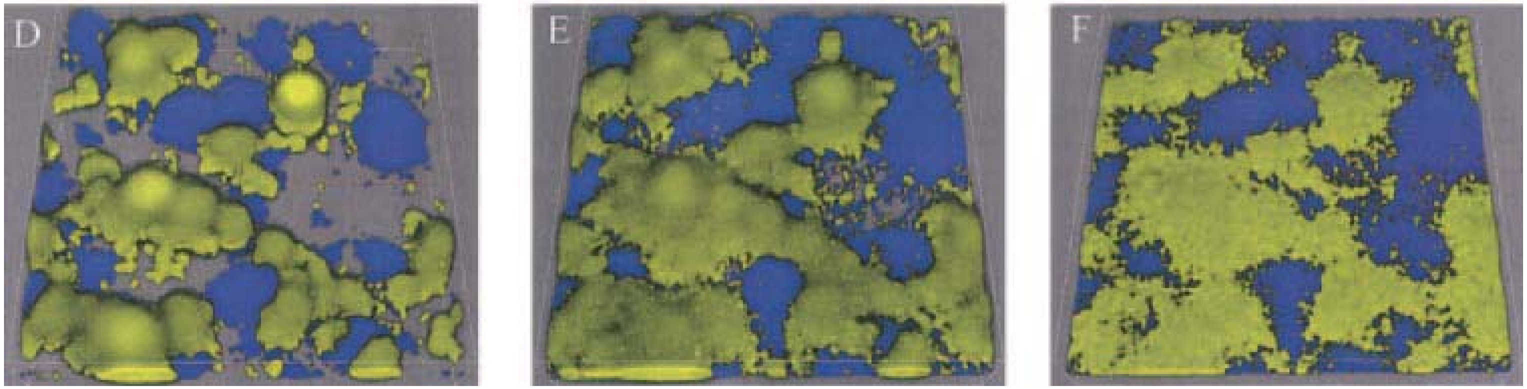
Structure in biofilms: How does it develop, and what roles does it play?

Vernita D. Gordon

Department of Physics, Center for Nonlinear Dynamics, Institute for Cellular and Molecular Biology, University of Texas, Austin

Talk given at Beijing University, May 24, 2012

Why study biofilms?

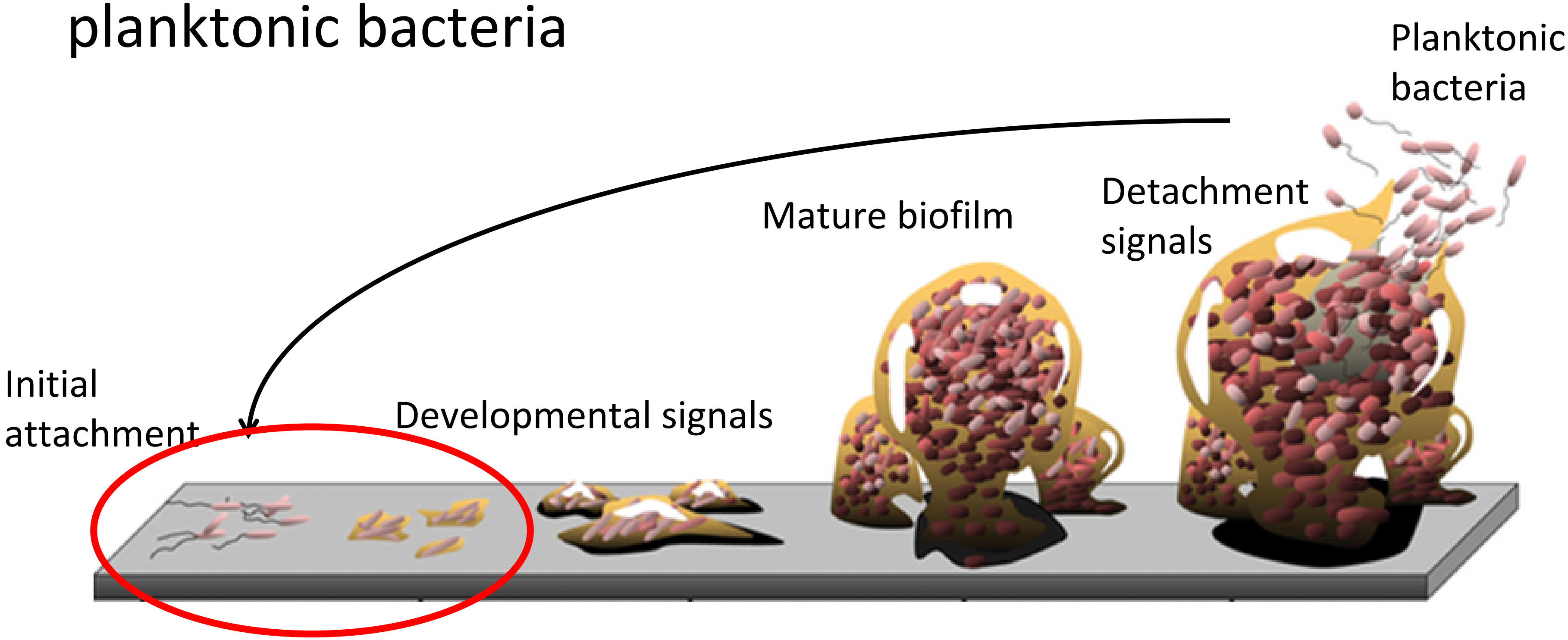


Klausen, M. *et al.*, *Molecular Microbiology* **48**, 1511–1524 (2003).

- Biofilms are multicellular communities of single-celled organisms that form at surfaces
- Very common! Most wild bacteria are found in biofilms
- Important in both medical and industrial settings
  - Increased antibiotic resistance and virulence
  - Biofouling of medical devices, pipes, ship hulls
- Model system for multicellularity
  - Simple, easy to tweak

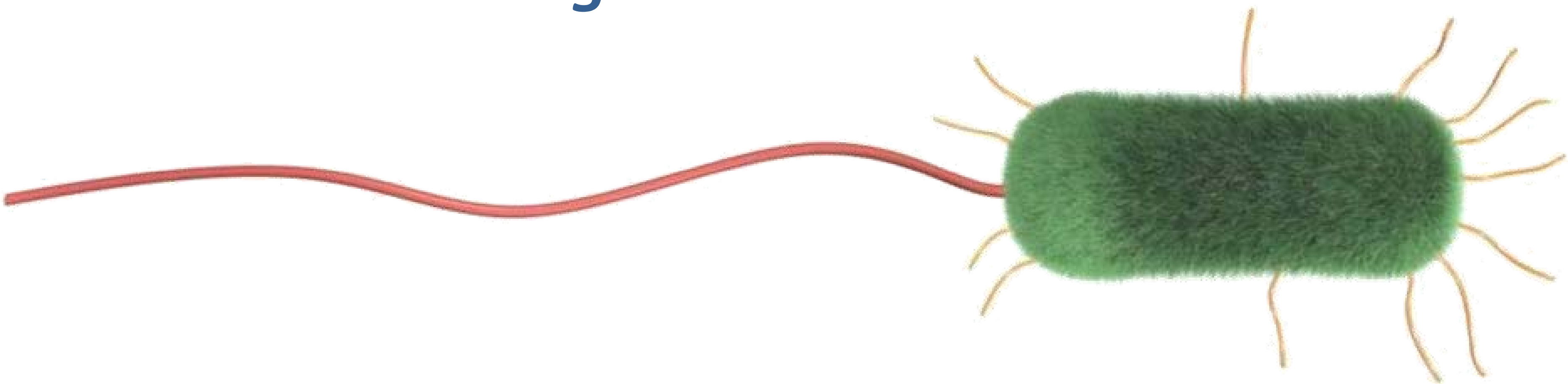
Biofilms development involves several stages

- Early stages include attachment to a surface and production of extra-cellular polysaccharides (EPS)
  - Pel and Psl are two main EPS elements for *P. aeruginosa*
- Complex mature biofilms structured by EPS
- Distinct phenotypes (gene expression) from planktonic bacteria



Monroe D (2007) Looking for Chinks in the Armor of Bacterial Biofilms. PLoS Biol 5(11): e307

Pseudomonas aeruginosa

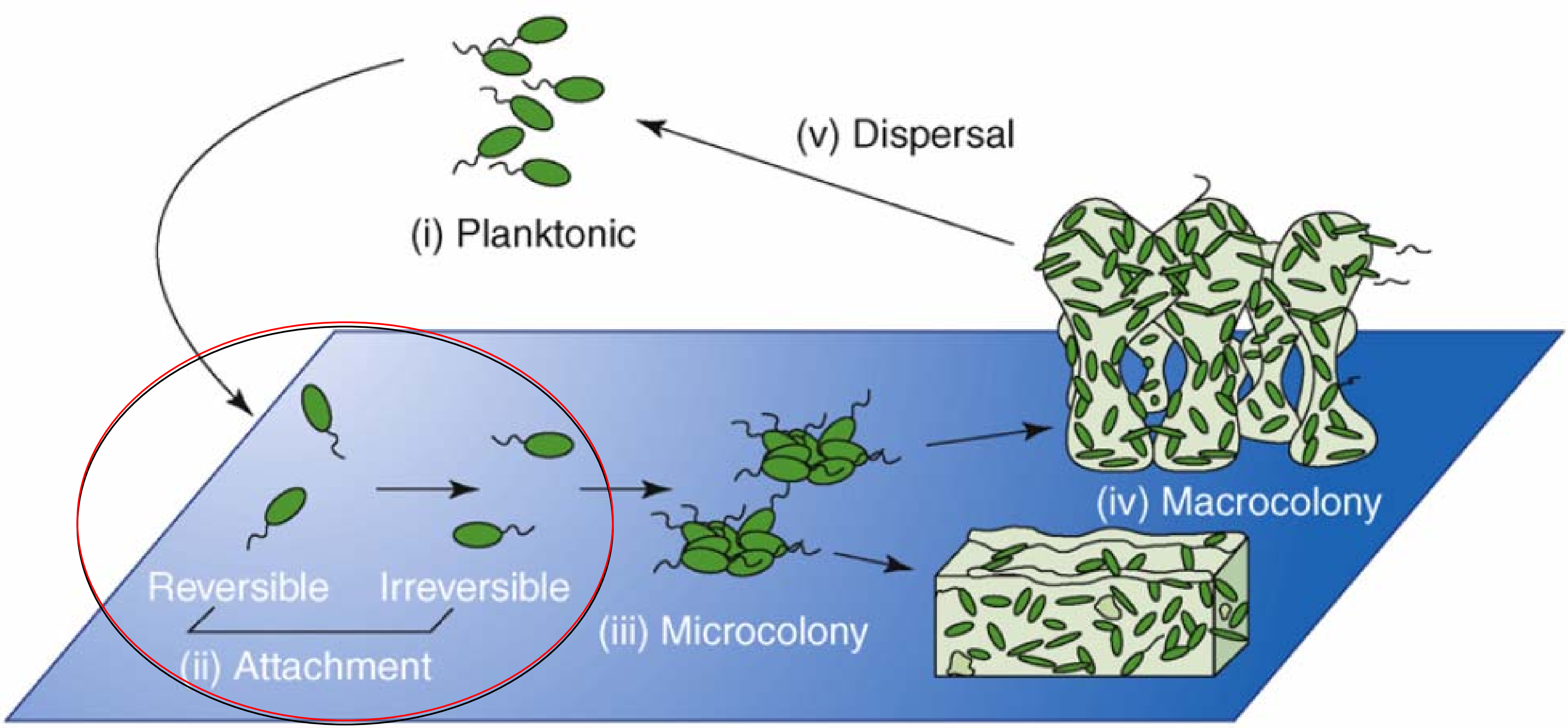


- Ubiquitous bacteria: found in/on water, soil, skin, etc.
- Opportunistic human pathogen, common in hospitals
- Causes serious lung infection is cystic fibrosis patients
  - Most common genetic disease in U.S.
  - Life expectancy ~30 years
- Gram negative, rod shaped bacteria (~1 µm x ~2 µm)
- Single polar flagellum, type IV pili
- Readily forms biofilms

Question 1

WHAT ARE THE TYPES OF SURFACE MOTILITY LEADING TO BIOFILMS?

Canonical Picture of Biofilm Formation



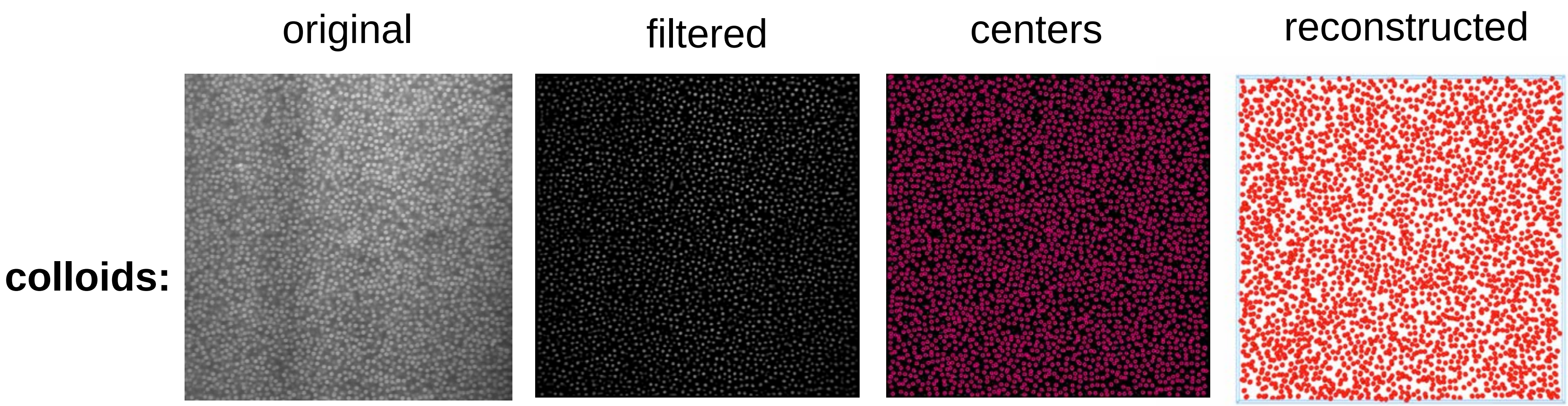
These cells are motile.

Figure from Monds and O'Toole, Trends in Microbiology 2009

High throughput tracking and biometric analysis of bacterial surface motility

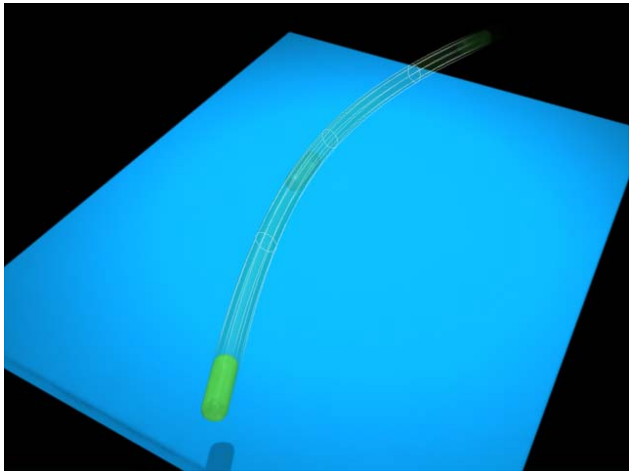
- Codes developed for colloid physics :

- Find centers (& characteristics - orientation, aspect ratio, etc.)
- Link coordinates and characteristics to form trajectories.
- Trajectories reconstruct the original movie's moving bacteria

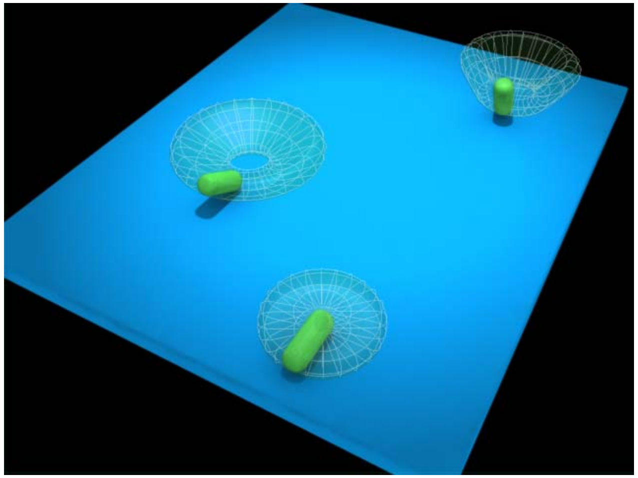


Particle-tracking reference: J. C. Crocker and D. G. Grier, J. Colloid Interface Sci. **179**, 298 (1996)

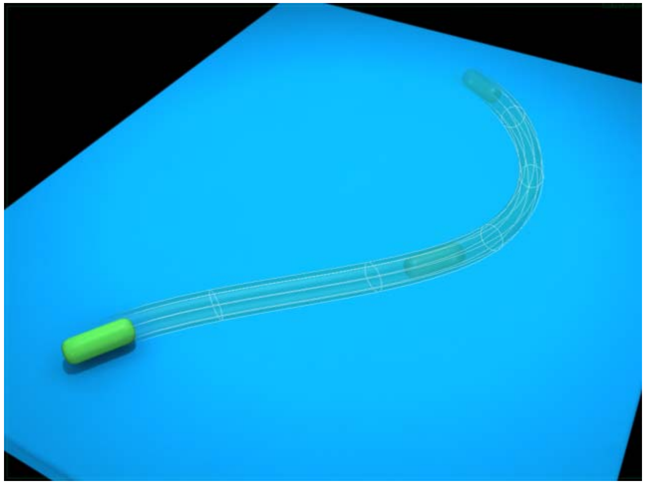
Tracking identifies distinct motility modes



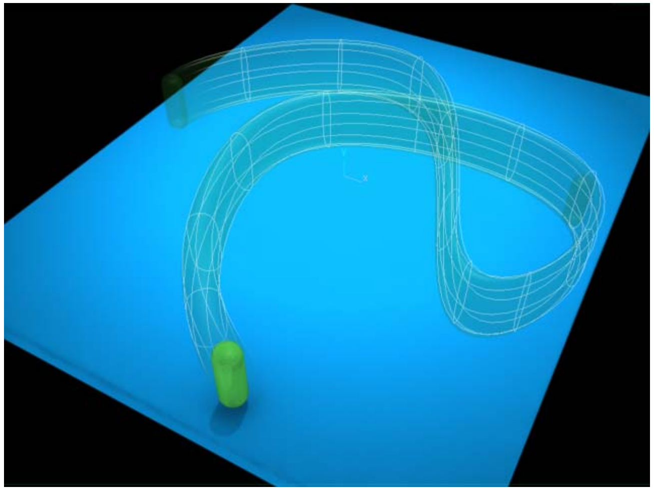
Flagellum-based "skimming"



Flagellum-based "Spinning"



Pili "Crawling" motility

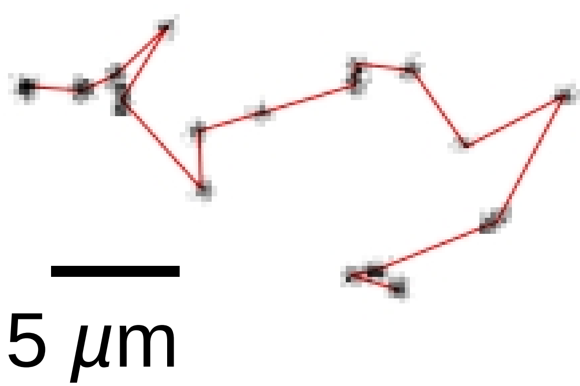


Pili "Walking" motility

M. Gibiansky, J. Conrad, et al., Science 2011

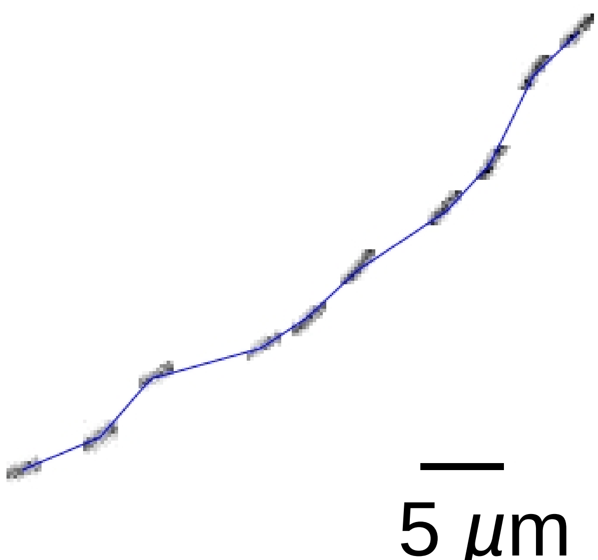
"Walking" motility

- Oriented perpendicular to the surface.
- No preferred direction of motion.
- *'short persistence length'* trajectories



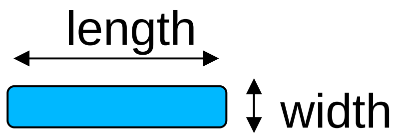
"Crawling" motility

- Oriented flat on the surface.
- Move along their body axis.
- *'long persistence length'* trajectories.



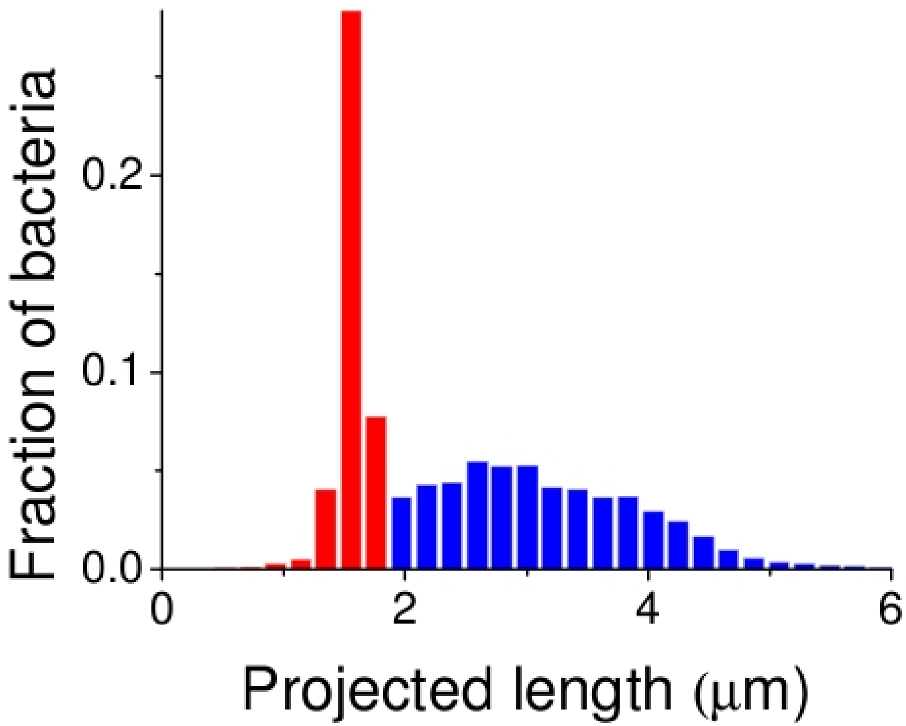
Motility modes have signature orientations as well as trajectories

- Two peaks in the X-Y projected length
  - correspond to the average width and length of a bacterium.



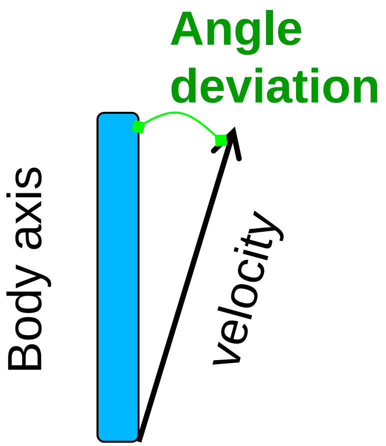
- Up to 50% of bacteria are “walking.”

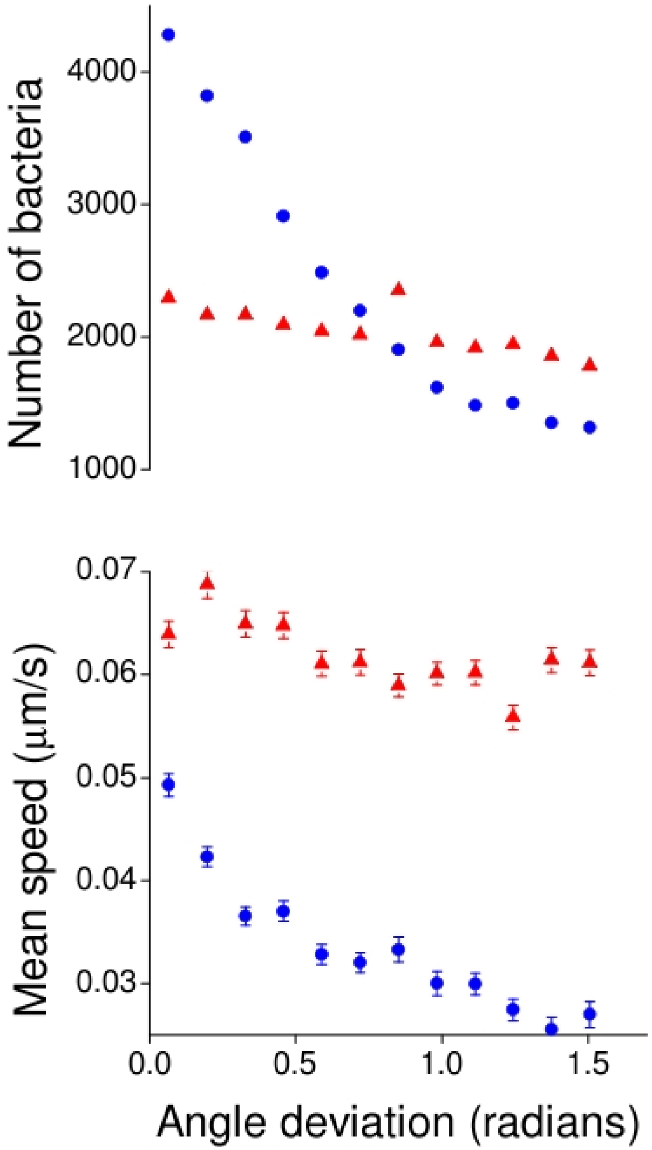
in the flagella-deficient fliM strain



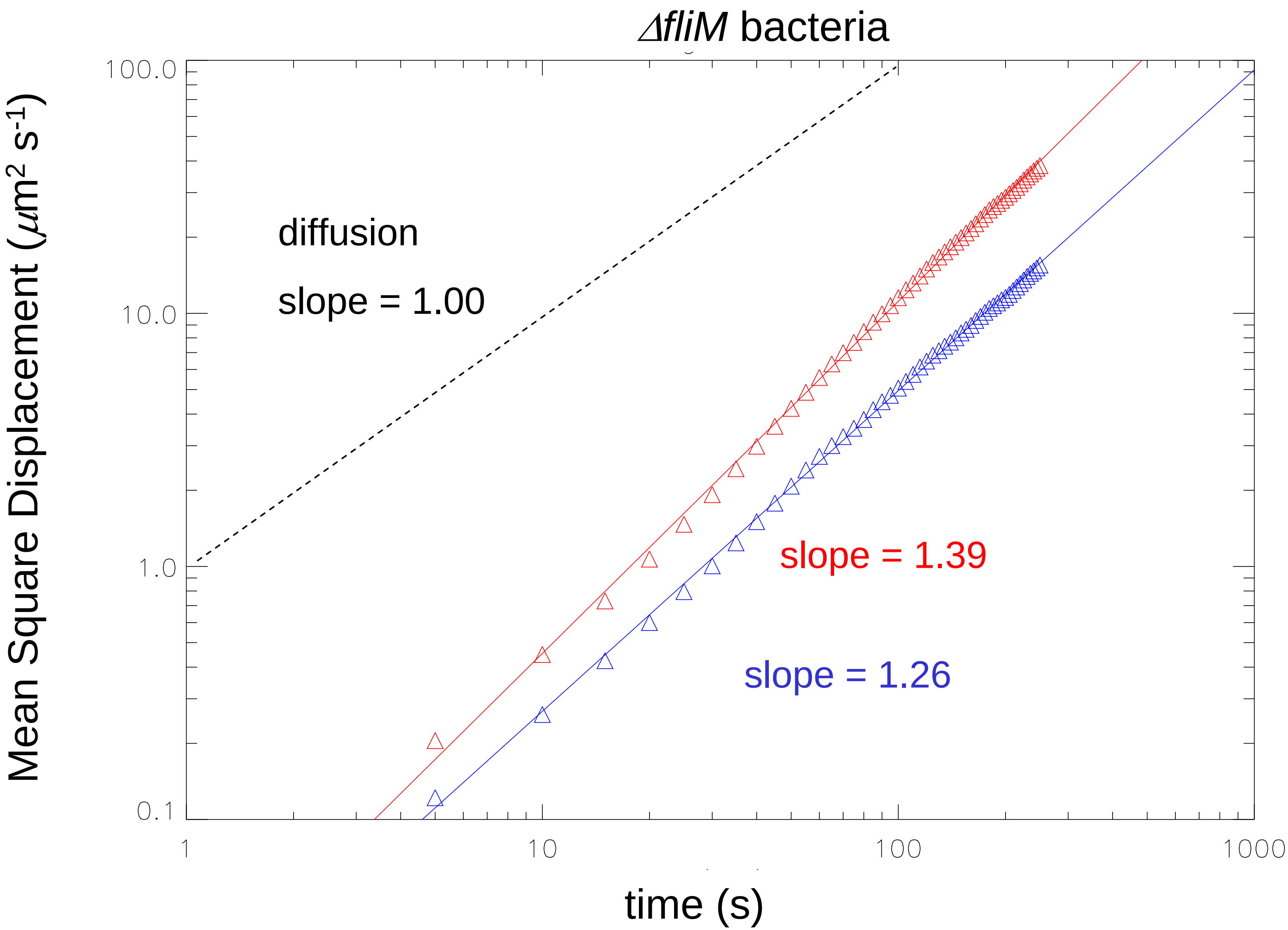
Motility comparison

- "Crawling" has a preferred direction
- "Walking" has a higher average instantaneous velocity





Walking, Crawling both superdiffusive



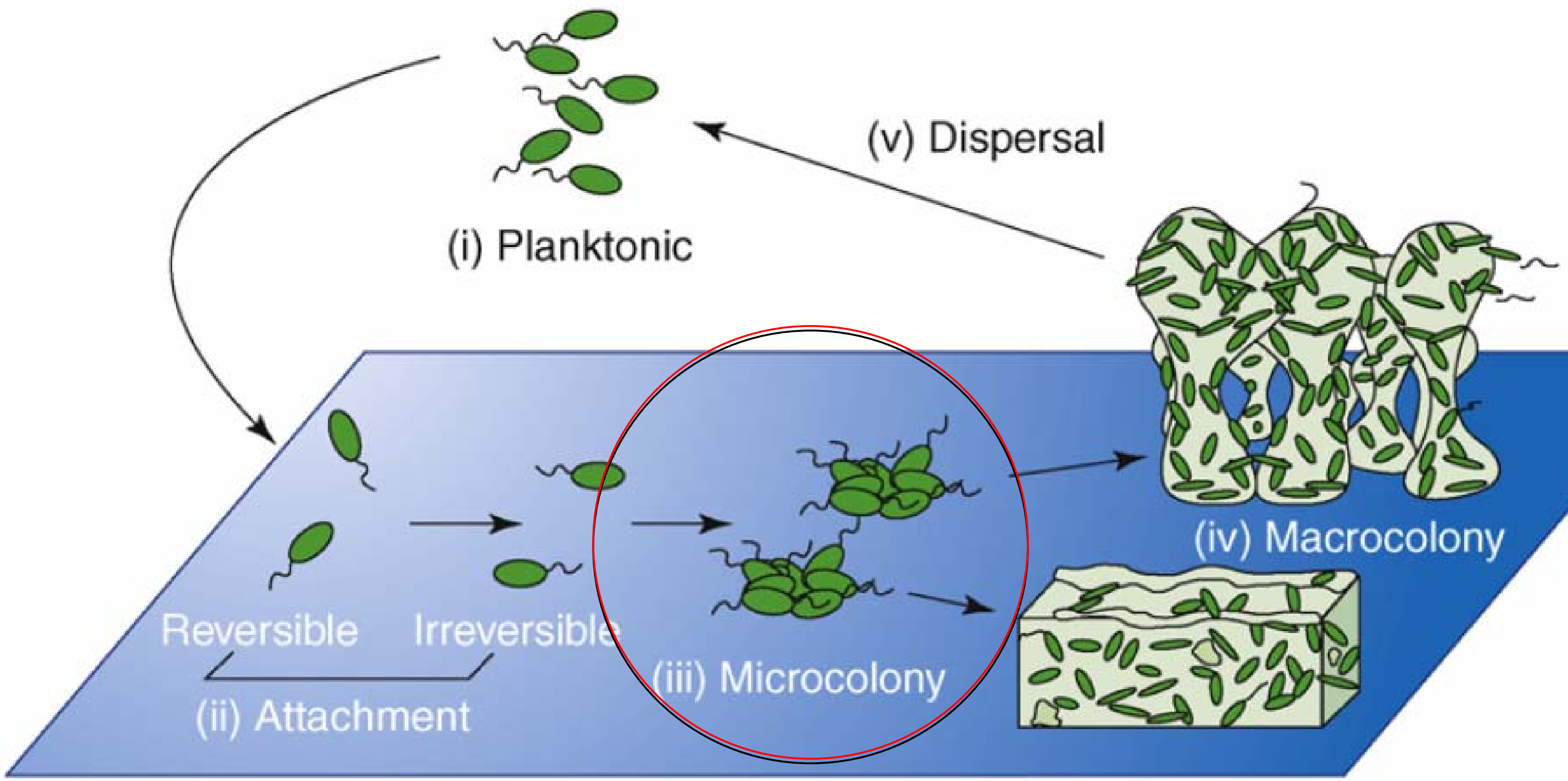
What we've learned:

- There are two pili-driven surface motility modes, flat "crawling" and vertical "walking".
- "Walking" is not directional (short persistence length), and allows the bacterium to explore its local environment.
- "Crawling" has a preferred direction.

Question 2

WHAT ARE THE ROLES OF EXTRACELLULAR POLYSACCHARIDES IN BIOFILM FORMATION?

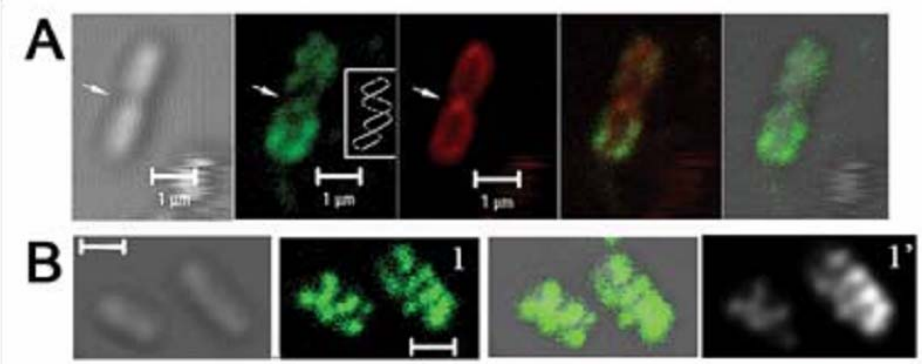
Canonical Picture of Biofilm Formation



Cells in microcolonies stick to each other.

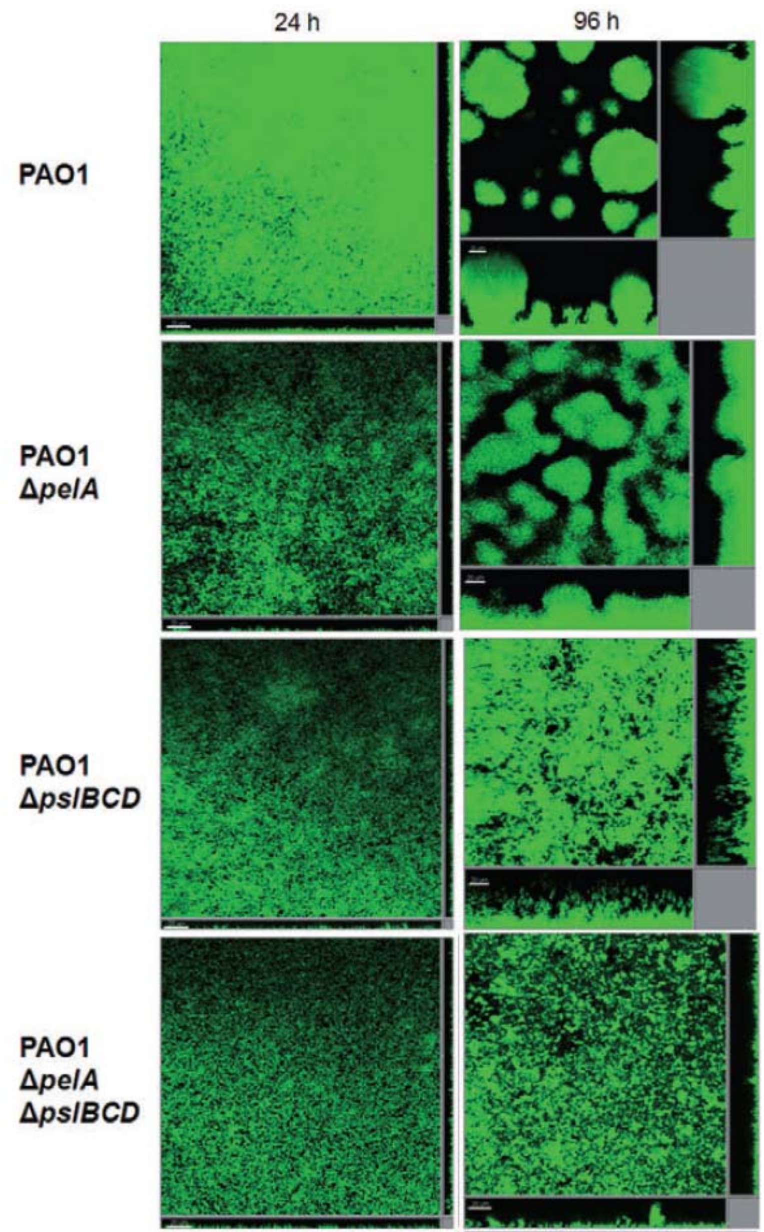
Figure from Monds and O'Toole, Trends in Microbiology 2009

Previous work: Psl -> surface adhesion, Pel -> self cohesion



Ma, *et al.*, PLOS Pathogens **5**, 1000354 (2009)

- Psl (above) forms helical structures around surface of bacteria
- Structure Pel makes is unknown
- Previous studies showed two distinct roles for Pel and Psl in biofilm formation



Yang, *et al.*, Environmental Microbiology **13**, 1705 (2011)

Open question:

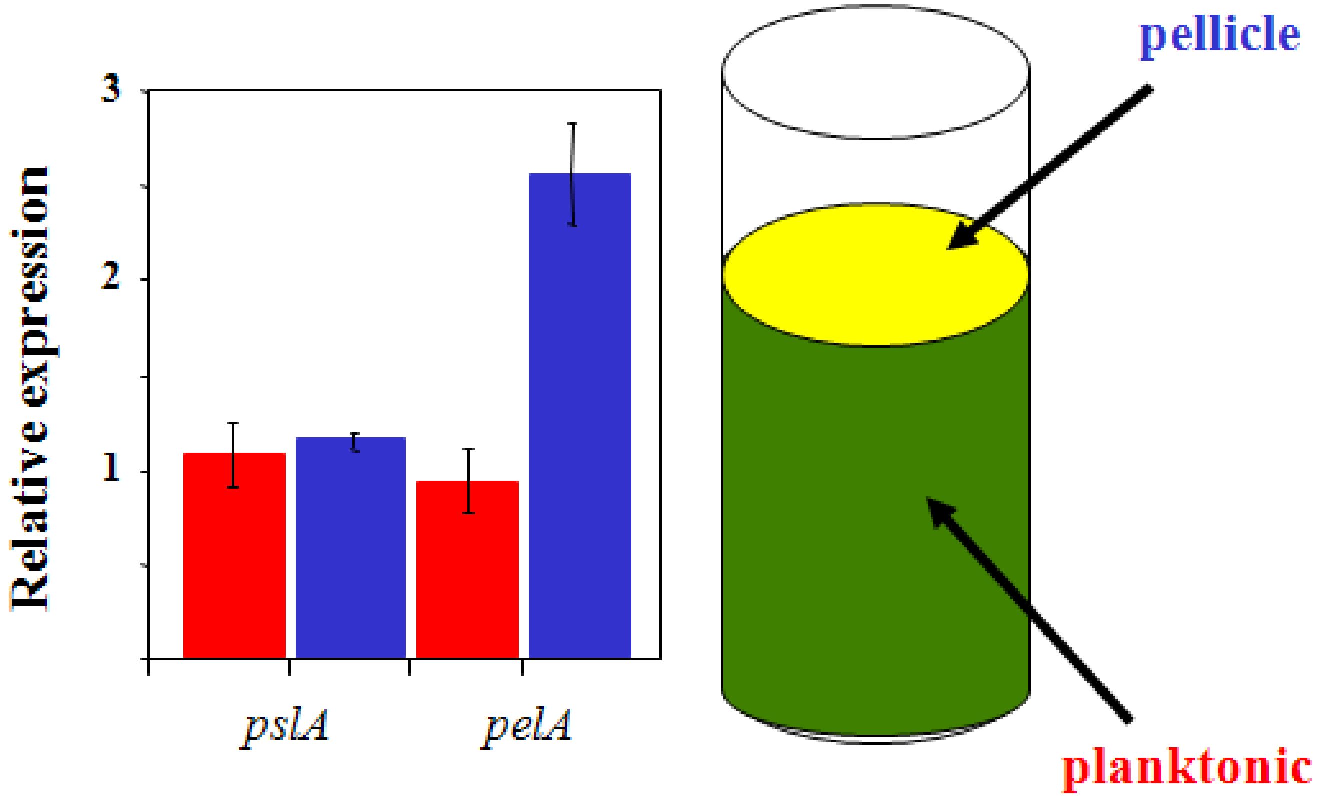
What are the key initial steps for microcolony formation and biofilm initiation?

Bacteria must

- **sense they are at a surface**
- **initiate production of some EPS**

  many possible candidates
- **interact specifically with other bacteria**

pel expression is induced in pellicles formed in standing liquid cultures



Data from Borlee and Parsek, University of Washington, Seattle

pelA expression induced after surface adhesion

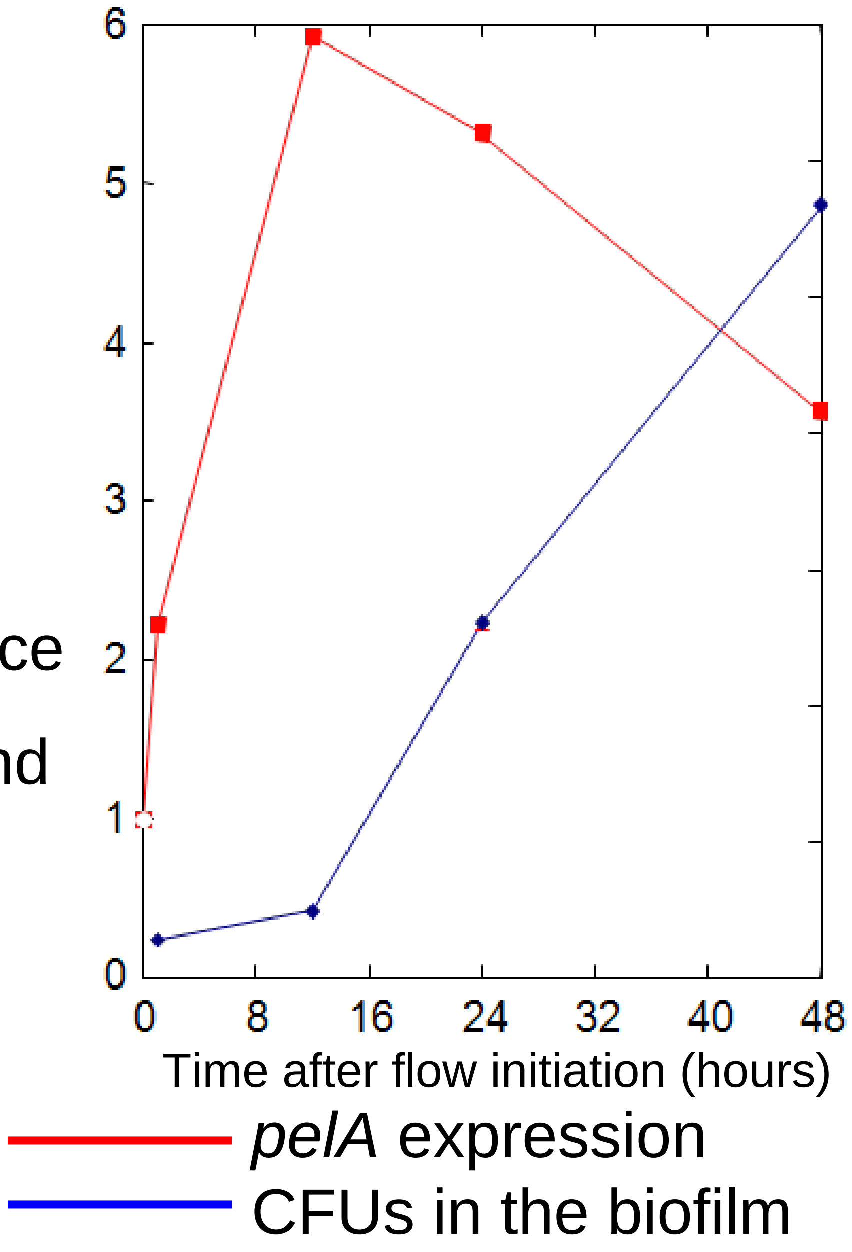
P. Aeruginosa biofilm grown in a silicone tube:

- incubate statically for 30 min
- begin flowing fresh medium
- adherent cells harvested off surface

monitor gene transcription levels and viability of cells in biofilm:

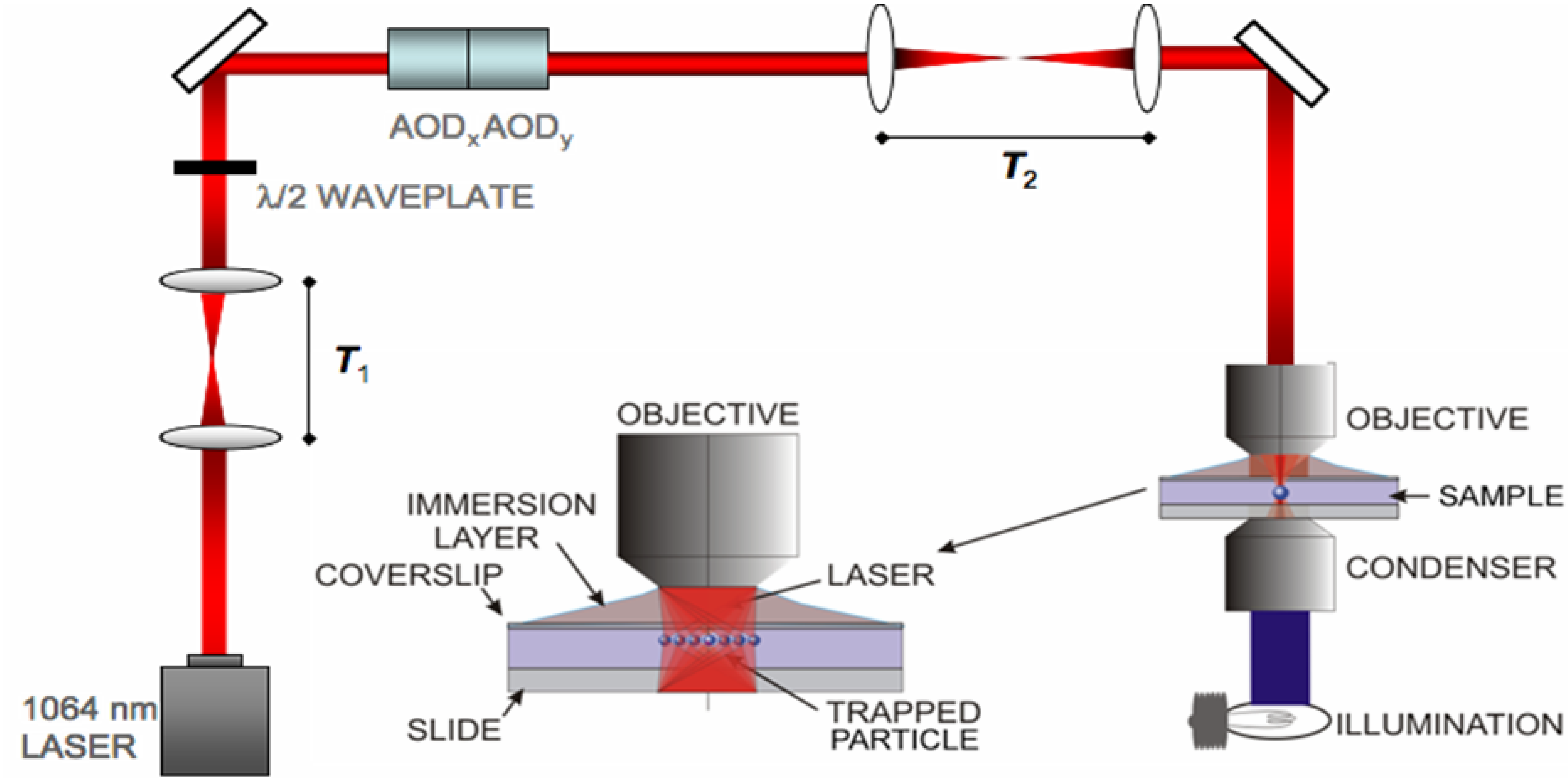
Pel turns on early in biofilm development, but turns off as the biofilm matures.

Data from Borlee and Parsek, University of Washington, Seattle



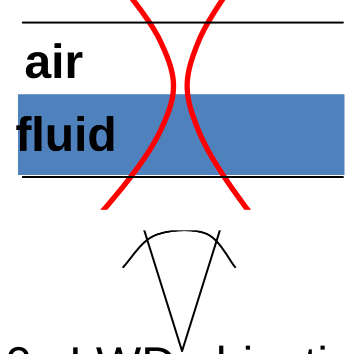
CFU = colony-forming unit (typically 1 cell)

Laser-trapping setup



- Built on inverted microscope
- Simultaneous trapping and imaging in brightfield transmission or fluorescence

Laser-directed aggregation

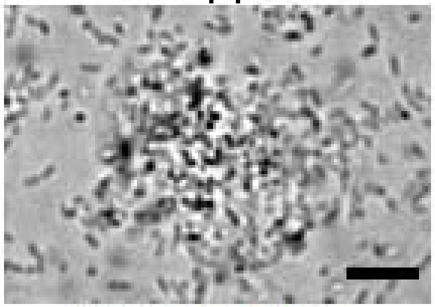


40x LWD objective

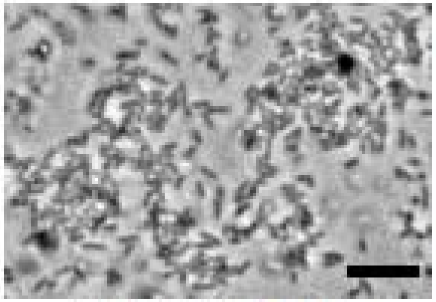
Making Pel is essential for bacteria aggregation on short timescales!

trapped | released

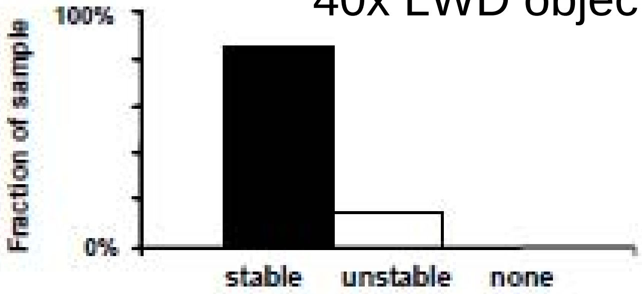
Pel



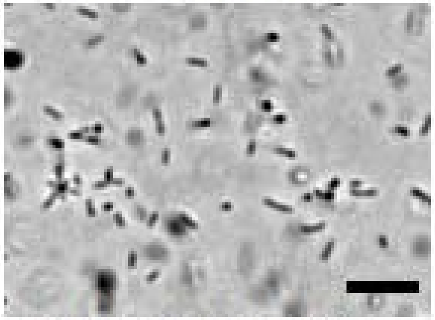
PA14 trapped 20 min



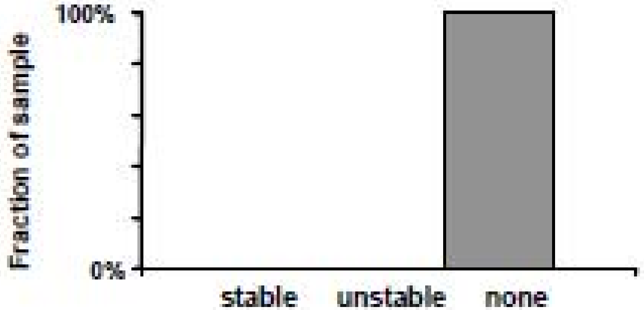
PA14 released 5 min



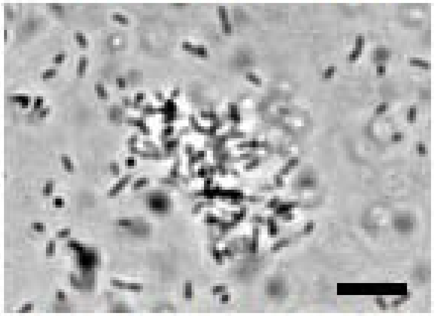
No Pel



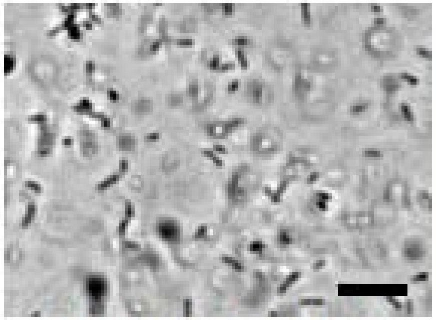
PA14Δ*pelB* trapped 20 min



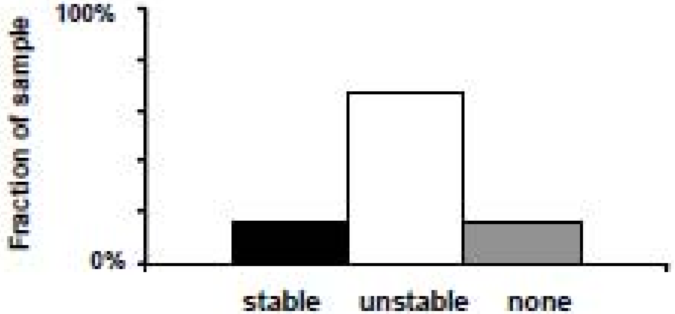
No Pel



PA14Δ*pelB* trapped 45 min



PA14Δ*pelB* released 5 min

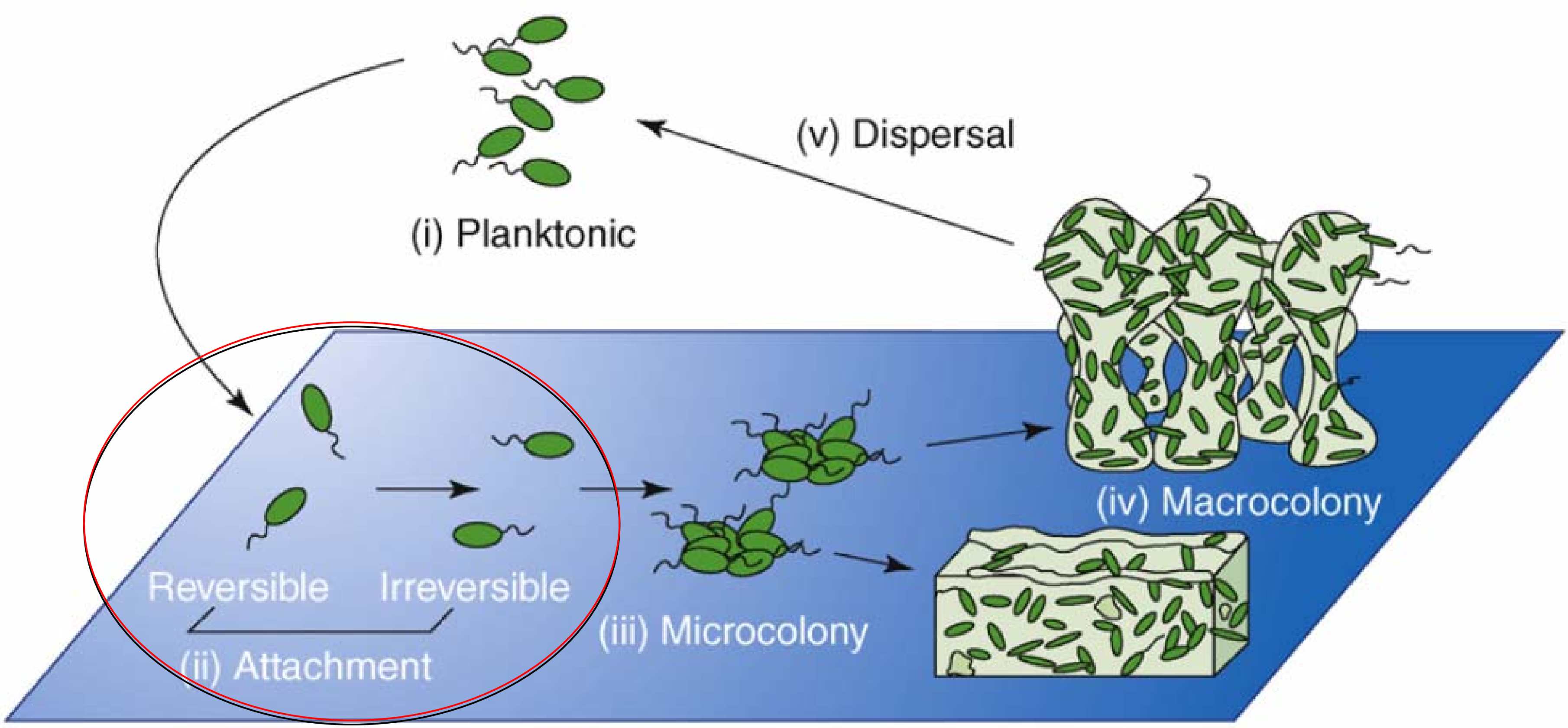


What we've learned:

- *pel* is the molecular glue first activated
- *pel* is responsible for inter-bacterial adhesion early in biofilm development

K. M. Colvin, V. D. Gordon, *et al*, PloS Pathogens 2011

Canonical Picture of Biofilm Formation



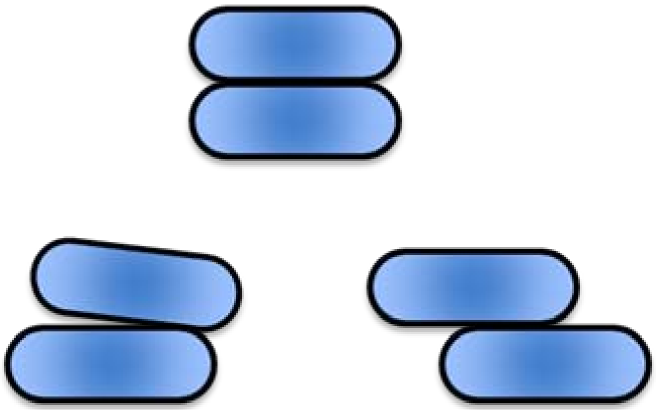
Cells land end-on, and lie down flat as part of irreversible attachment.

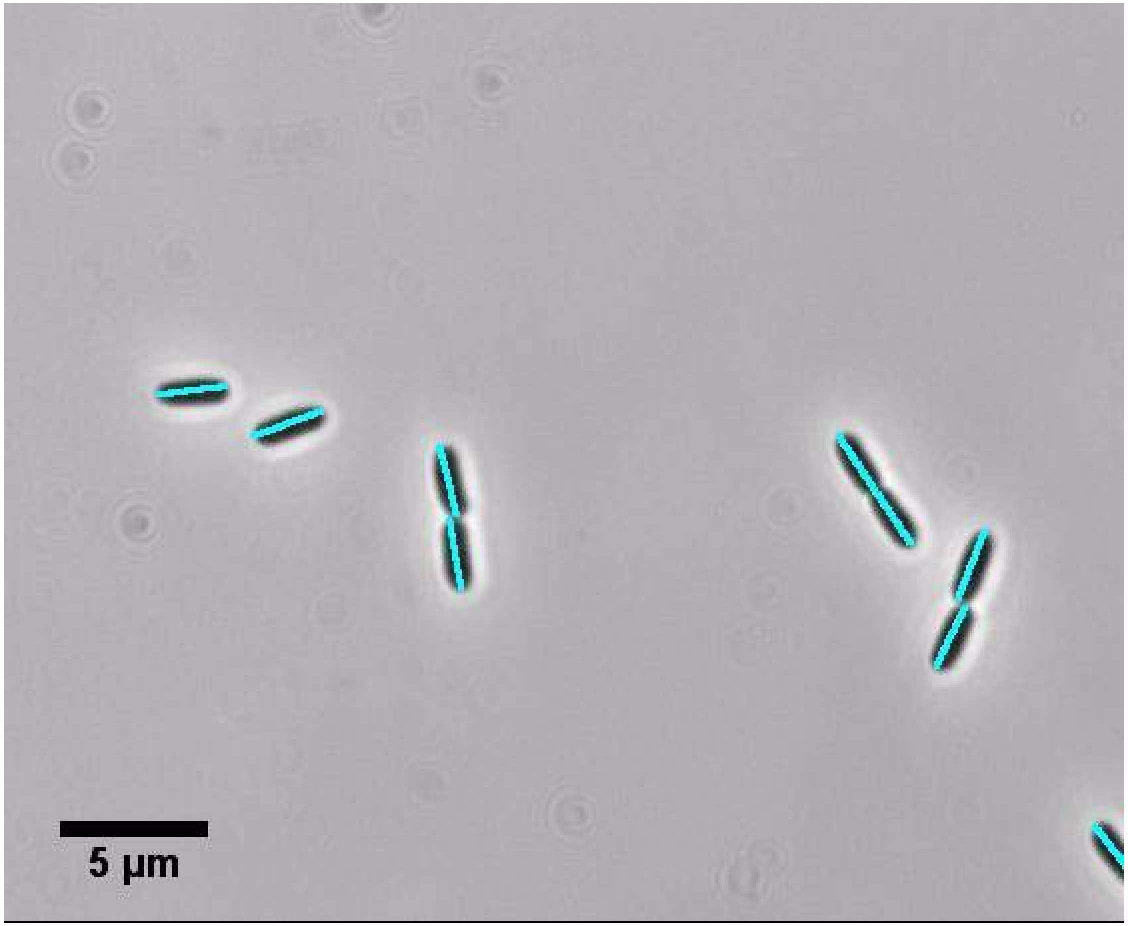
Figure from Monds and O'Toole, Trends in Microbiology 2009

Measuring effects of EPS in very early biofilms

- Tracking code identifies individual bacteria and outputs position, speed, direction, length, aspect ratio

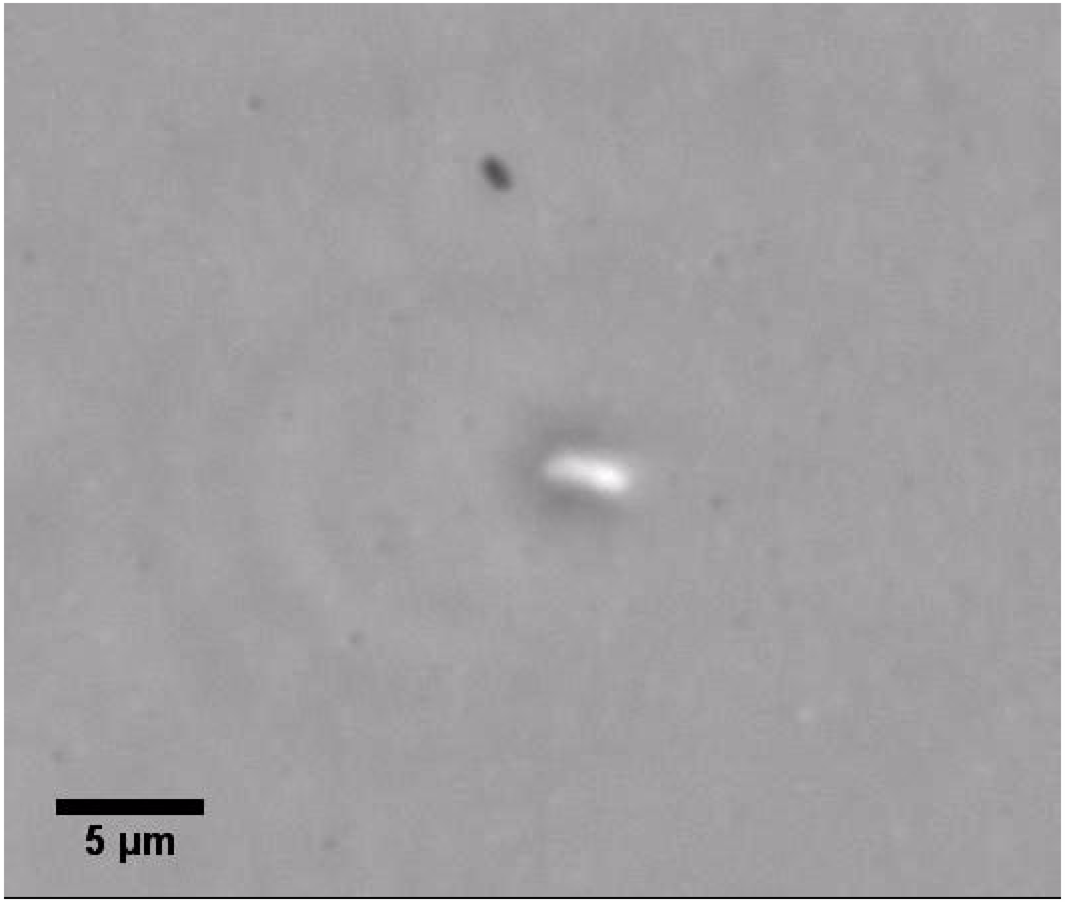
Identify self-cohesive bacteria (side-by-side)

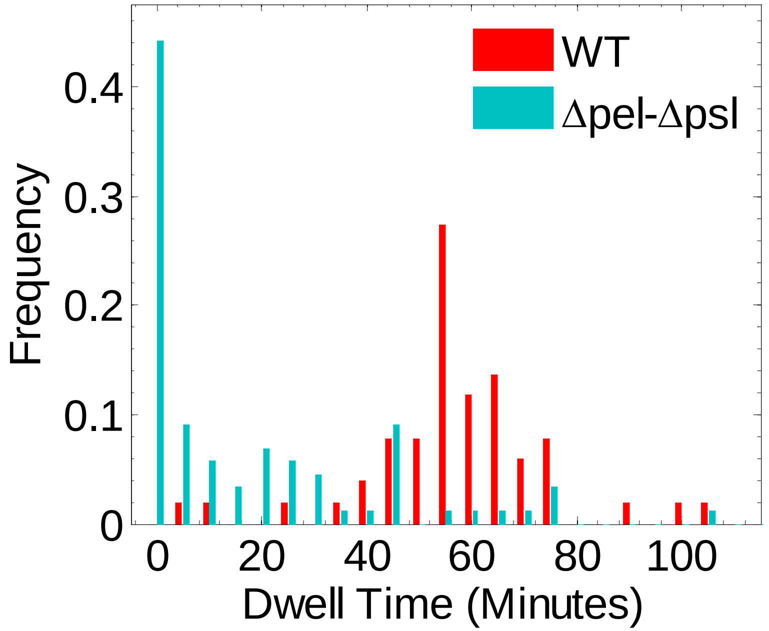


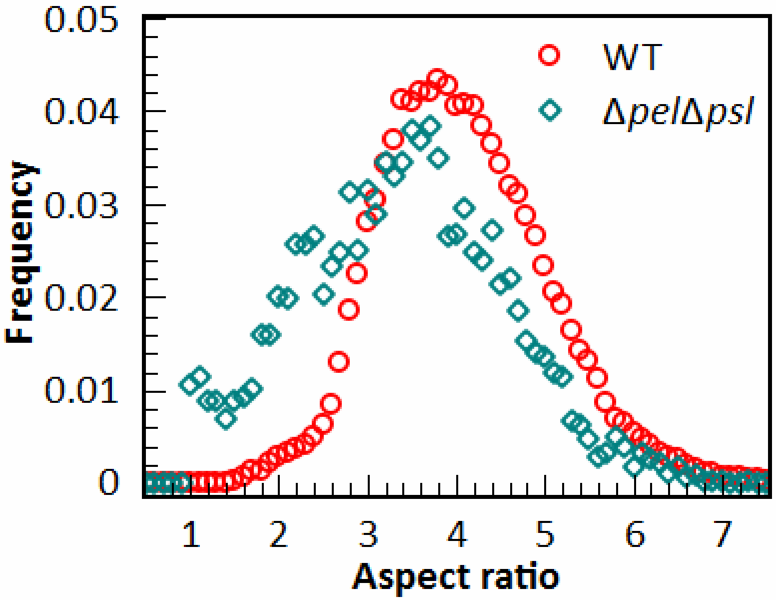


Δ*pel*Δ*psl* has severely impaired surface adhesion

- Agrees with previous results

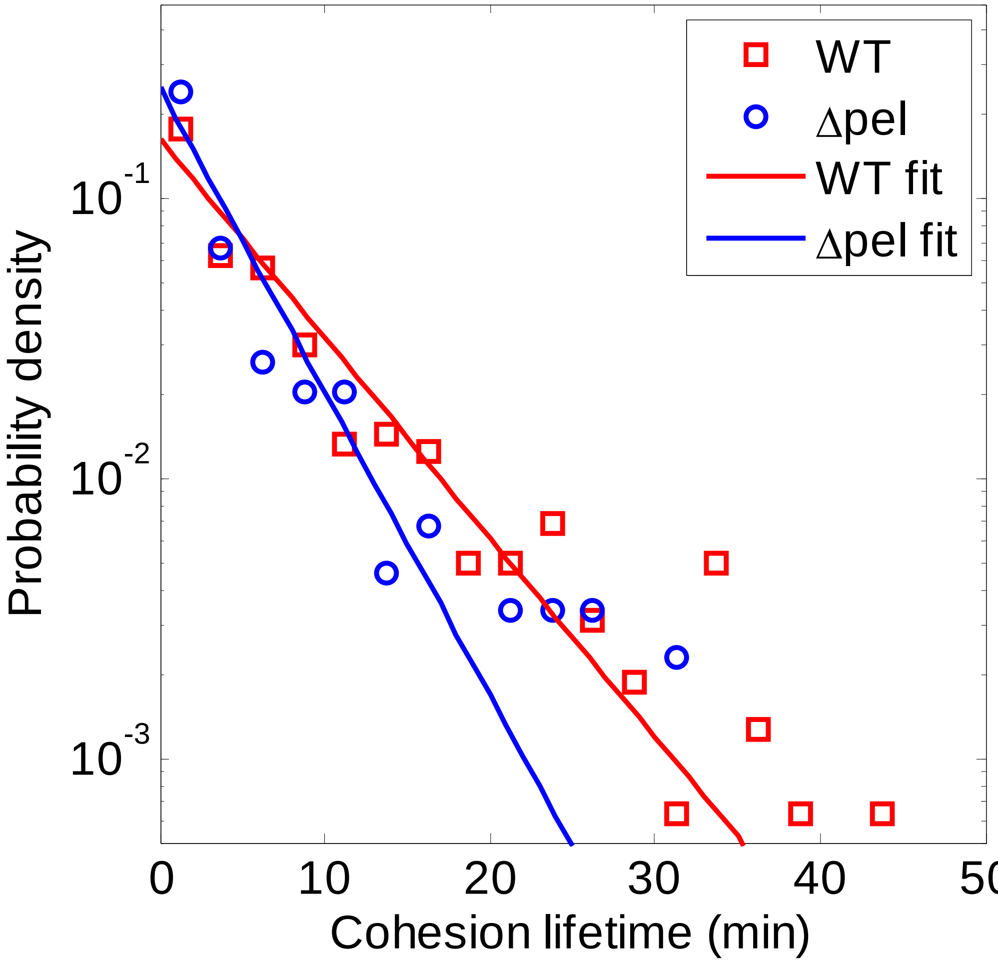




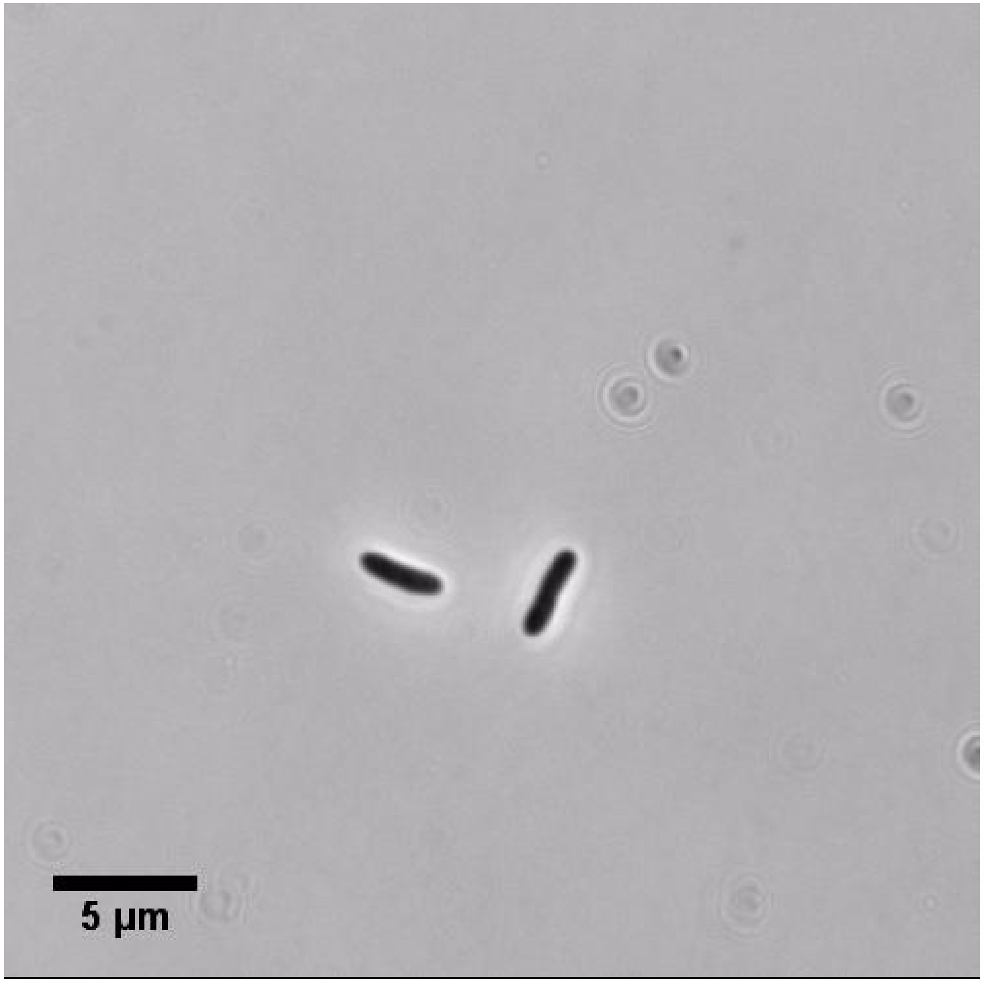


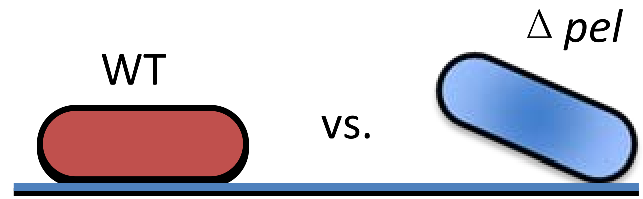
Pel increases self-cohesion lifetime

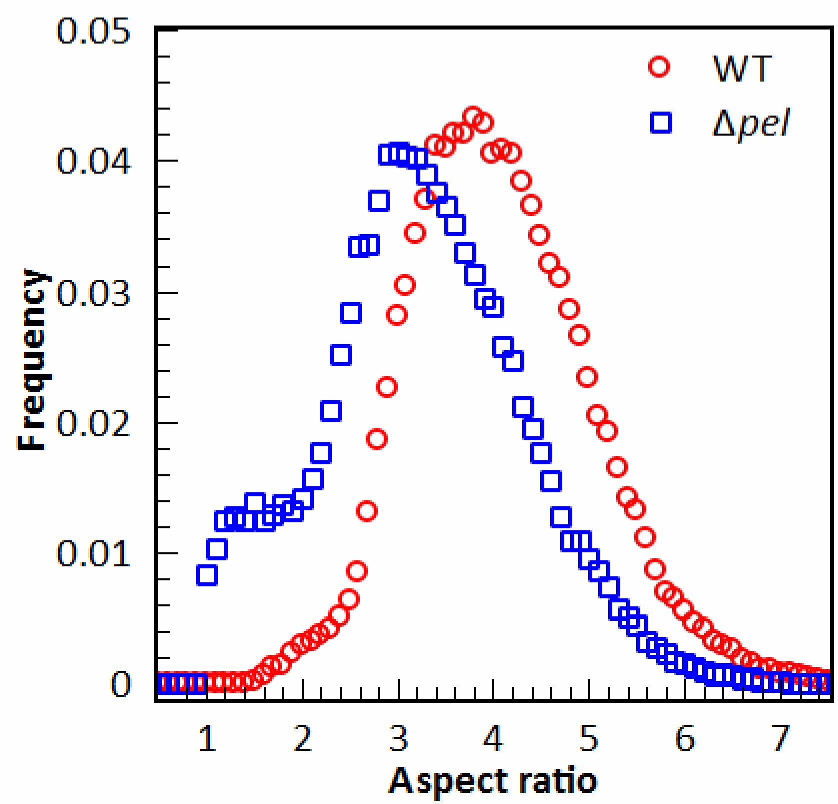
- WT cohesions last longer than those of Δ *pel* mutant
- Exponential fit decay constants:
  - WT: 6.1
  - Δ *pel*: 4.0
- Number of cohesions greater than 30 min:
  - WT: 15
  - Δ *pel*: 0
- Percentage of cohesions longer than 5 min:
  - WT: ~42 %
  - Δ *pel*: ~25 %



Surprise! Pel also mediates surface adhesion!

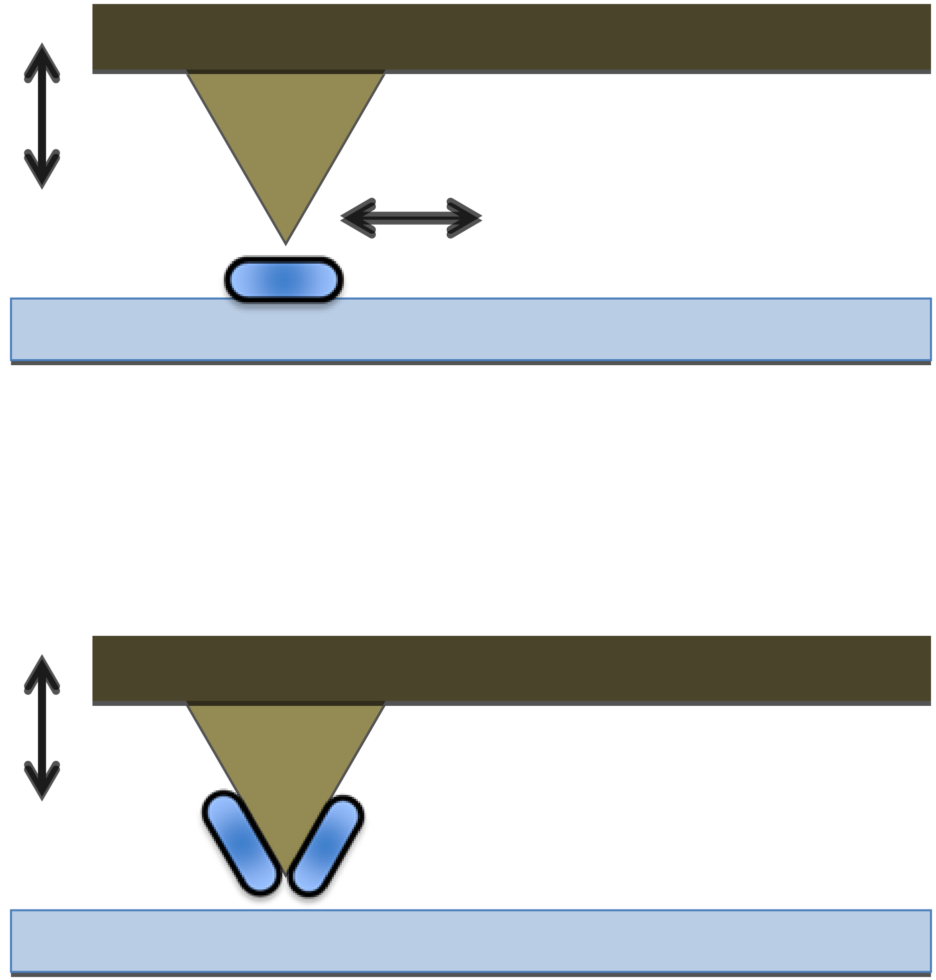




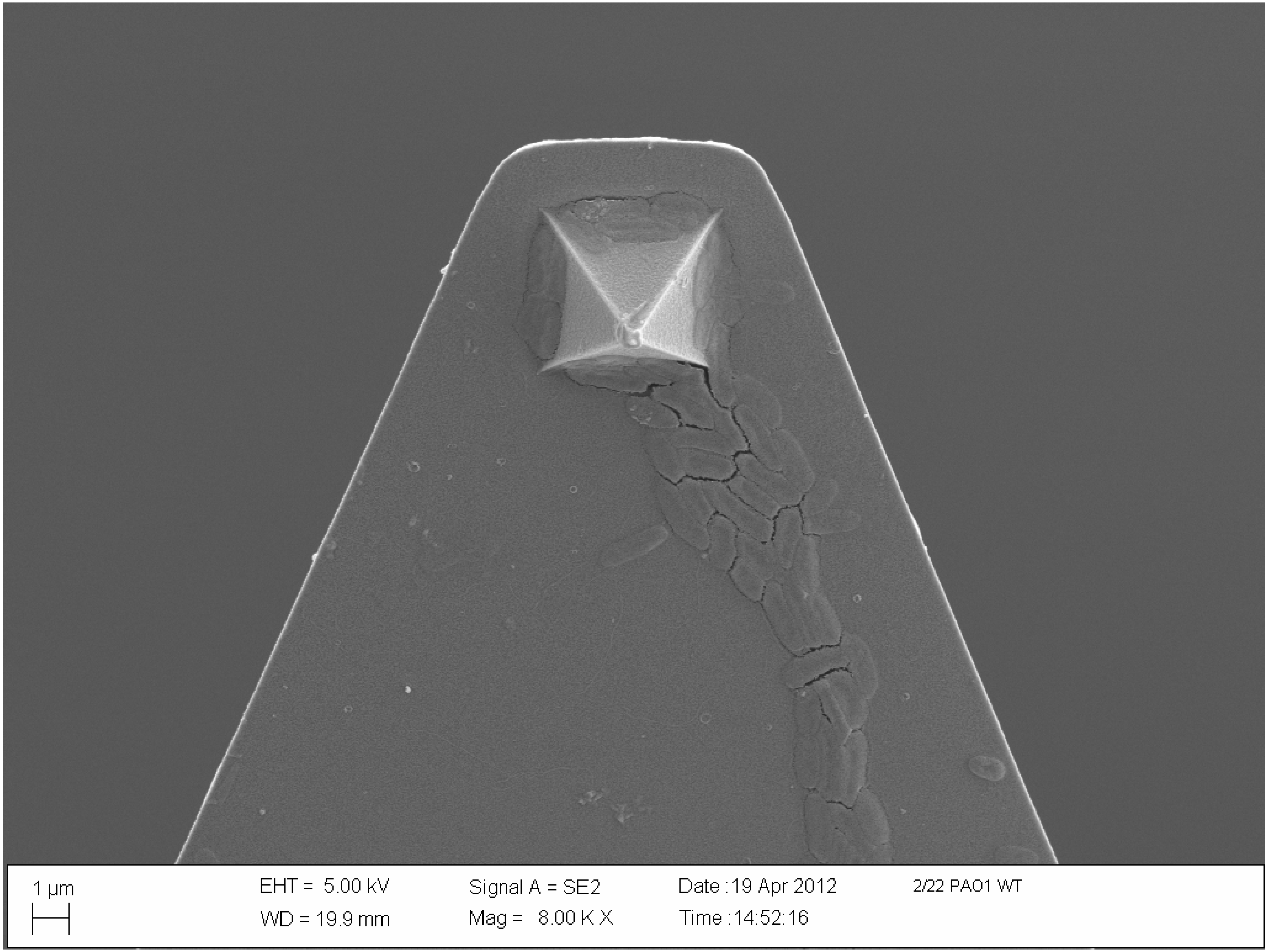


AFM adhesion force measurements

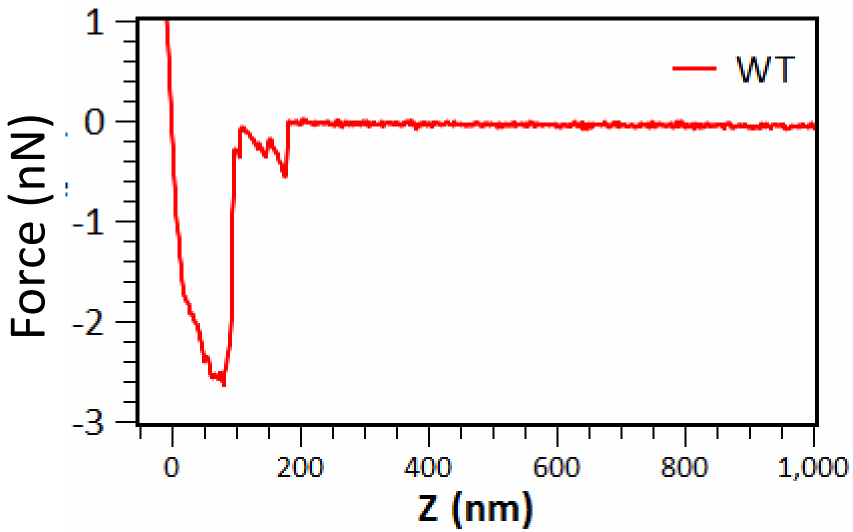
- Directly measure difference in adhesion between WT and mutants
- Two methods
  - Attach bacteria to surface
  - Attach bacteria to tip
- All measurements done in liquid with live bacteria



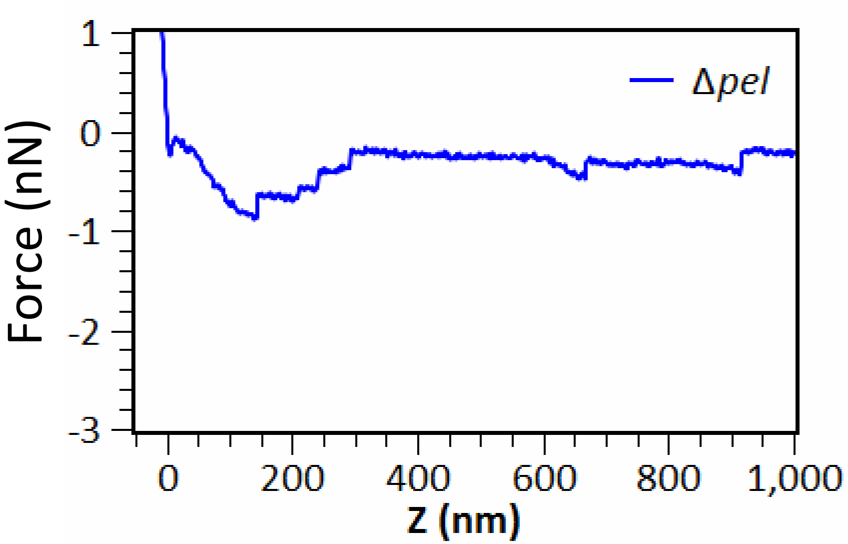
Our method: bacteria attached to tip

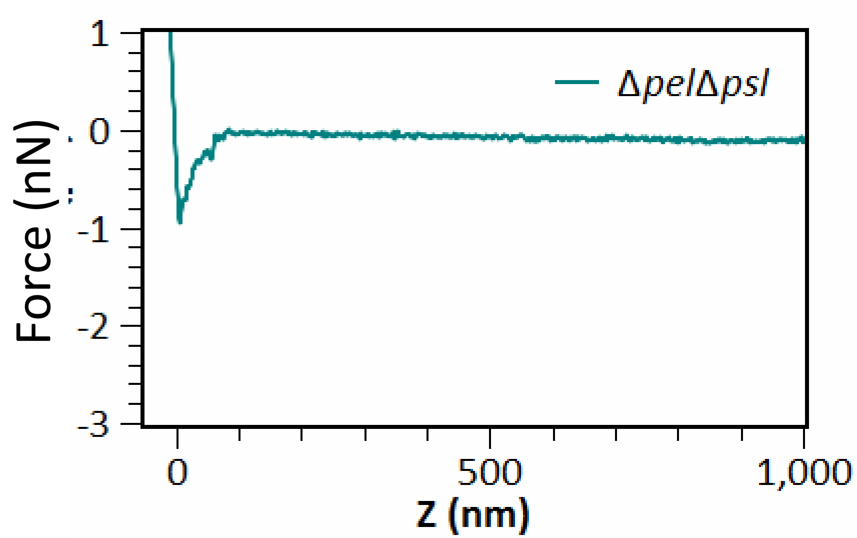


AFM measurements support inferred roles of EPS

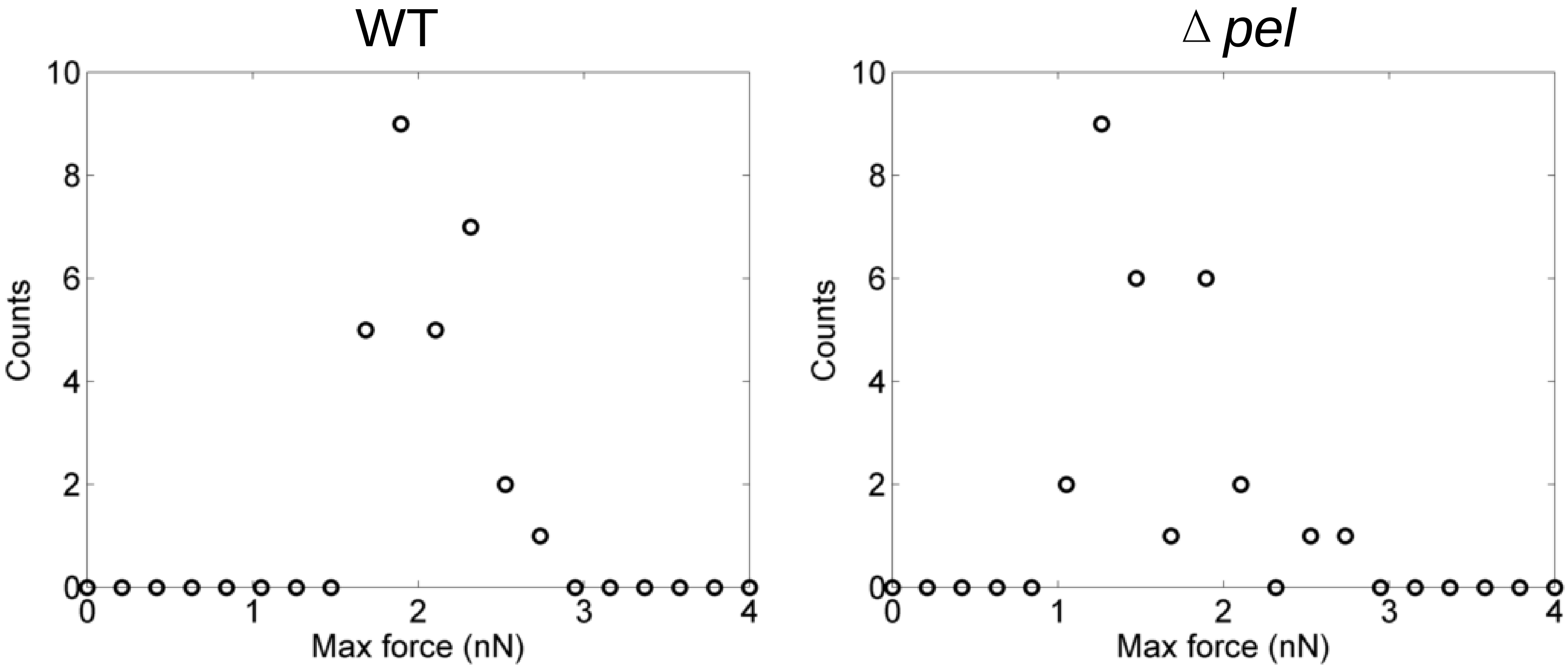


- First time to measure adhesion strength for EPS elements
- Measure adhesion force between bacteria and glass substrate



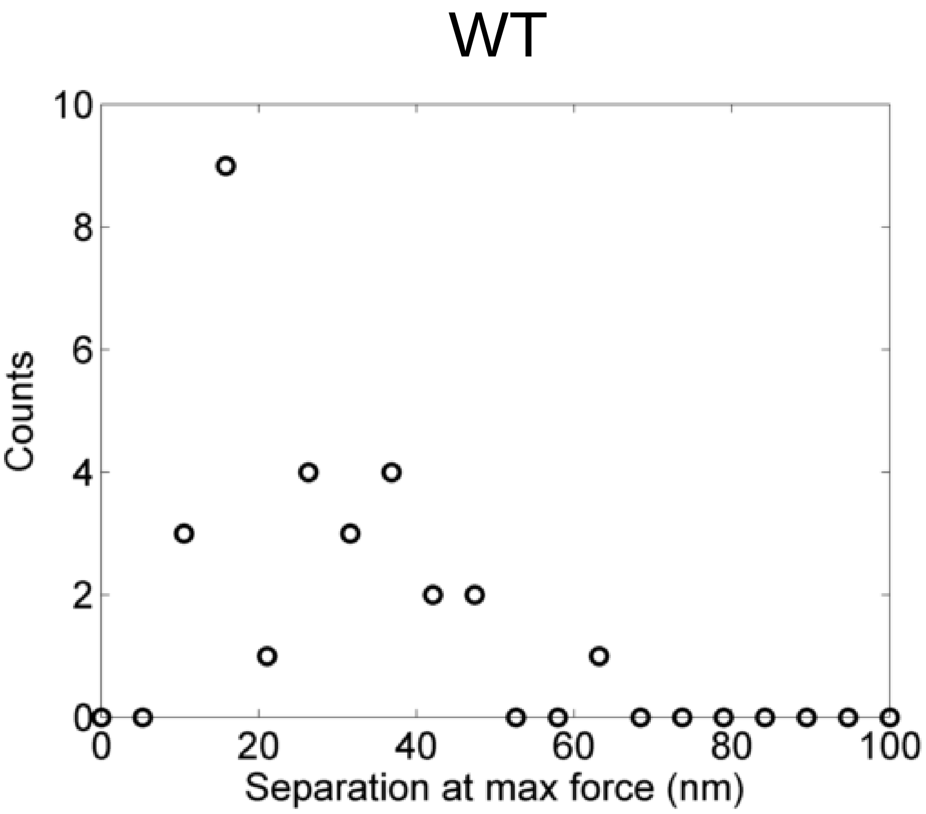


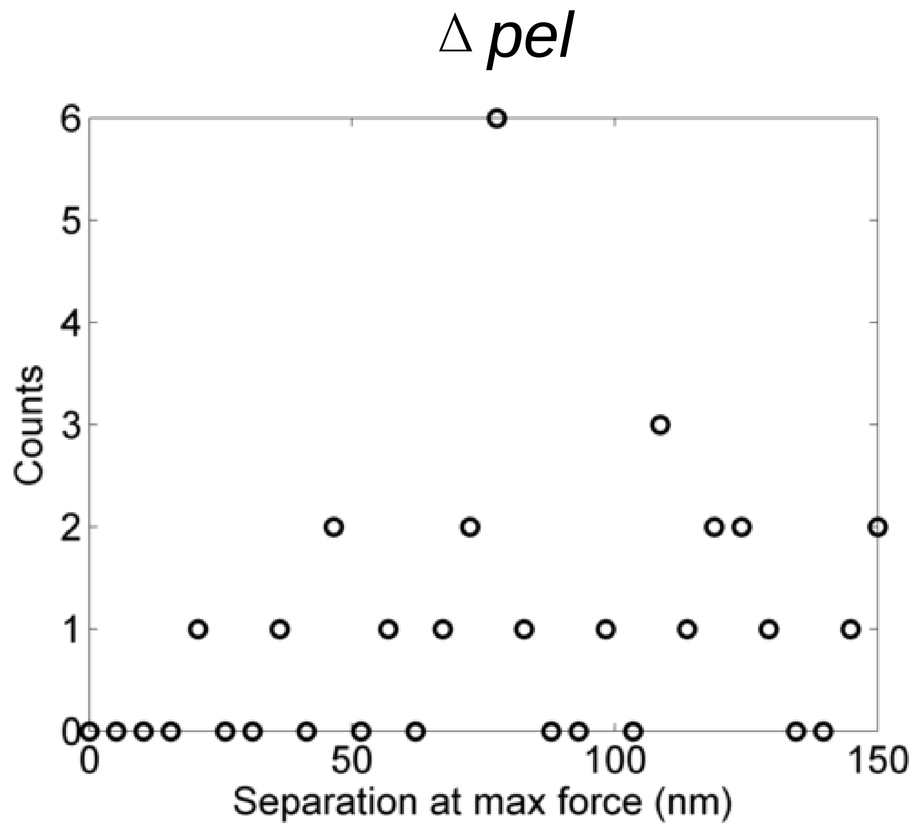
Peak force measurements



Pel contributes about 25% of the maximum adhesion force.

Peak force location measurements

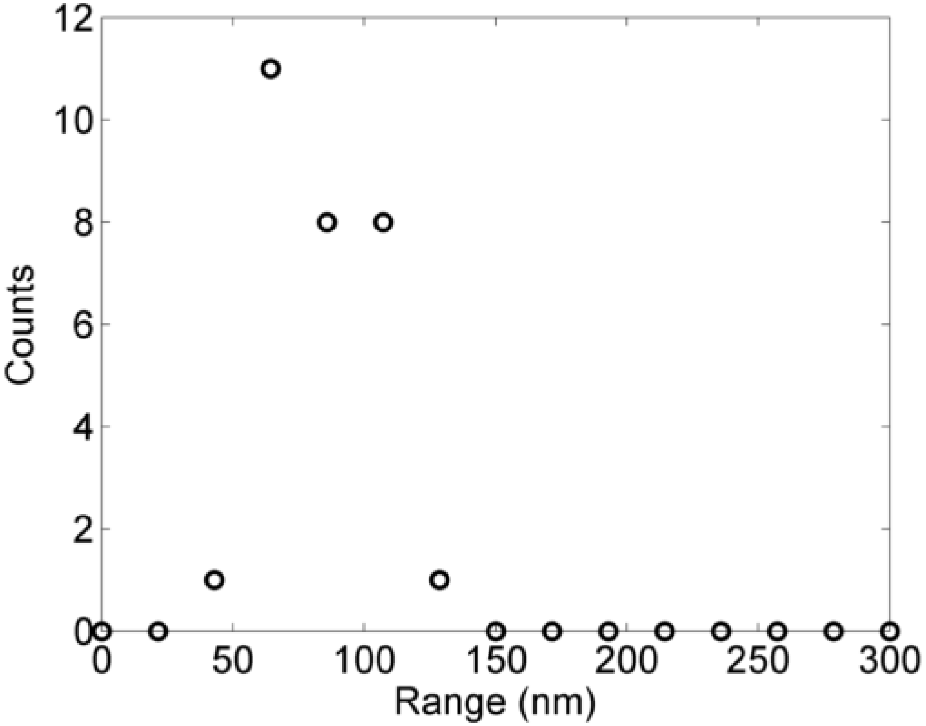




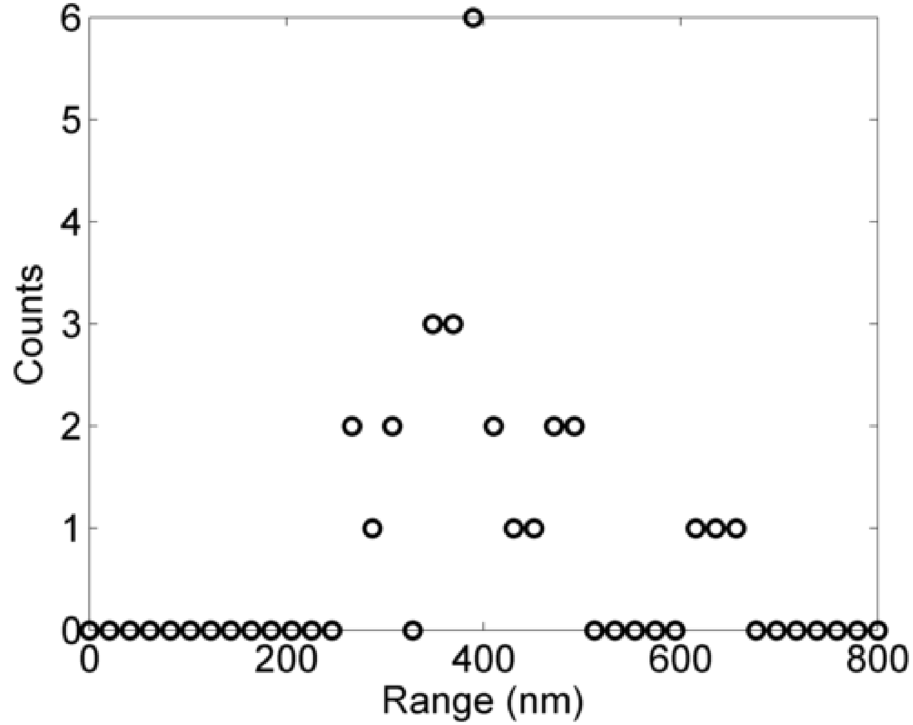
Pel makes the maximum adhesion force location ~4x more short-ranged.

Force range measurements

WT



Δ *pel*



Pel decreases the extent of
the adhesion force ~4x.

What we've learned:

- Pel helps mediate the lying-down associated with irreversible attachment
  - Pel symmetrizes bacterial attachment to surfaces
- Quantitative measurements of EPS-mediated adhesion force.
  - Pel makes adhesion short-ranged.
- (Implicit: Psl mediates non-symmetric attachment – why?)

Summary

- Bacterial biofilms are important medically, and good model systems for multicellularity.

- Distinct surface motility modes allow bacteria to explore space differently.

- Specific molecular glues mediate surface attachment and intercellular cohesion in distinct ways.

Acknowledgements

CNLD
BJ Cooley
Karishma Kaushik
Nalin Ratnayeke
Travis Thatcher
Guillaume L'Her
Erin Reed
Jamie Stuart
April Kissinger
Travis Cormier
Henry Le

Whiteley group
Marvin Whiteley
Aimee Wessel
Matt Ramsey
Aishwarya Korgaonkar

UT Brownsville
Ahmed Touhami
Daniele Provenzano
Boris Ermolinsky

Yale
Sara Hashmi

UCLA
Gerard Wong
Fan Jin
Maxsim Gibansky

NOTRE DAME
Joshua Shrout

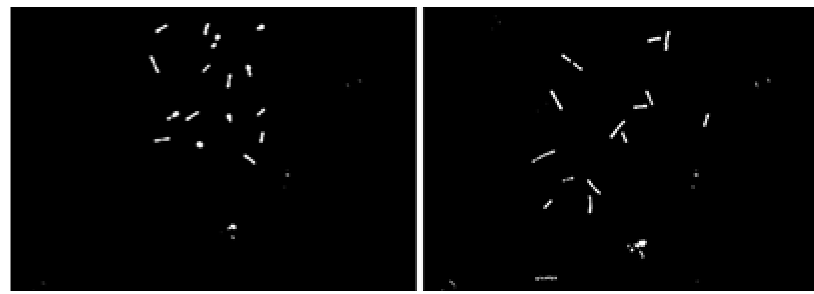
UNIVERSITY OF HOUSTON
Jacinta Conrad

UNIVERSITY OF WASHINGTON
Matthew Parsek
Bradley Borlee
Kelly Colvin

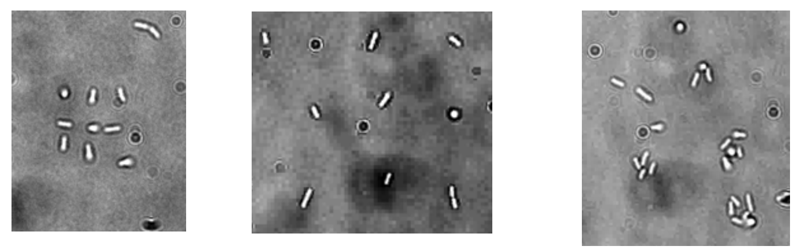
CYSTIC FIBROSIS FOUNDATION

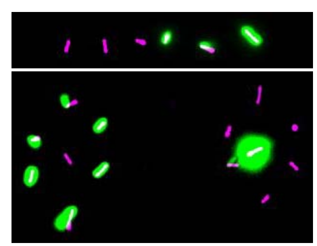
Advertisment

- **Postdoc** to work on a bacteria experiment: how does spatial structure develop in biofilms, and how does this impact cooperation?
  - This 4-investigator collaboration is funded by the Human Frontiers Science Project and is a great opportunity to train across disciplines.



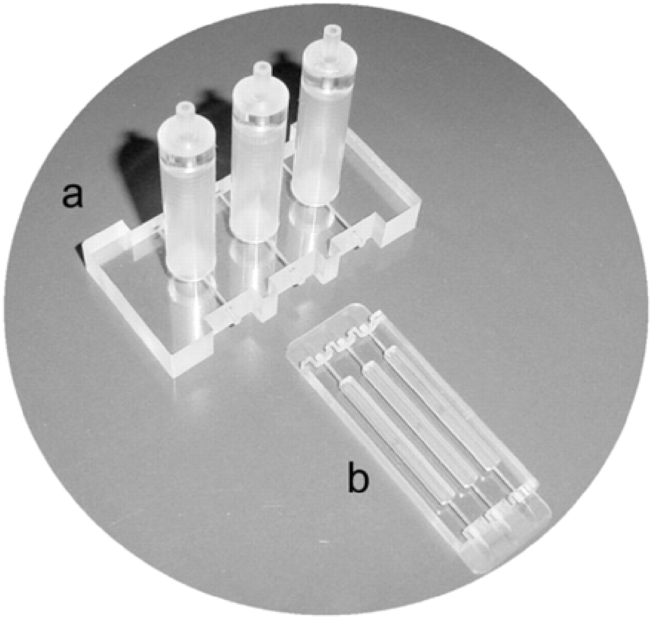
- gordon@chaos.utexas.edu



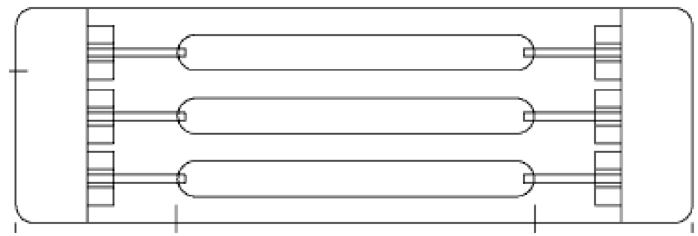


Flow cell experiment

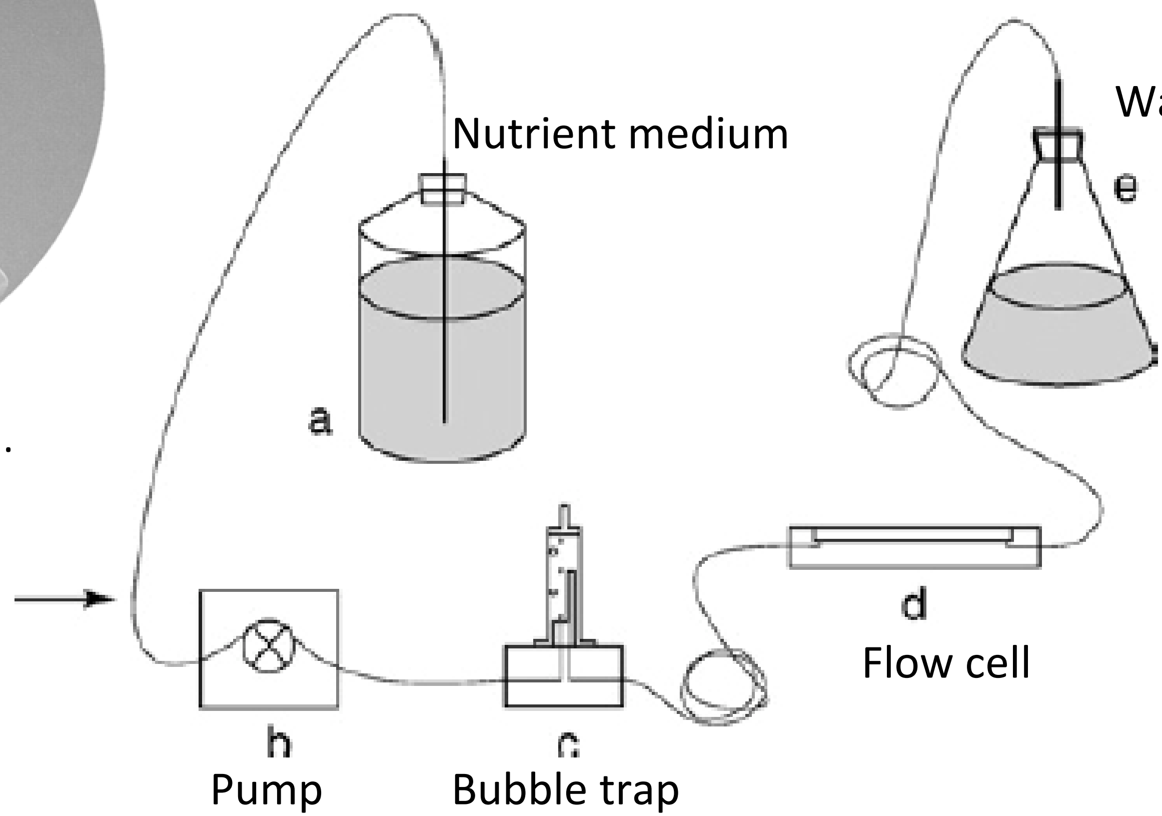
- Static sample chamber useful, but time-limited
- Flow cell provides constant nutrient and oxygen supply



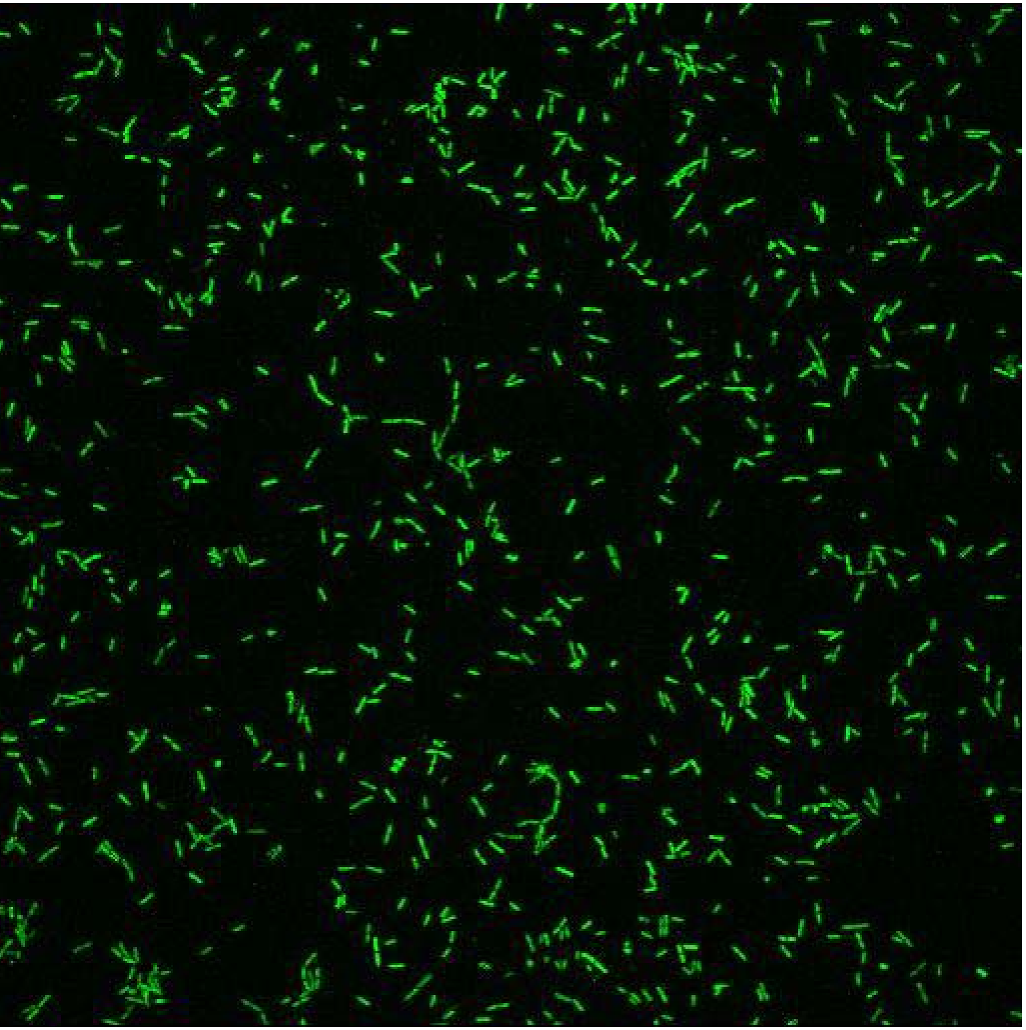
Stapper, *et al*., J. Med. Microbiol. **53**(7): 679–690 (2004)



Sternberg & Tolker-Nielsen, Curr. Protocols in Microbiol. 1B.2.1–1B.2.15 (2005)

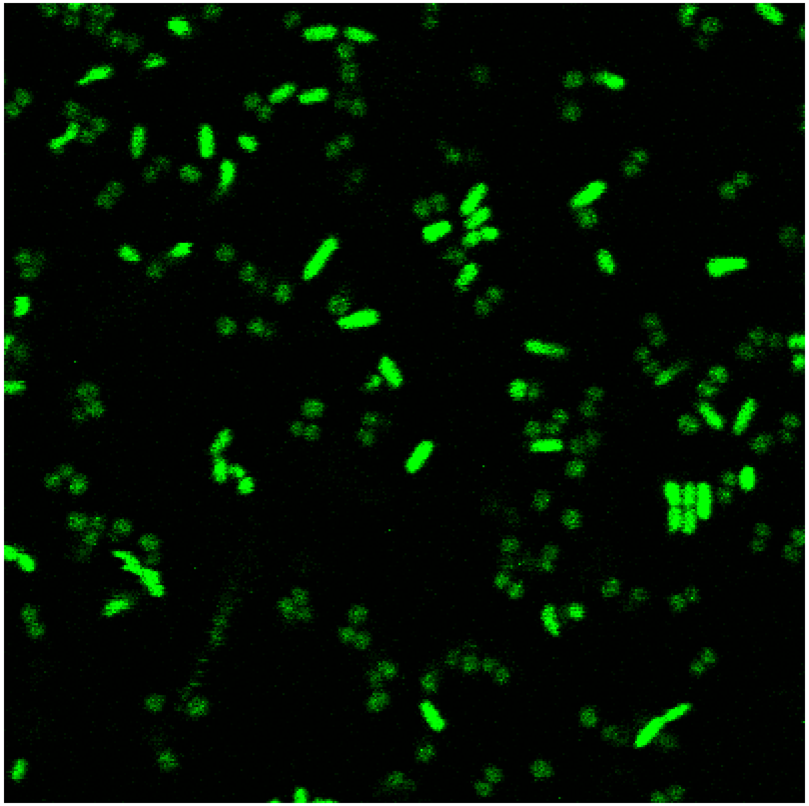


Flow cell plans

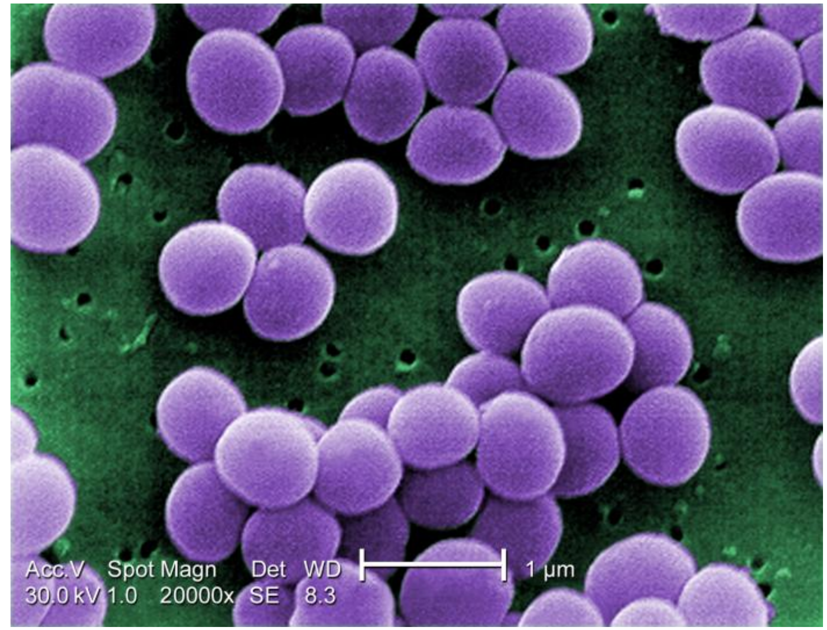


- Use confocal microscopy
- ~18 hour runs (not oxygen limited)
- Start with denser culture than static experiments
- Initial idea: look for similarities to colloid condensation transition
- New ideas and techniques

Staphylococcus aureus coculture



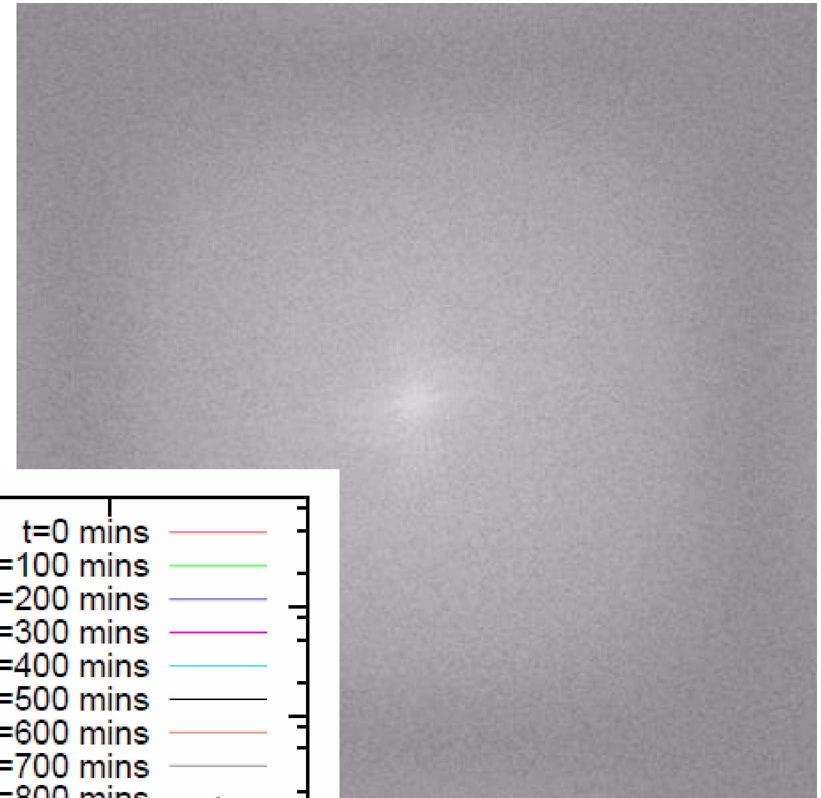
CDC Public Heath Image Library
http://phil.cdc.gov/phil/details.asp?pid=11157

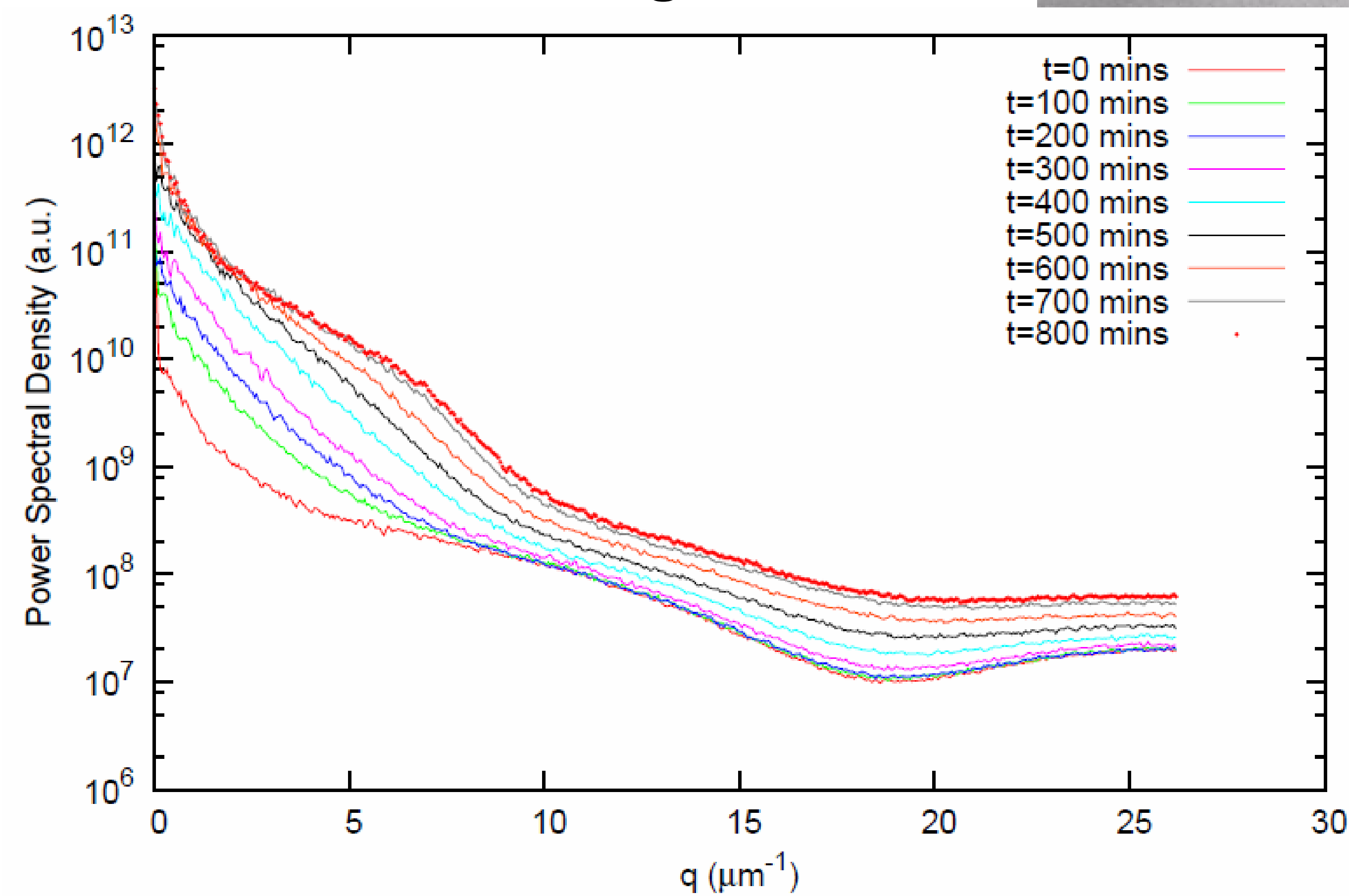


- S. aureus and P. aeruginosa both present in CF lung
- Evidence that P. aeruginosa can lyse Staph for iron
  - Mashburn, et al. *J. Bacteriol.* **187**, 554–566 (2005)
- How does P. aeruginosa biofilm growth change in the presence of Staph?

New analysis

- Power spectrum of each frame
- Azimuthal avg shows features related to cluster growth





With Laurence Wilson, Rowland Institute at Harvard

Flow cell plans

- Testing strains for use in coculture experiments
- Learn to grow Staph.
- Work on analysis (old & new)

Surprise #2: adhesion leads to faster growth

- Faster doubling on surface vs. liquid culture