Emergence of spatial structure in growing biofilms and its implications for evolution

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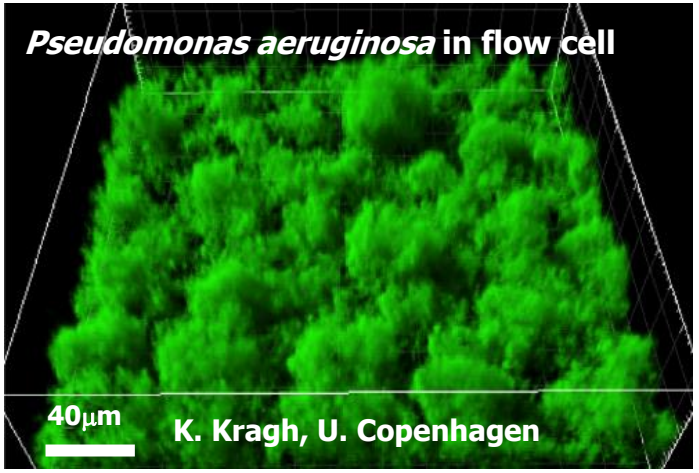
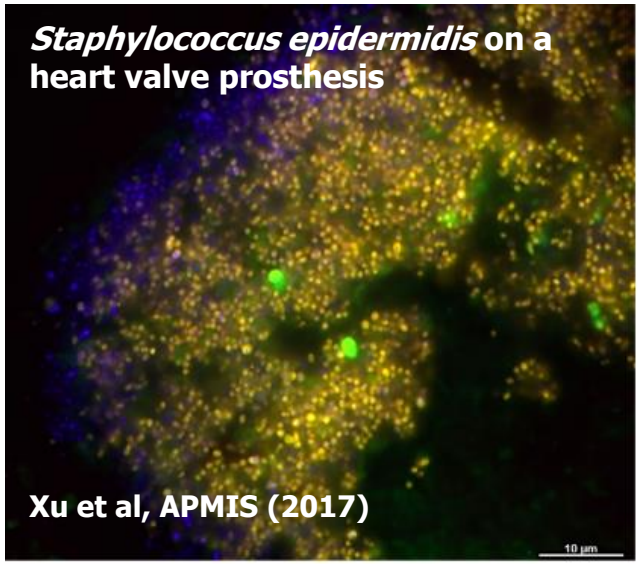
Bacterial biofilms

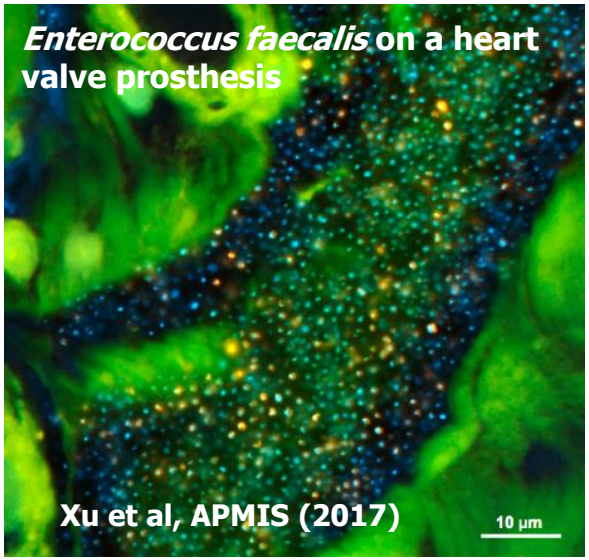
A beautiful example of multicellular self-assembly driven by non-equilibrium processes

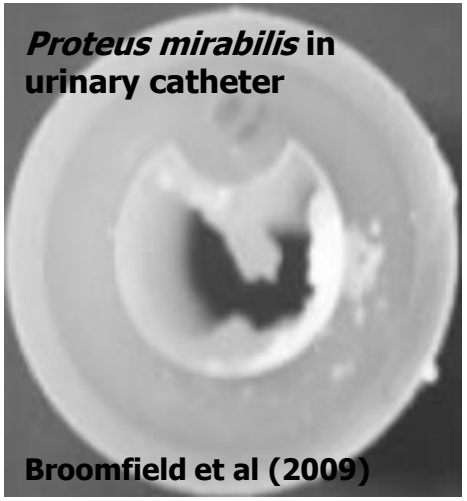
and

a major cause of clinical infections that may be a source of antibiotic resistance





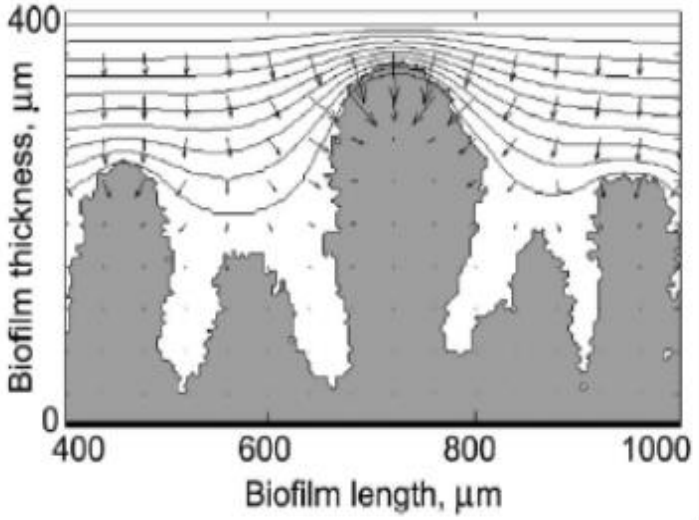




Question 1:
what controls biofilm spatial structure?

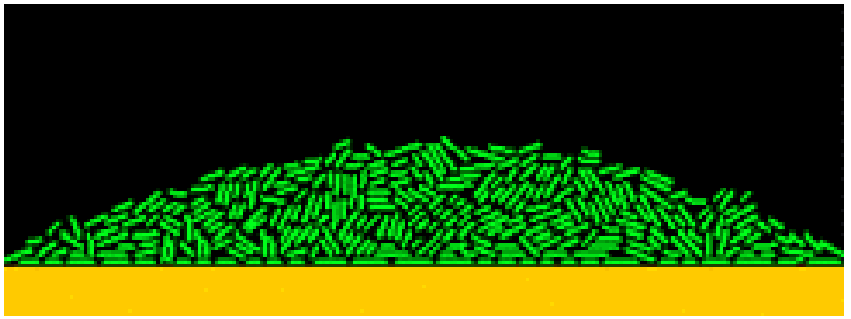
i.e. whether interface is rough or smooth

Nutrient limitation



Dockery and Clapper 2002
SIAM J. Appl. Math.

Mechanical interactions

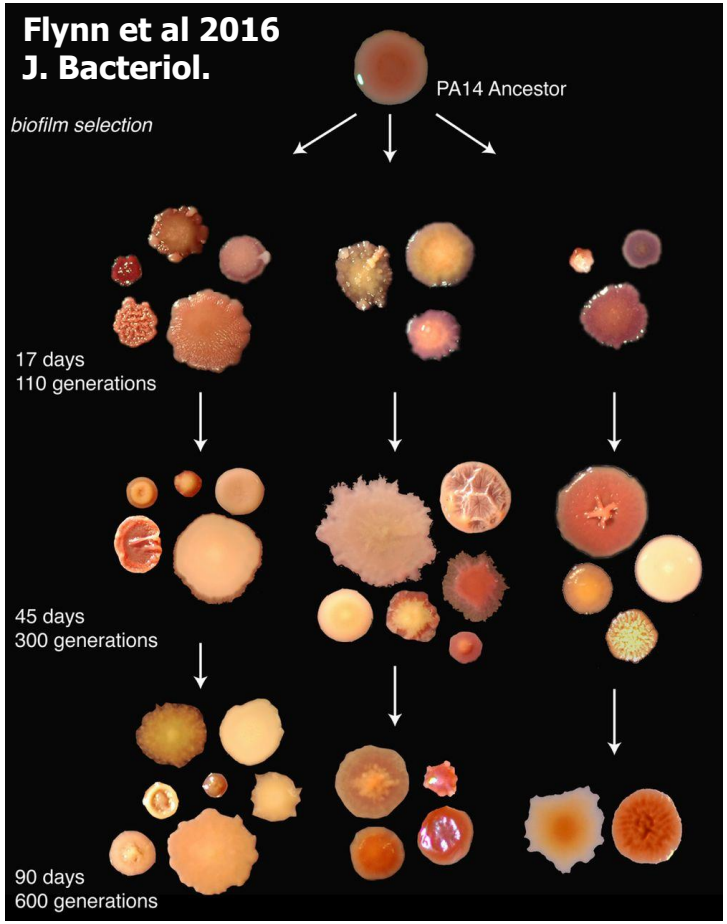


Farrell et al 2017
Phys. Rev. Lett.

Question 2:
what controls biofilm genetic diversity?

how does biofilm structure influence evolution?

are biofilms a source of antibiotic resistance?



"Individual-based computer simulations"

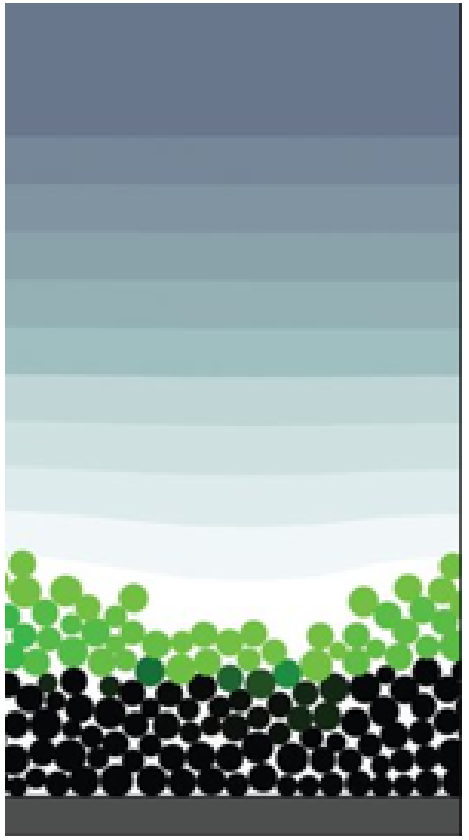
Bacteria are represented as disk-shaped particles (2D)

Nutrient is represented as a concentration field

Bacteria consume nutrient, grow and divide

Bacteria push each other out of the way

Nutrient diffuses from above and is consumed by bacteria



L.A. Lardon *et al.*, *Environ. Microbiol.*, **13** (2011) 2416

Bacterial dynamics

Growth

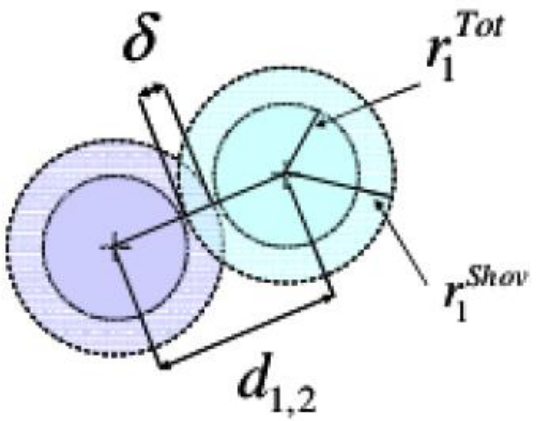
$$\frac{dX}{dt} = \mu_{max} \frac{S}{k_S + S} X$$

- Cells expand as they consume food
- Growth rate is function of local nutrient concentration
- Divide in 2 once they reach a threshold radius (with some stochasticity)

Mechanical interactions

- At each timestep, detect cell-cell overlaps
- Move cells apart to resolve overlaps

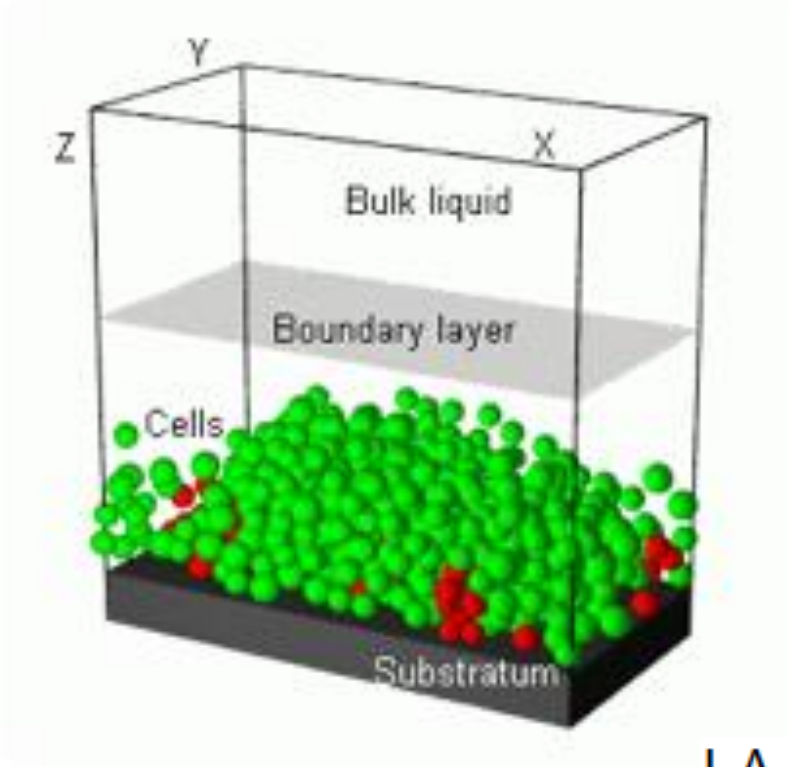
  (random order)



L.A. Lardon *et al.*, *Environ. Microbiol.*, **13** (2011) 2416

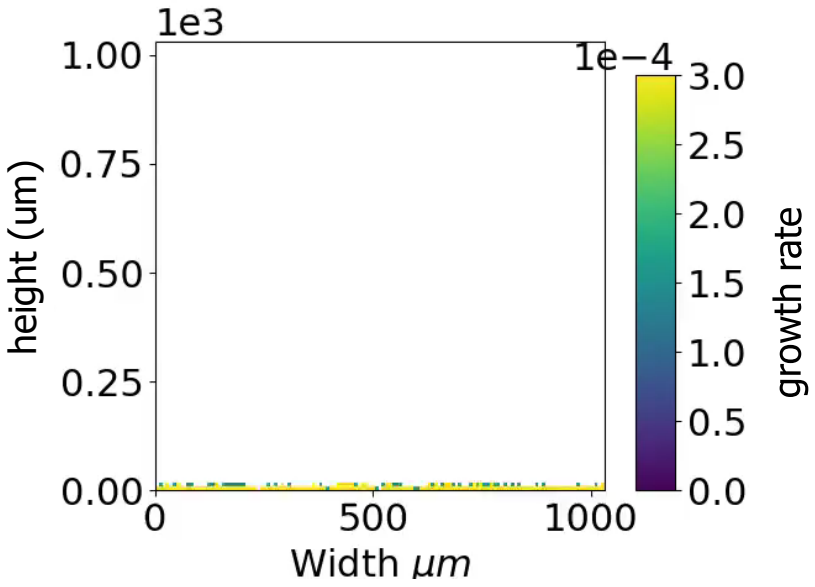
Nutrient dynamics

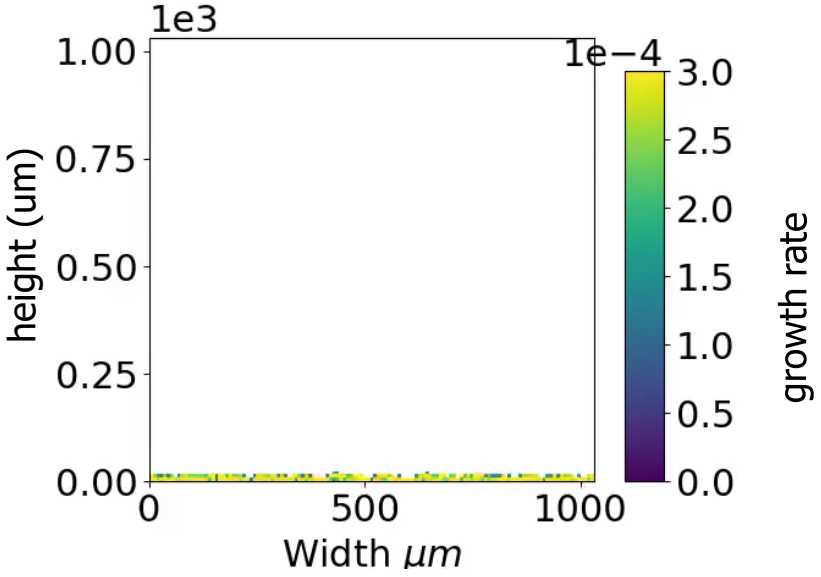
- Nutrient diffuses from above and is consumed by bacteria
- Assume nutrient diffuses fast compared to bacterial growth
- Solve steady state of reaction-diffusion equations (multigrid method)



L.A. Lardon *et al.*, *Environ. Microbiol.*, **13** (2011) 2416

Typical simulation output



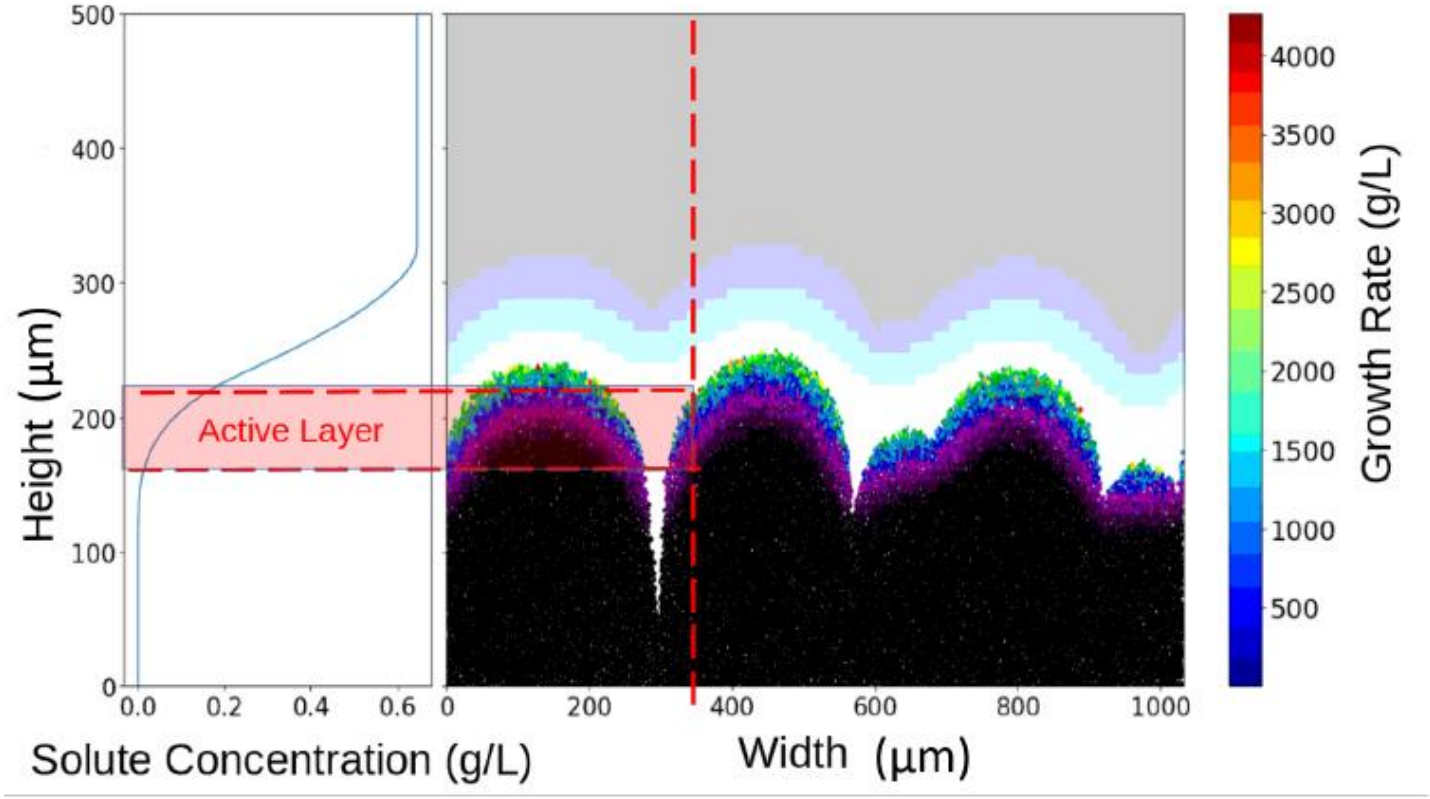


"steady state" of growth reached after initial period

only a few bacteria are growing

biofilm morphology depends on parameters

Active layer: a key concept in biofilm growth



Does the thickness of the active layer control biofilm structure?

thick active layer: smooth biofilm

thin active layer: rough biofilm

Nadell 2010 PLOS Comp. Bio.

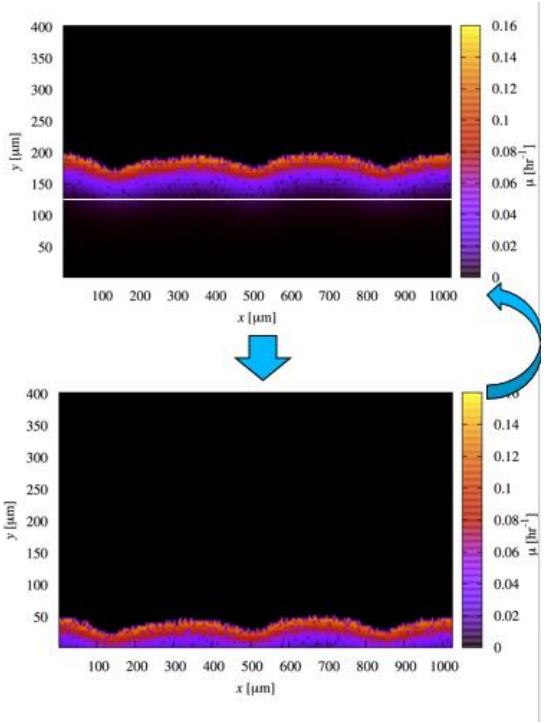
Computational challenge

need long-time simulations to find steady-state behaviour

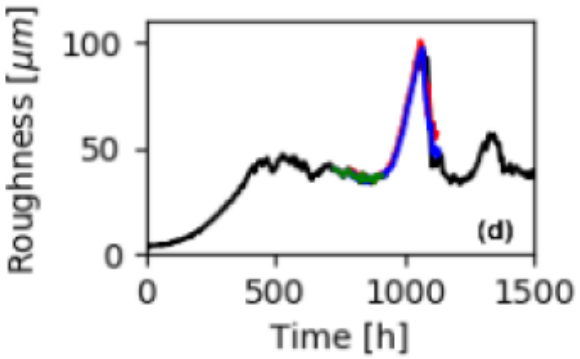
but number of bacteria quickly becomes unmanageable

solution: periodic "clipping"

- define lowest point in active layer and interface
- clip the configuration, keep only bacteria above this height
- re-initiate the simulation



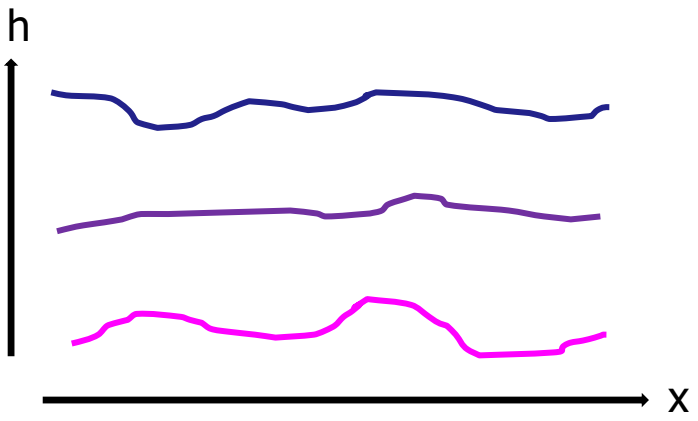
makes long-time biofilm simulations computationally feasible

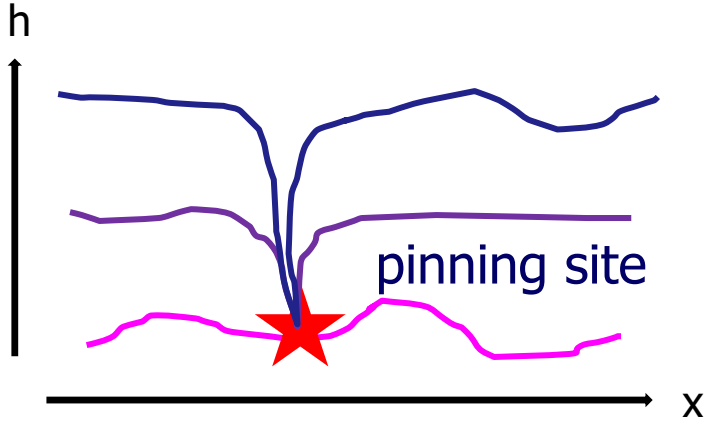


Question 1:

what controls biofilm spatial structure?

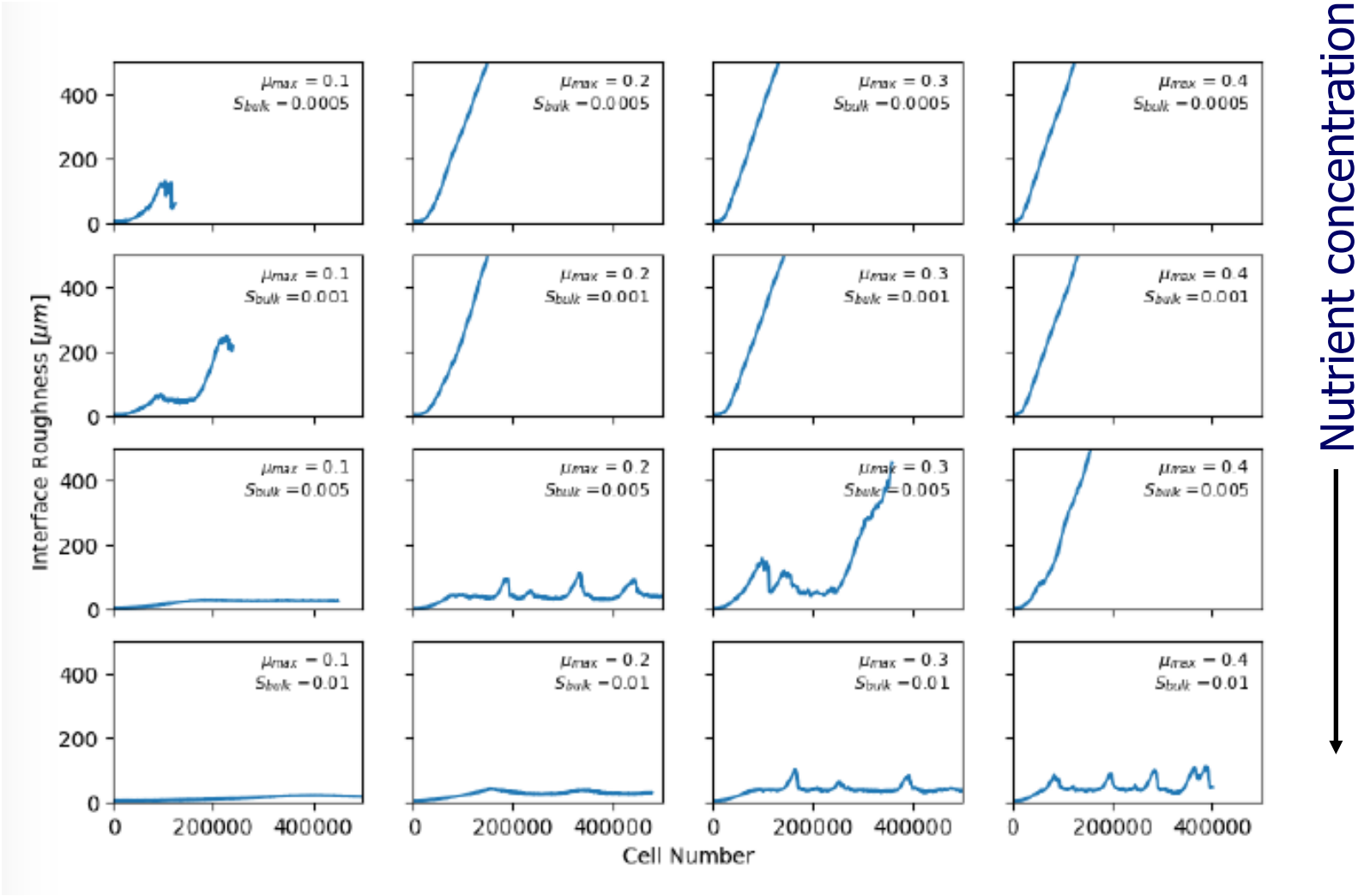
key concept: interface pinning





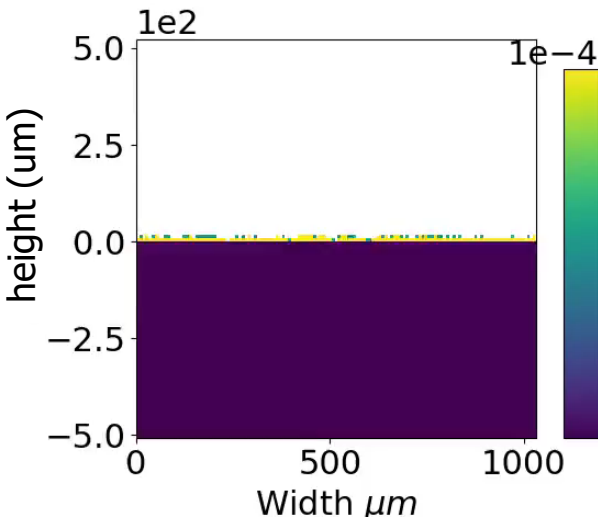
Run long time simulations varying nutrient conc and max growth rate

Max growth rate →



Key result: we see three distinct "phases" of spatial structure

Unpinned Phase

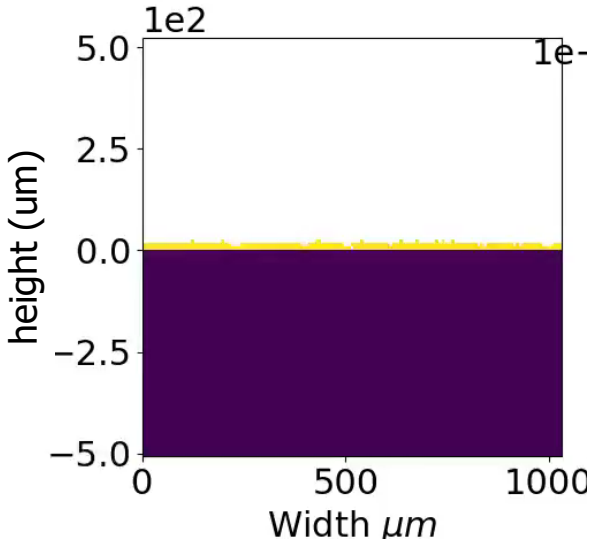


no gaps in active layer

interface does not pin

roughness is low

Transiently pinned Phase

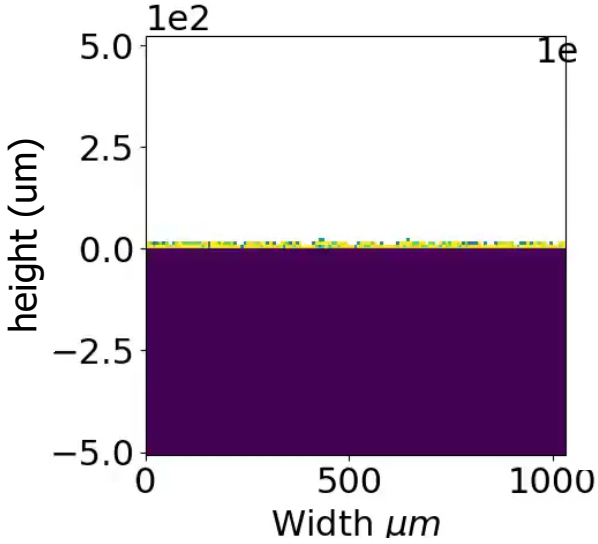


transient gaps

transient pinning

roughness fluctuates

Pinned Phase

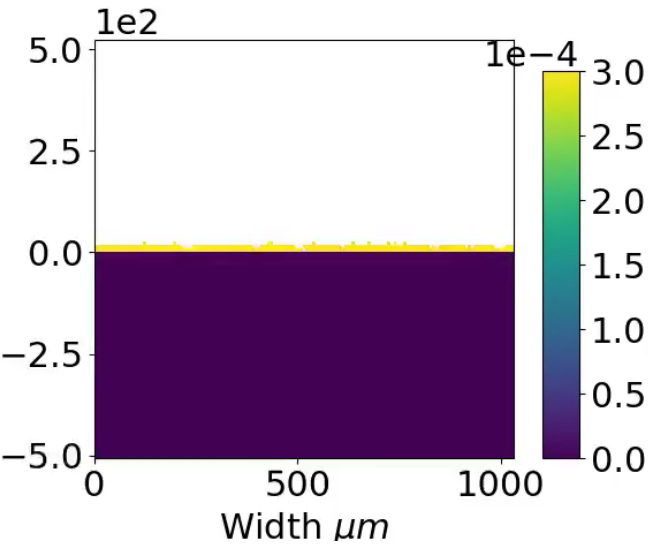


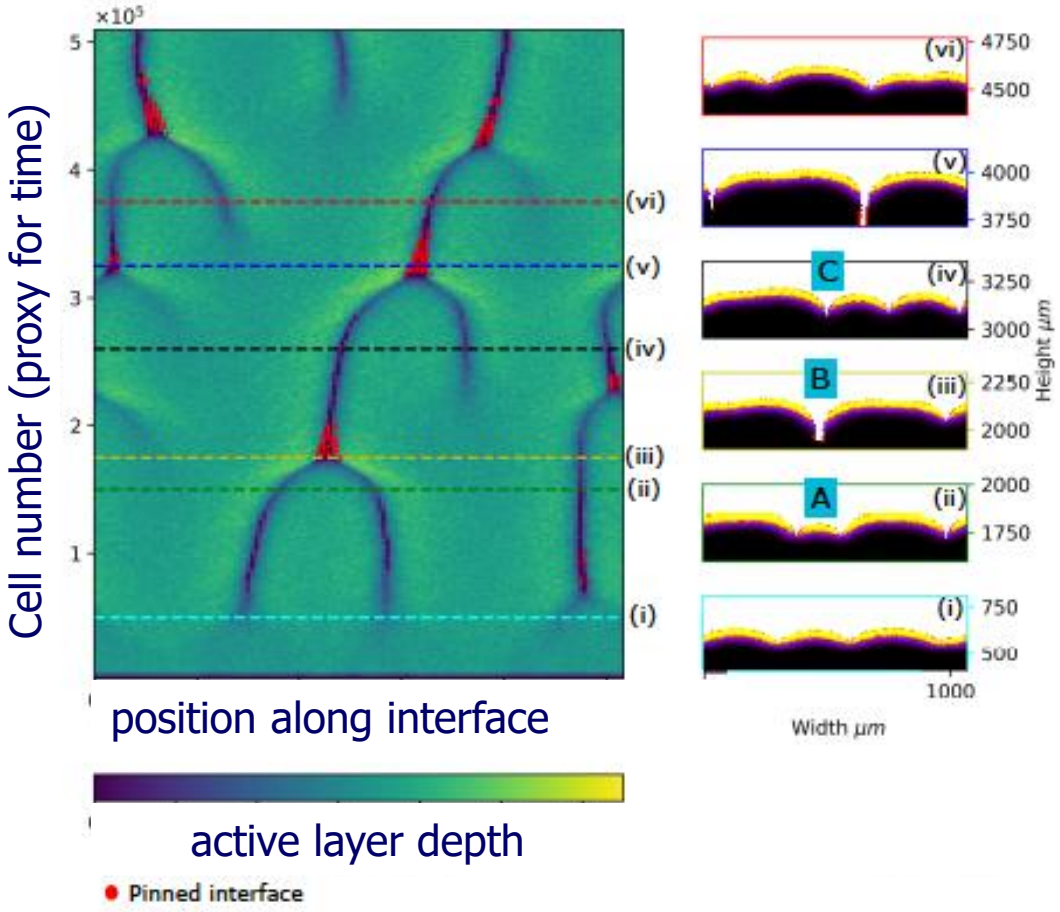
permanent gaps

sustained pinning

roughness increases

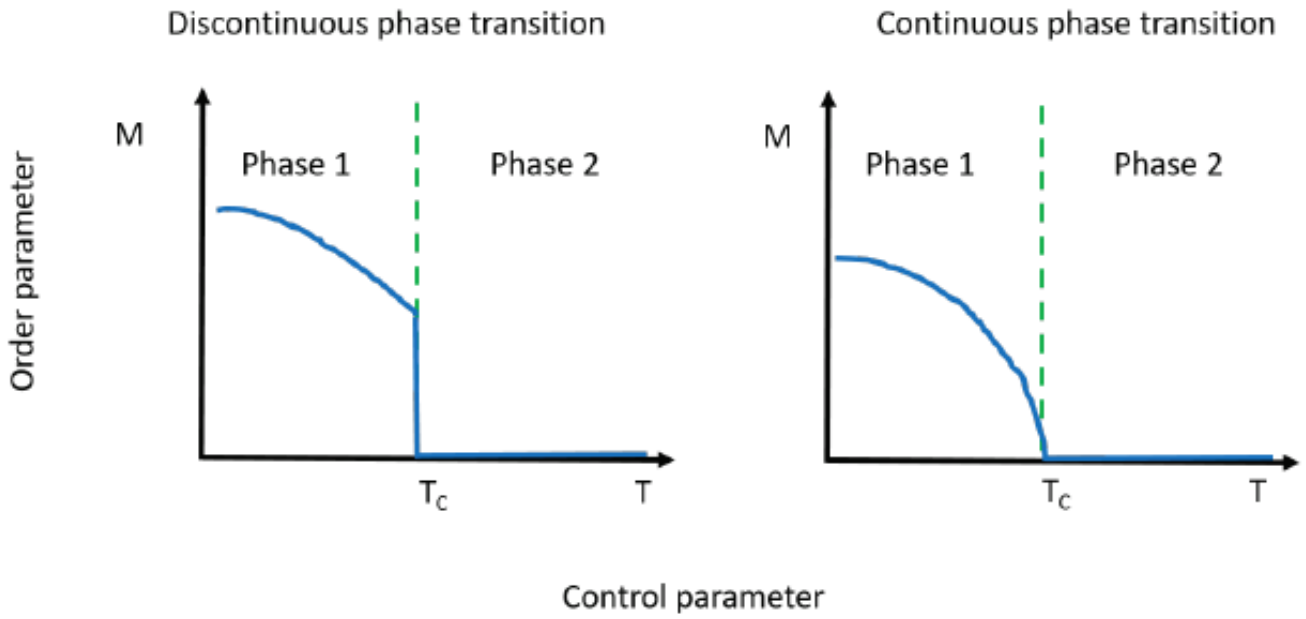
Interface pinning arises from active layer gap dynamics





interface pins when a small bulge is engulfed

Can we make a phase diagram for interface pinning?

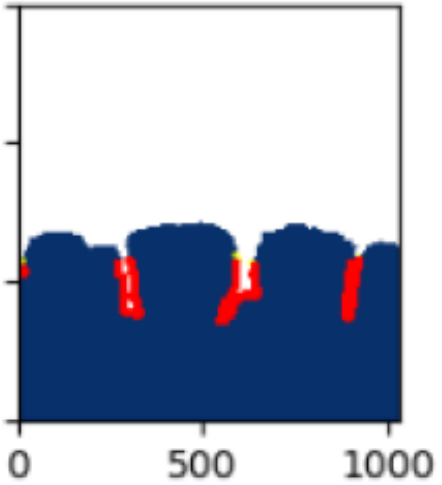


what's the control parameter?

what's the order parameter?

Order parameter: quantify difference between the phases

$$\text{Pinned interface fraction} = \frac{\text{length of non-moving interface}}{\text{total interface length}}$$



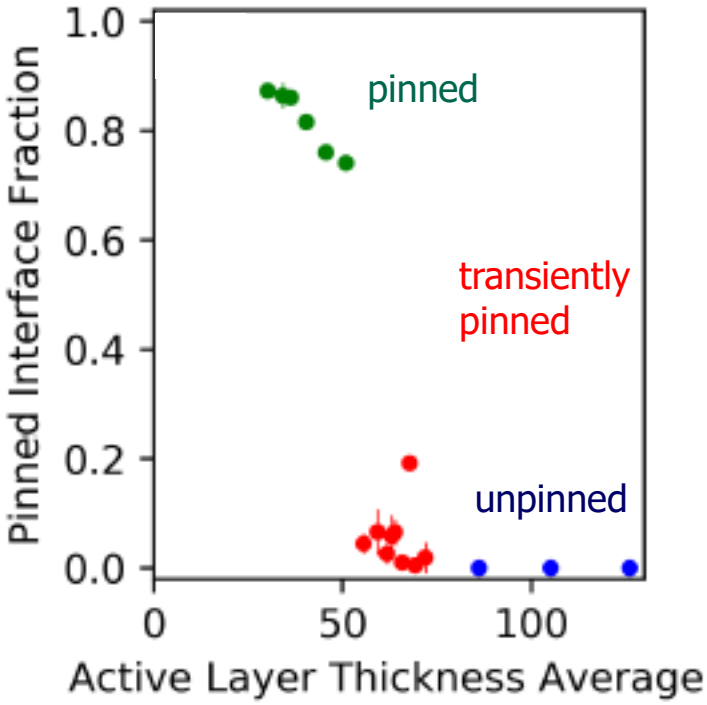
Control parameter: tune the transition

multiple simulation parameters are involved

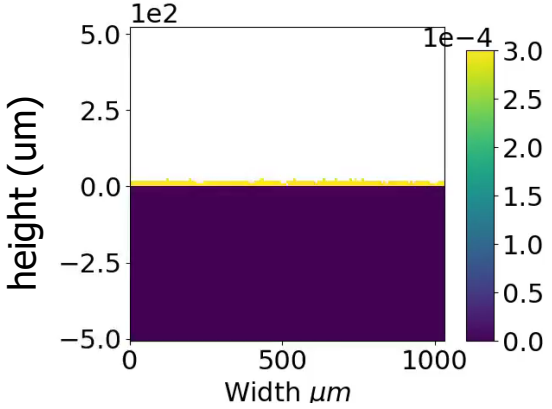
-> do they act via the active layer?

-> active layer thickness as a possible control parameter?

Candidate phase diagram: active layer thickness – pinned interface fraction

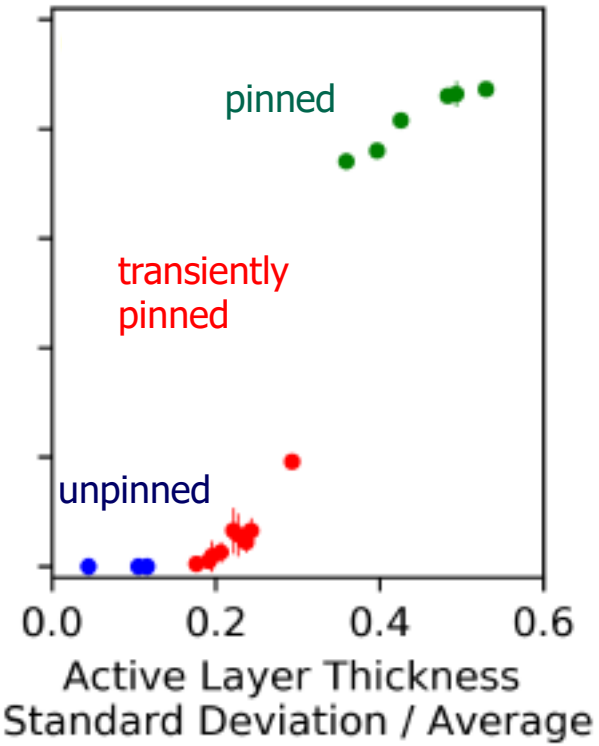


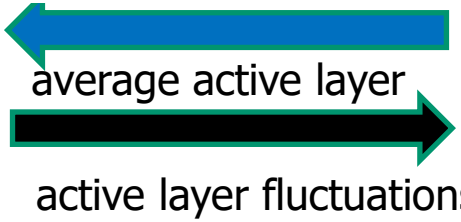
the data doesn't collapse very well



But the active layer is also dynamic. Maybe fluctuations are important?

Candidate phase diagram: active layer thickness stdev/mean



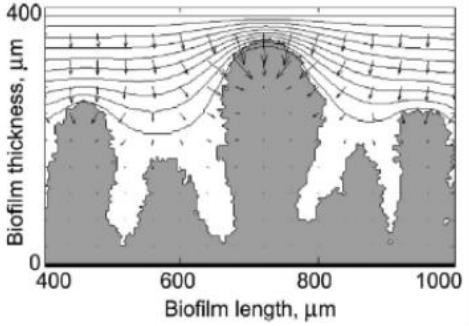


active layer thickness stdev/mean is a better control parameter
suggests active layer fluctuations are important

-> a spontaneous interface pinning transition in a non-equilibrium system

apparently driven by dynamical fluctuations in the active layer

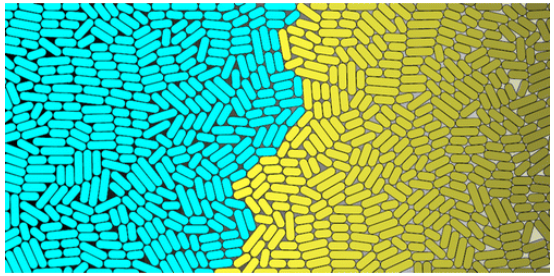
nutrient limitation



creation of active layer gaps?

closure of active layer gaps?

mechanical interactions



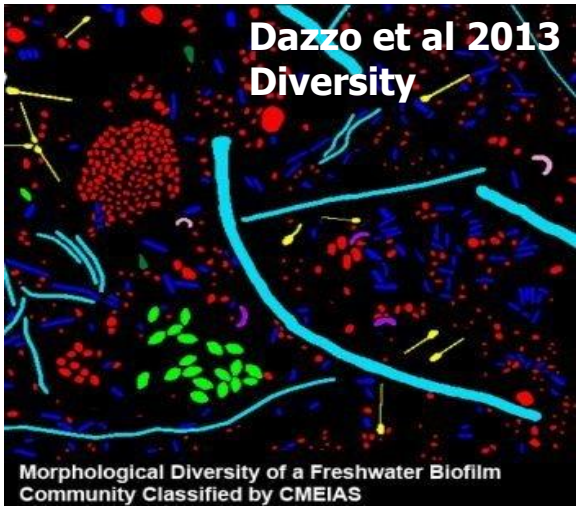
Question 2:

what controls biofilm genetic diversity?

Standing diversity = genetic variation of founder cells

Biofilms seeded from diverse populations
e.g. marine or soil environments

standing diversity decreases in time

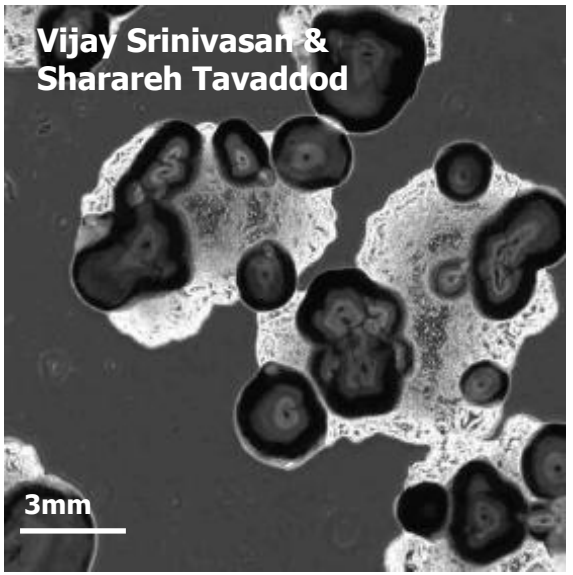


De novo diversity = genetic variation due to mutations

might encode antibiotic resistance

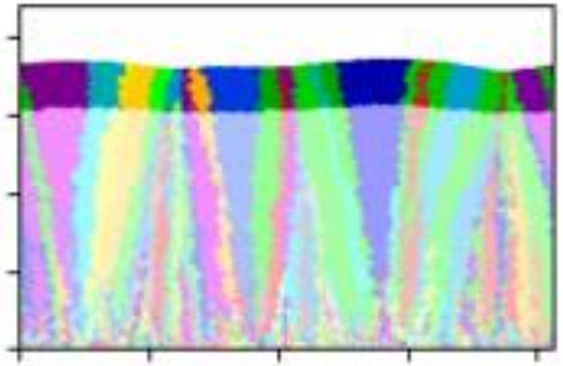
de novo diversity increases in time

how does spatial structure affect the balance between standing and *de novo* diversity?



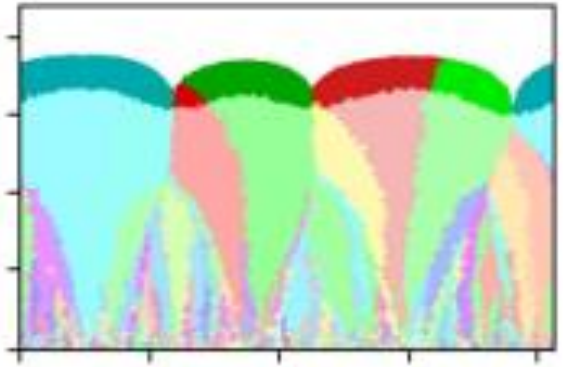
Standing diversity: colour the lineage of each founder cell

unpinned phase

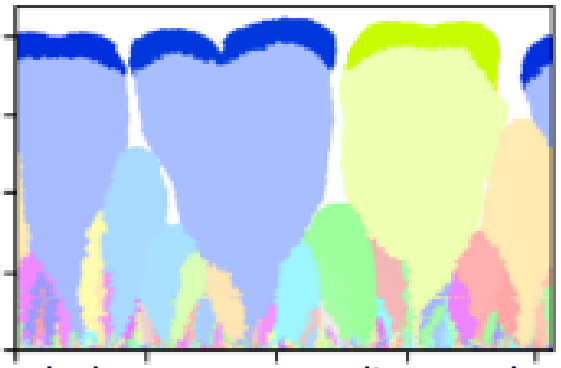


75,000 cells

transiently pinned phase

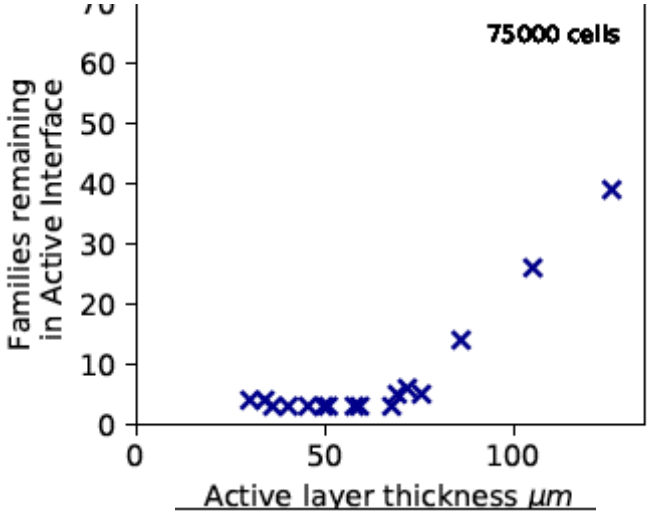


pinned phase

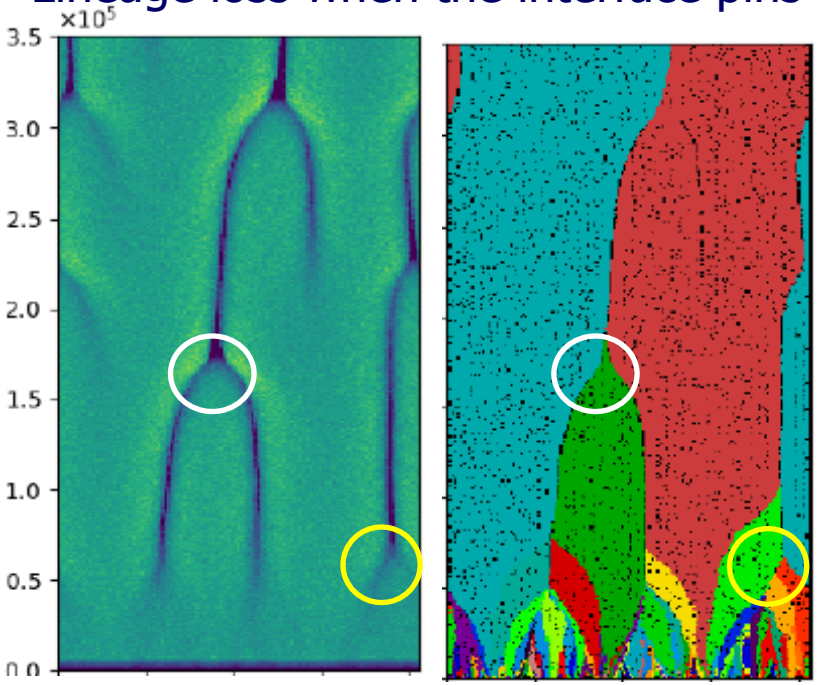


pinned phase -> more lineage loss

Thin active layer:
more stochastic fluctuations



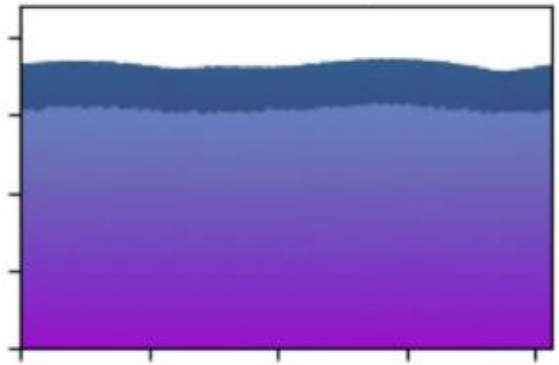
Lineage loss when the interface pins



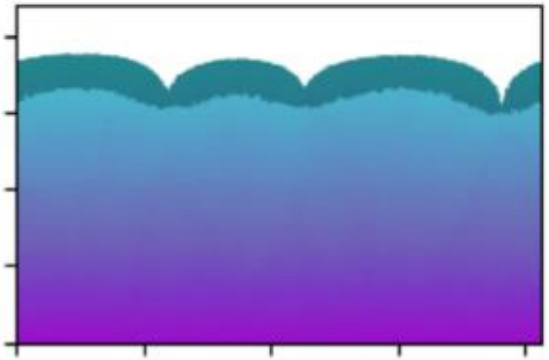
De novo diversity:

use lineage length as a proxy - long lineages accumulate more mutations

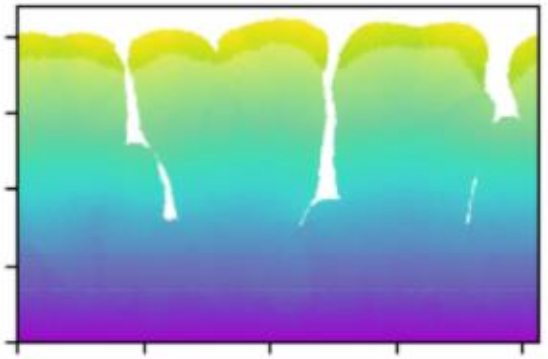
unpinned phase



transiently pinned phase



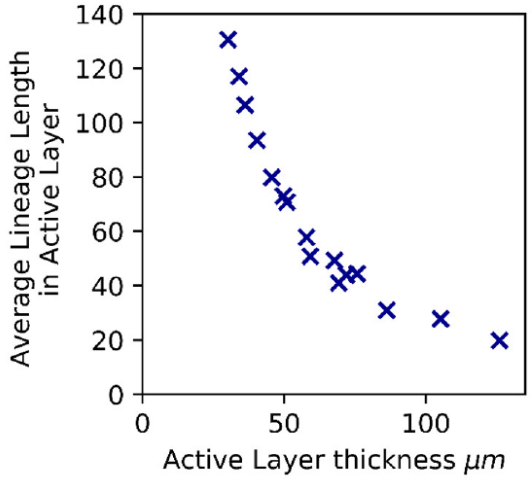
pinned phase

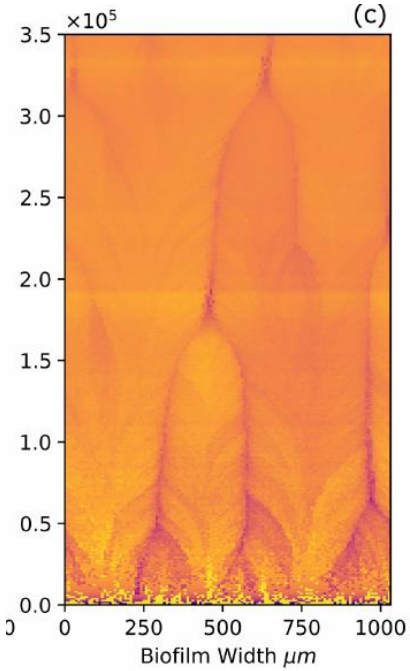


75,000 cells

pinned phase -> longer lineages

Thin active layer:
longer lineages
division events concentrated in fewer cells





also some
local effects

Conclusions

Biofilms undergo a spontaneous pinning transition

Active layer dynamics are important in this

Patterns of genetic diversity are also controlled by the active layer

Ongoing questions

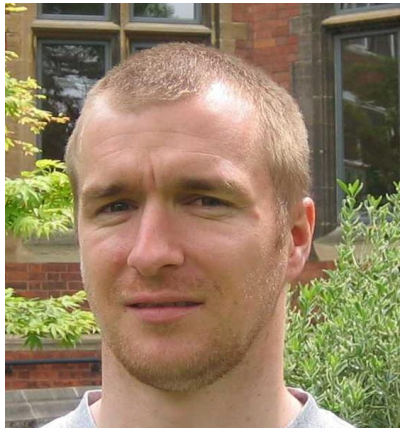
Statistical physics of populations with “active layer”

More realistic biofilm models

flow, polymer matrix, mutant fitness advantage…

Ellen Young

Gavin Melaugh



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BALANCE OF THE MICROVERSE

DFG Deutsche Forschungsgemeinschaft