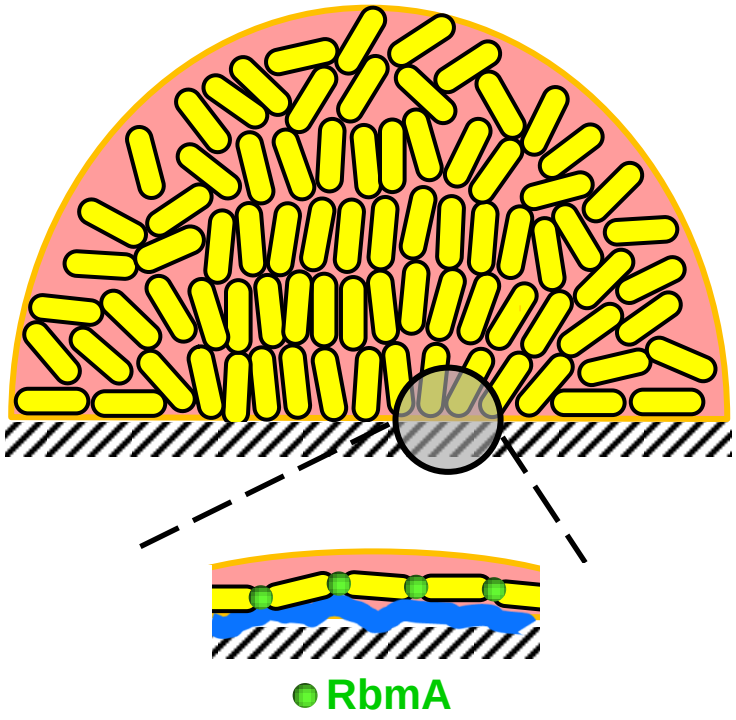


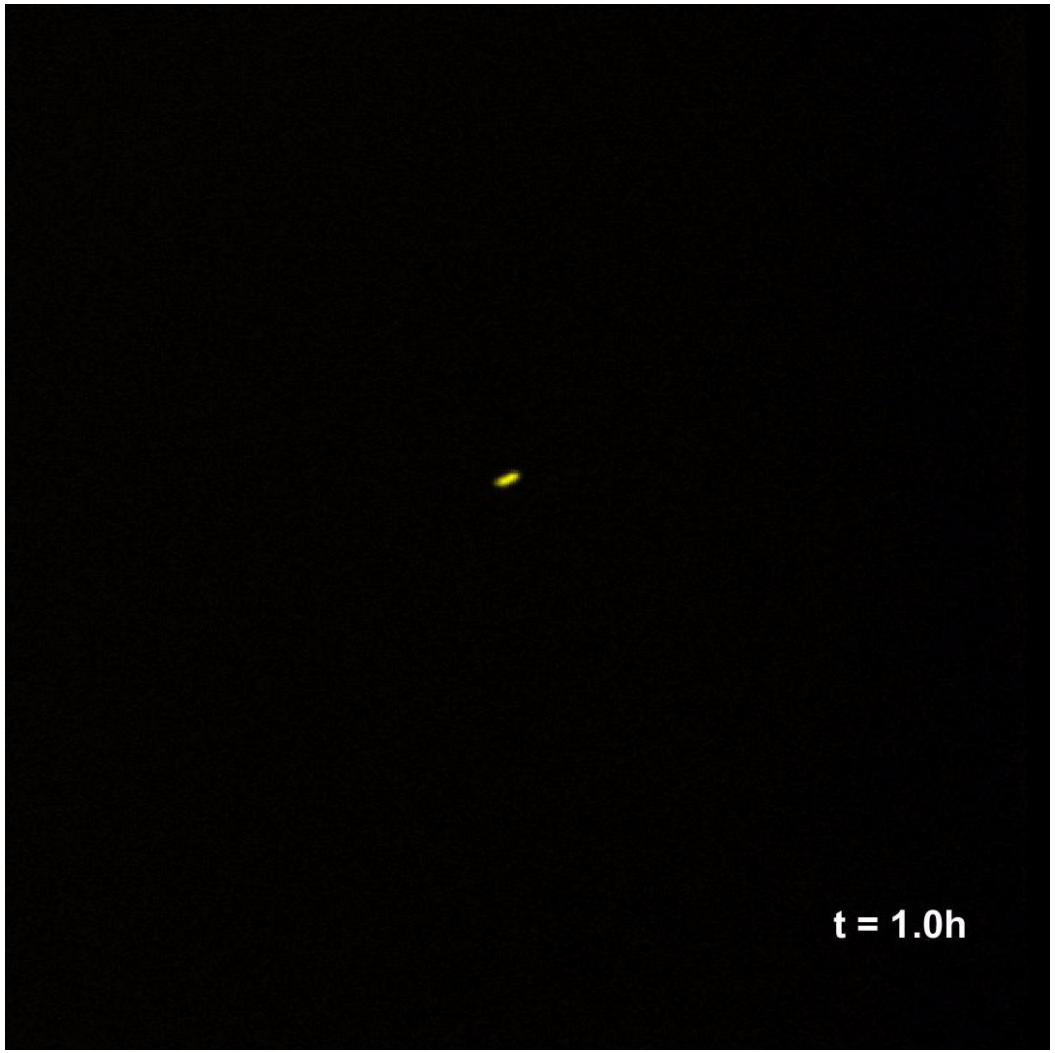
$L_{\mathrm{div}} = 3$



$L_{\mathrm{div}} = 7$

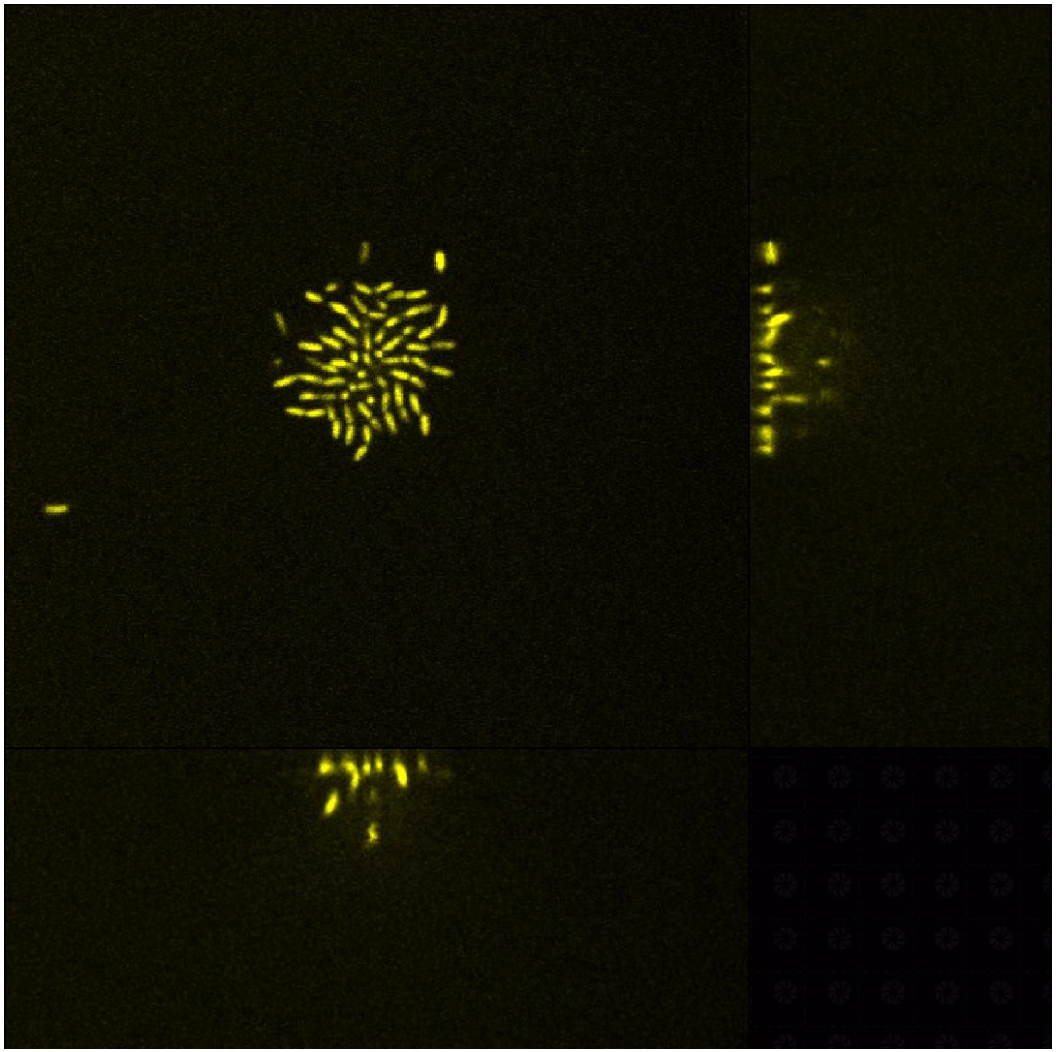
Role of cell-to-cell adhesion





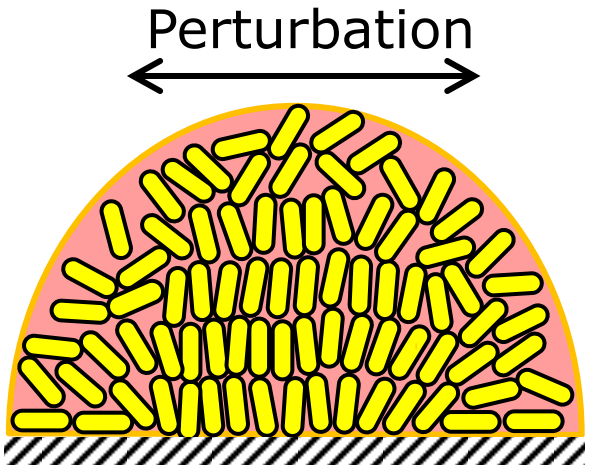
Δ*rbmA* mutant

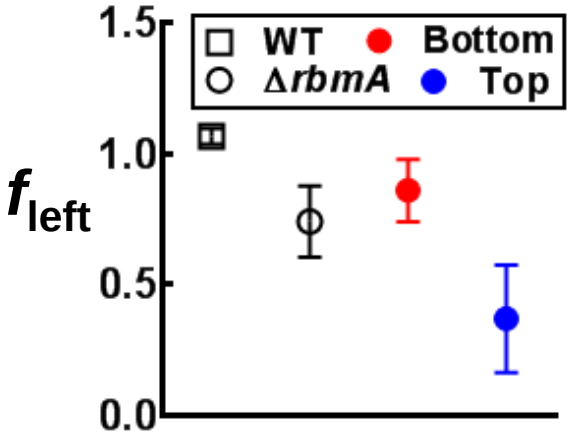
A different mode of biofilm growth



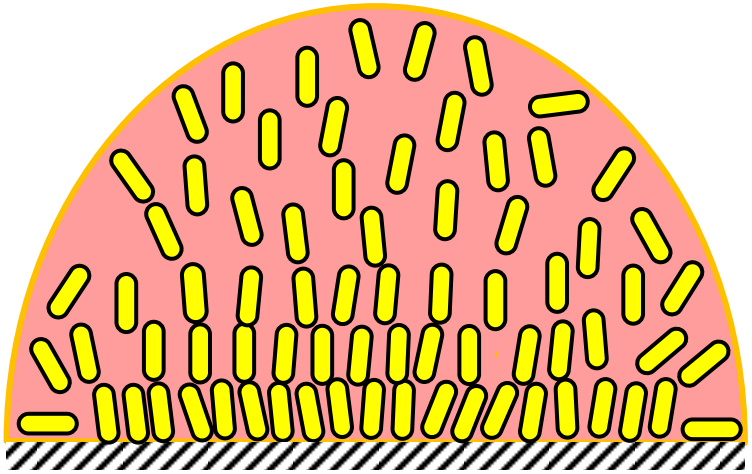
Resistance to mechanical perturbation

RbmA^{+}



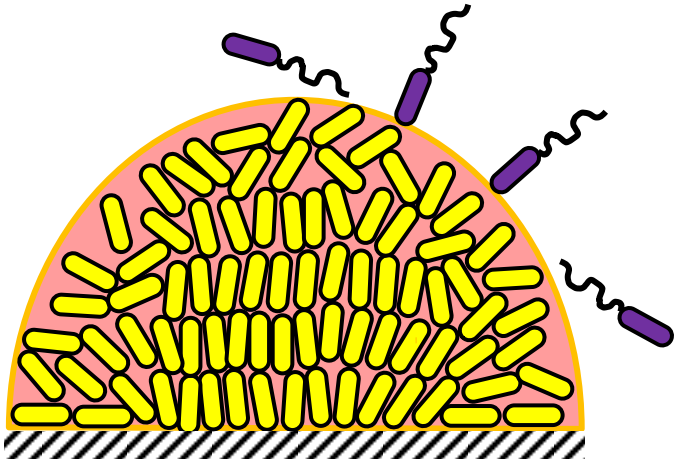


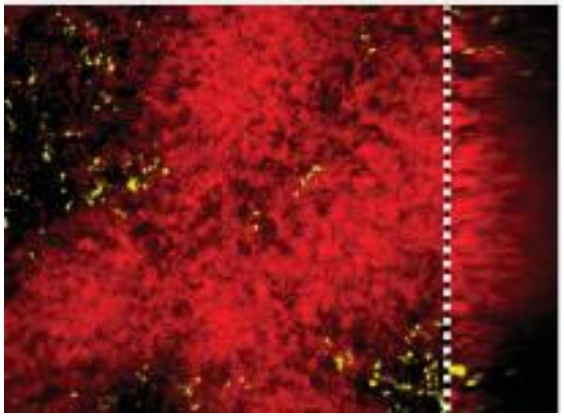
RbmA^{-}



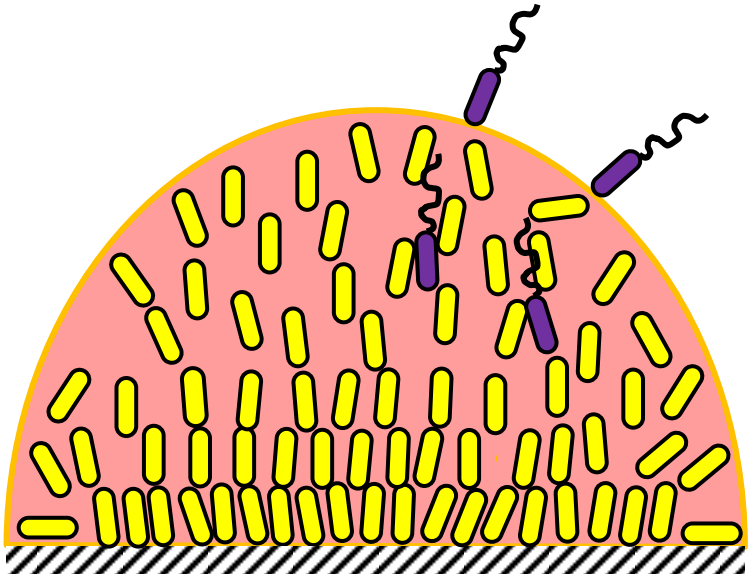
Resistance to invasion

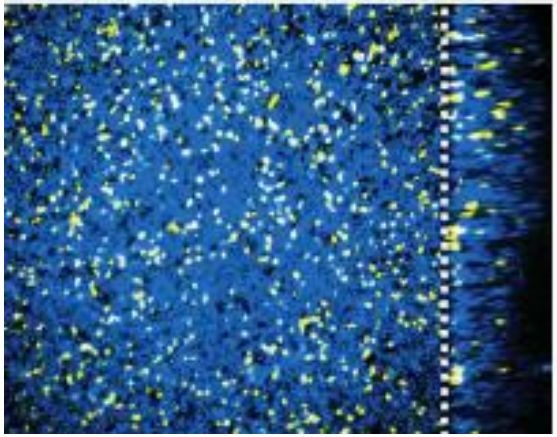
RbmA^{+}



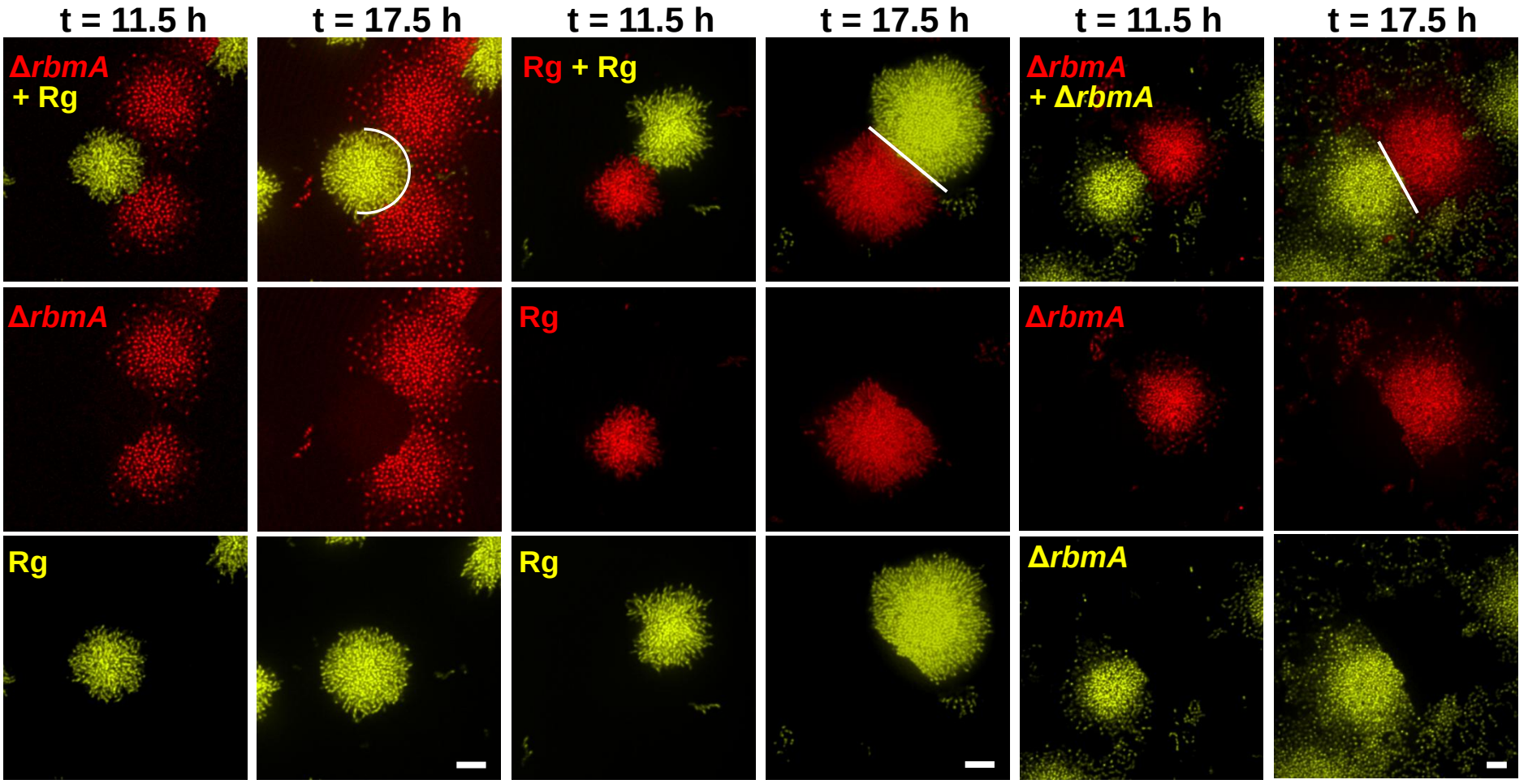


RbmA^{-}

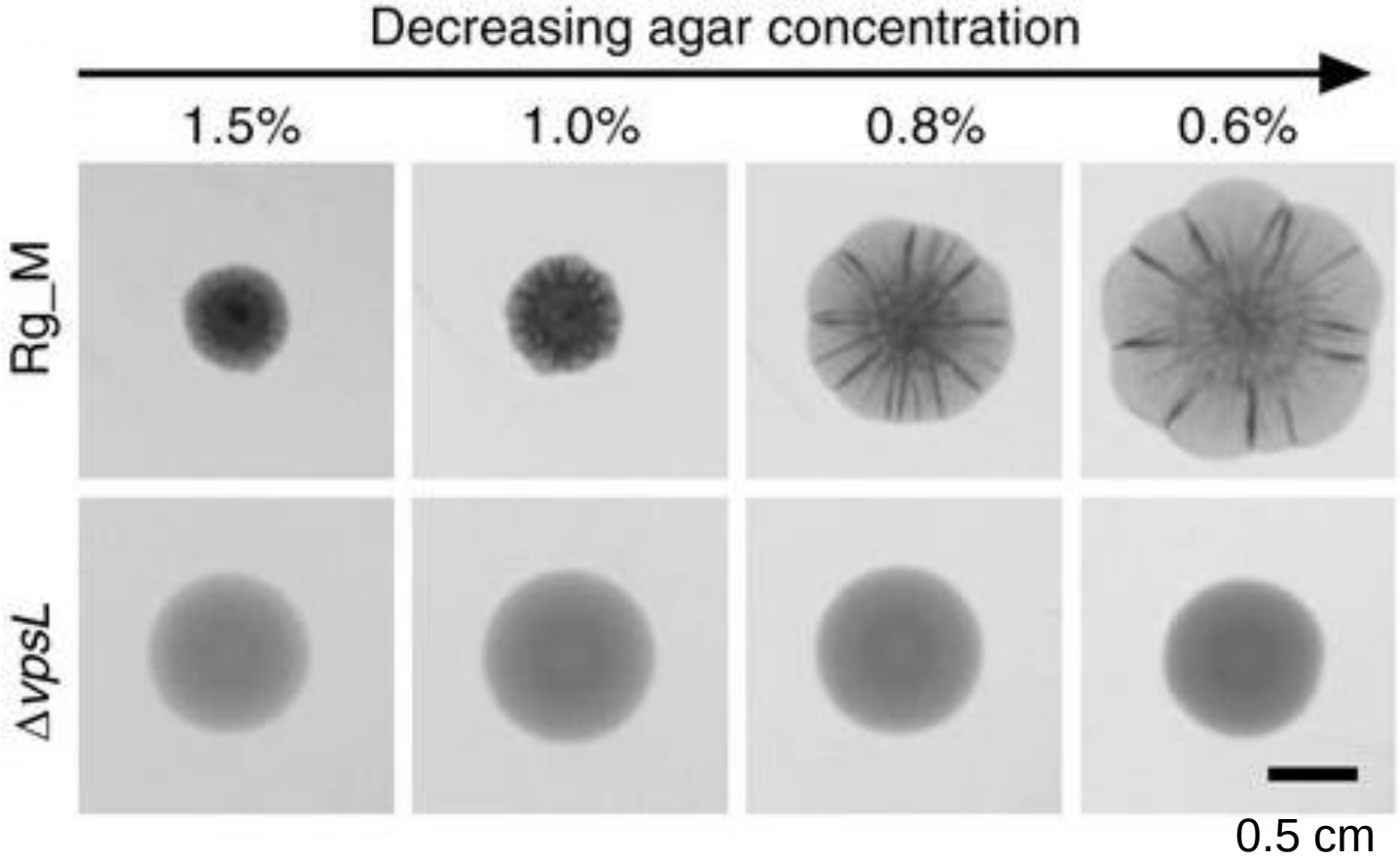




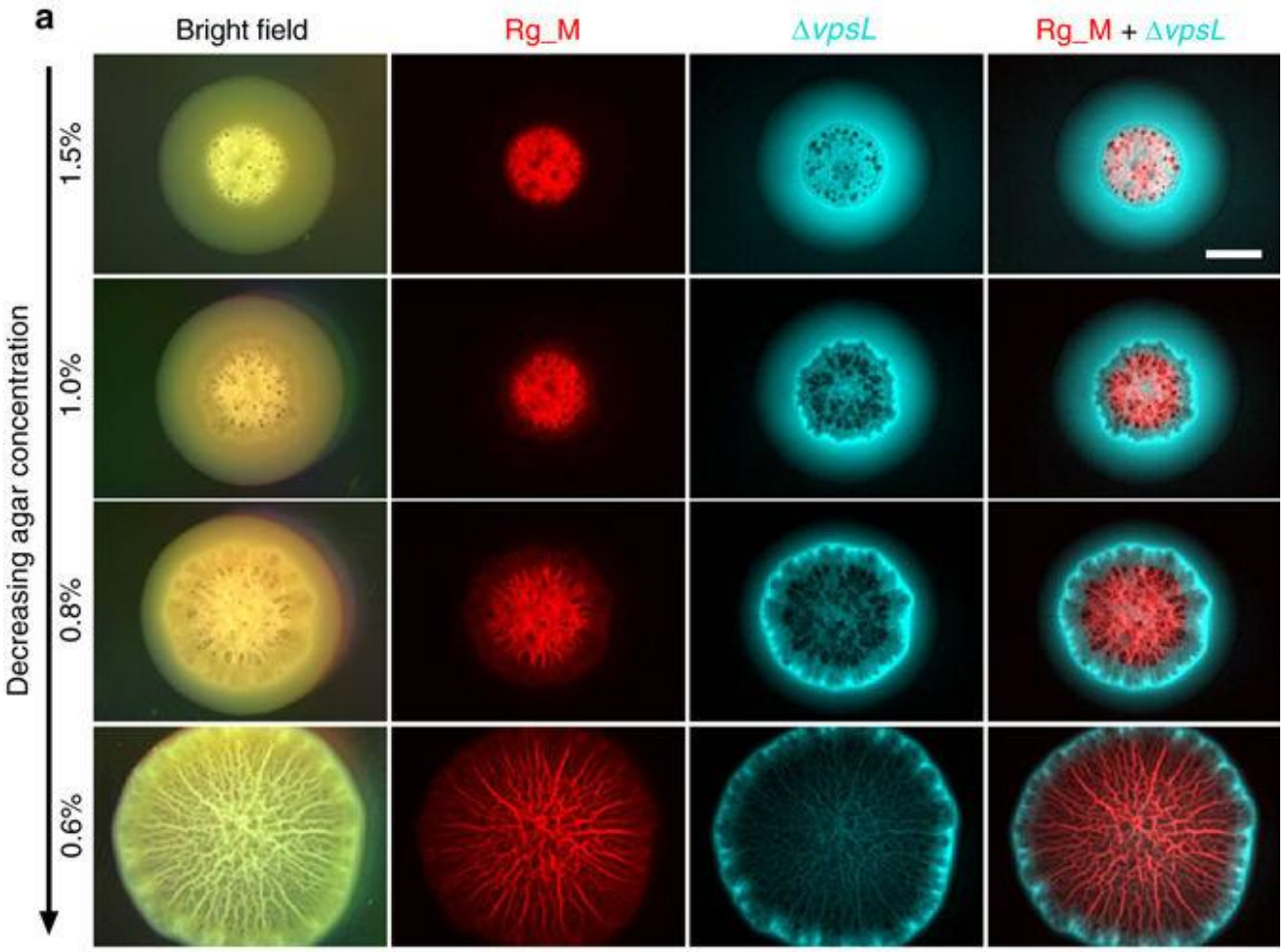
Contact between two clusters



Osmotic pressure drives biofilm expansion



Osmotic swelling enables matrix producer to exclude non-producer



Summary

- Biofilms are ubiquitous – bacteria live in communities
- Single-cell imaging reveals single founder, 2D → 3D transition, and internal order
- Agent-based and continuum modeling reveal mechanism of verticalization, influence of cell size, etc.
- *V. cholerae* biofilms control territory:
  - Resist invasion
  - “Bulldoze” competitors
  - Exploit osmotic swelling

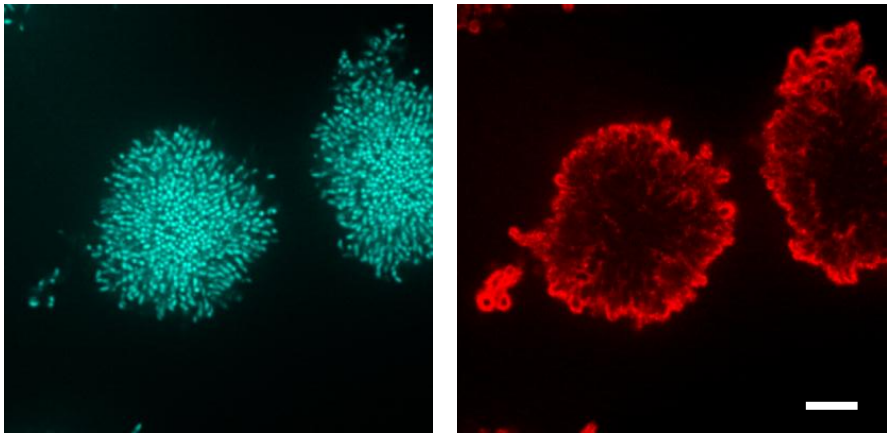
Drescher *et al.,* PNAS (2016)

Yan *et al.*, PNAS (2016)

Yan *et al.*, Nature Comm (2017)

Future directions

- Role of cell curvature?
- Role of the matrix “envelope”?



- Quorum sensing & gene expression
- How do different species build biofilms?
- Implications for disease

Acknowledgements

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Yigal Meir (Ben-Gurion)

Eric and Wendy Schmidt
Transformative Technology Fund

Colocalization of matrix components